# How do shuttle and slow-release effects of zinc fertilizers alter zinc diffusion in soil and its uptake by wheat plants?

by

Sevendeep Kaur

B.S., Punjab Agricultural University, 2019

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2021

Approved by:

Major Professor
Dr. Ganga Hettiarachchi

# Copyright

# Abstract

Zinc deficiency is a widespread problem. To correct these deficiencies, different organic and inorganic Zn fertilizers have been used. Various sorption-desorption reactions control the Zn concentration in soil solution and Zn availability from these different sources to crops. The effectiveness of Zn fertilizers varies with soil type. Very soluble Zn fertilizer sources can undergo adsorption and precipitation losses upon soil application. Although these transformations are relatively minor, the chelated Zn source is not cost-efficient and could also be sensitive to leach in alkaline soils. In contrast, sparingly soluble Zn fertilizers can show poorer performance in neutral and low pH soils. Considering all these factors, a new granular Zn mix (mixture containing 60% ZnO, 25% $ZnSO_4$, and EDTA to $ZnSO_4$ 1:8 ratio) fertilizer was recently introduced. The objectives of this thesis work were to compare the efficiency of different Zn only sources in four different soils, two calcareous, one neutral and one acid, with varying pH with varying pH; and compare different Zn-coated urea fertilizers for two soils, one calcareous and one acid soil. Zinc diffusion from the point of application was measured using incubation, visualization, and greenhouse studies. Zinc plant uptake was measured in the greenhouse study using a high-Zn responsive wheat line. Along with the wet chemical analysis, advanced synchrotron-based X-ray absorption near-edge structure technique was also used to understand the Zn fertilizer reaction products better when added as different sources in soil. We found that Zn diffusion and Zn plant uptake were greater for $ZnSO_4.7H_2O$ and ZnEDTA for neutral pH soil. Zinc mix performed similarly to the two most effective ZnO and ZnEDTA treatments in terms of plant biomass and plant Zn uptake in both calcareous soils, which are well known for Zn fixation. There were no significant differences in the Zn sources concerning plant

uptake for acid soil. Regarding Zn-coated urea treatments, ZnO-U and ZnEDTA-U performed well in plant Zn uptake in acid soil. However, no significant differences were observed between any Zn-coated urea sources in calcareous soil and Zn-coated urea sources increased plant biomass and uptake irrespective of the source. Zinc-coated urea sources seemed to provide more plant available Zn due to the enhanced solubility facilitated by its urea co-additive. Results from our studies suggest that the suitable Zn source should be carefully selected for efficient mitigation of Zn deficiencies in different soils depending upon their physicochemical properties.

# Table of Contents

# List of Figures

# List of Tables

# Acknowledgements

I would like to express my deepest appreciation to my advisor, Dr. Ganga Hettiarachchi for all her excellent guidance, care, and encouragement. I would also like to thank her for giving me the opportunity to experience research in soil chemistry and patiently correcting my writings. Thank you to my committee members; Dr. Nathan Nelson, and Dr. Mary Guttieri for their valuable inputs and time for my research. I appreciate all my lab members; Manjot Kaur Rekhi, Dr. Madhubhashini Galkaduwa, Dr. Zafer Alasmary, Kasuni Gamage, Chandima Wekumbura, Ruwandi Kumarasinghe, and Dr. Mohammad Almutari for their considerable support. Special thanks to Dr. Bvandeep Shergill, James Lin, and Kaila Carnley for their love, friendship, and emotional support.

I would like to acknowledge the Department of Agronomy, the Soil Test Laboratory, and the staff for their assistance. A portion of this work was performed at DND-CAT located at Sector 5 BM-D of the APS. DND-CAT is supported by Northwestern University, E.I. DuPont de Nemours & Company, and The Dow Chemical Company. This research used resources of the APS, a USDOE Office of Science User Facility operated for the USDOE Office of Science by Argonne National Laboratory under Contract no. DE-AC02-06CH11357. I would like to acknowledge the funding support that made my research possible from Compass minerals and Kansas State University Research and Extension. I am grateful to the Soil and Environmental Chemistry Research Program, for giving me this wonderful opportunity to pursue my M.S. studies.

.

# Dedication

To my family who has supported me at every step of the way.

# Chapter 1 - Introduction

Zinc plays a vital role in many biological processes and is an essential trace element for the proper growth and reproduction of plants and the health of animals and humans (Lebourg et al., 1998; Cakmak et al., 1999; Alloway, 2009). Poor diet quality in humans is correlated with Zn-deficiency exacerbated by Zn deficient soils (Barber, 1995). Zinc deficiency in humans is an important malnutrition problem world-wide. Epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems are the organs most affected clinically by zinc deficiency (Roohani et al., 2013). Soils susceptible to Zn-deficiency are calcareous, sandy, and saline soils with high organic matter, nitrogen, and phosphate content (Cakmak et al., 1999). Alkaline calcareous soils are most susceptible to Zn-deficiency (Thind et al., 1990). The high pH and presence of active $CaCO_3$ cause Zn insolubility because of Zn adsorption in the presence of carbonates (Khoshgoftarmanesh et al., 2004; Lindsay, 1979; Udo et al., 1970) and then precipitates as zinc hydroxide or carbonate (Singh and Sekhon, 1977), or forms the insoluble calcium zincate (Sharpless et al., 1969). The presence of active $CaCO_3$ in the soil can also produce an excess of Ca absorption by the plant preventing the Zn transport and uptake (Kabata-Pendias and Pendias, 1985).

Zinc concentration ranges from 10 to 300 mg $kg^{-1}$ in an unfertilized and uncontaminated soil (overall mean of around 50-55 mg/kg) (Alloway, 1995; Barber, 1995). The relative distribution of Zn varies with different types of soils and their physiochemical properties (Viets, 1962). It is estimated that nearly half of the world's land is Zn deficient. Zinc deficiency is prevalent in cereal-growing regions, leading to low yields, poor grain quality, and low Zn concentrations (Cakmak et al., 1999; Cakmak, 2008). For example, Zn concentrations in cereals can be reduced by 80% when cereals (rye (*Secale cereale*, cv. Aslim), bread wheat (*Triticum*

*aestivum*, cvs. Dagdas) and durum wheat (*Triticum durum*, cv. Kunduru-1149) are grown on soils deficient in Zn or have low plant-available Zn (Cakmak et al., 1997a). The availability of Zn to plants is regulated by the dynamic balance between the different forms of Zn in soil and soil physicochemical properties, and not by the total Zn content (Elsokkary and Lag, 1978; Loué et al., 1988). Among the factors that determine the mobility and availability of Zn in soil are pH and concentration of $CaCO_3$ (Adriano, 1986). The bioavailability decreased when the pH of the soils increased (Lindsay, 1979). Methods like DTPA and Mehlich-3 extraction are commonly used procedures to determine the Zn bioavailability for plant uptake and used as alternatives to chemical fractionation (Mortvedt and Gikes, 1993).

Zinc deficiencies are traditionally remediated by the application of inorganic fertilizers (Nayar et al., 1980). The cost of Zn fertilizer application is much lower than the loss of production and yields due to Zn-deficiency. Due to lack of awareness and negligence regarding losses occurring due to Zn-deficiency, Zn fertilizers are not widely used. Therefore, an adequate Zn supply is essential to achieve cost-effective crop yields worldwide. Wheat grain yield has been shown to increase by up to 32% when Zn-deficient soils (i.e., in central Anatolia, Turkey) are altered by Zn-enrichment (Kalayci et al., 1999). The response of plants to Zn fertilization varies depending on the source of Zn fertilizer, soil chemical and physicochemical properties, and fertilization method (Boawn, 1973; Rehm et al., 1980, Anderson, 1972). The Zn fertilizer must be chosen depending upon the relative agronomic efficiency of the fertilizer source for a particular soil type. Zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO) are commonly used as inorganic Zn fertilizers to overcome Zn-deficiency.

Zinc oxide is an inorganic compound nearly insoluble in water but soluble in acids (Montalvo et al., 2016). Therefore, in alkaline soils, ZnO may act as a slow-releasing Zn

fertilizer in calcareous soils. Slow-release and controlled release are designed to release nutrients gradually and per the plant's nutritional needs (Ge et al., 2002; Shavit et al., 2003). Therefore, because of the less precipitation and fixation, the nutrients are released for an extended period, the efficiency is more significant for slow-releasing fertilizer than conventional fertilizers (Dou and Alva, 1998). As a result, nutrient uptake efficiency is more remarkable for slow-release fertilizers than the forms of fertilizers that are readily available for plant uptake (Riandy and Sofyan, 2015).

When fertilizers with $ZnSO_4$ are added to adjust the deficiency in calcareous soils, reactions as

$$ZnSO_4 + CaCO_3 \leftrightarrow ZnCO_3 + CaSO_4$$

can be produced, reducing the effectiveness of the fertilizer. Other anionic species, such as sulfides, can also contribute to Zn insolubilization due to the low solubility of their salts (Mortvedt and Giordano, 1969).

These problems have led to synthetic chelates, which is an effective agricultural practice to overcome metal deficiencies with the limitation of being expensive (Lucena et al., 2006).

Metal chelates play an essential role in transporting Zn (acting as a shuttle) from the solid phase in the soil to the surface of plant roots (Dhillon, 1983). Both synthetic and natural chelates increase the availability of micronutrient cations to plants from the soil by improving both the diffusion and convective flow of nutrients to the plant surface. Chelating agents have been shown to contribute largely to Zn movement in soil (EI-Gawhary et al., 1970; Hodgson, 1969; Modaihsh, 1990). Complexed forms of Zn can be pointed out to be the major sources of available Zn (Hergert et al., 1984, Lahav et al., 1975), and their effectiveness depends on their rate of disappearance from the soil solution, which is related to their stability (McBride, 1989).

Moreover, Rico et al. (1996) reported the increase in yield and Zn concentration in plants with ZnEDTA in calcareous soil. Another series of experiments were performed under neutral pH conditions (Gangloff et al., 2002; Alvarez and Gonzalez, 2006; Gangloff et al., 2006), which showed that ZnEDTA is the more effective Zn source in comparison to ZnSO4. However, in some cases, the use of Zn chelates does not show an advantage over inorganic Zn fertilizers, the cases where Zn-deficiency can be easily overcome by Zn mineral forms (Nayyar, 1980). As stable chelates, ZnEDTA, when used on alkaline soils, can undergo leaching (Modaihsh et al., 1990; Alvarez et al., 1996). It is very necessary to select appropriate Zn sources for soil application is critical for improving Zn use efficiency, according to the soil type. Therefore, it is crucial to find new sources with increased efficiency and reduced cost.

A new Zn fertilizer has been recently introduced that is a mixture of inorganic and chelated Zn, Zn mix. Zinc mix is a mixture that contains 60% ZnO, 25% $ZnSO_4$, ratio of $ZnSO_4$ to EDTA is 8:1. The efficiency and performance for this fertilizer is yet to be tested but we hypothesize it to inherent the desired characteristics from its components and should remain effective in different soil conditions. If true, Zn mix would be a cost effective and efficient source that can be used under different prevailing conditions.

Additional way of reducing cost related to micronutrients is by doing combined application with macronutrients where macronutrients act as potential carriers. Solid Zn fertilizers are incorporated into, blended with, or coated onto macronutrient fertilizer, which is a cost-effective solution to provide uniform distribution of Zn. The effectiveness of Zn fertilizers for providing plants with Zn in Zn-deficient soils depends on several factors but the solubility of the Zn source in soil is the major one. Mortvedt and Giordano (1969) and Degryse et al. (2020)

reported that the availability of Zn from granular fertilizers is majorly controlled by water solubility.

Fertilizer reaction pathways, which affect Zn availability, are influenced by fertilizer characteristics, such as differences between Zn only and Zn coated urea, and the physicochemical properties of soils to which the fertilizers are applied. Adsorption and desorption of Zn is controlled by soil properties including soil texture, pH, cation exchange capacity, organic matter content, the presence of anionic species (such as carbonates, chloride, phosphates, chloride, and sulfides) that form complexes with Zn cation (Richardson et al., 2006). Thus, exploring the interactions between these soil properties and added Zn will allow us to identify the Zn species present and their contribution in Zn availability. Synchrotron techniques such as X-ray absorption near edge structure (XANES) with statistical methods such as linear combination fitting (LCF) and principal component analysis (PCA) can be used to elucidate Zn speciation in soils. These direct identification techniques in conjunction with wet chemical analyses will ensure a more accurate estimation. However, not much metal speciation research has been done to provide real data to complement incubation and greenhouse studies.

## Objectives

The objective of the first study is to use alkaline calcareous soil to investigate and compare the efficiency of different Zn fertilizers and Zn-coated urea fertilizers by incubation visualization and greenhouse studies.

The objective of the second study is to use neutral sandy soil to compare the fate, behavior, and speciation of different Zn fertilizers.

The objective of the third study is to compare the efficiency of different Zn fertilizers in an alkaline calcareous soil by incubation visualization and greenhouse studies and comparing various Zn and Zn-coated urea fertilizers in acidic soil by greenhouse studies.

## References


Adriano, D. C. 1986. p. 429- 448. Zinc In Trace Elements in the Terrestrial Environment; Springer-Verlag, NY.

Alloway, B.J. 1995. p. 284-305. Heavy Metals in Soils (second ed.), Blackie Academic and Professional, London.

Alloway, B.J. 2009. Soil factors associated with zinc deficiency in crops and humans Environ. Geochem. Health. 31:537-548.

Álvarez, J. M., M.I. Rico, and A. Obrador. 1996. Lixiviation and extraction of Zn in a calcareous soil treated with Zn-chelated fertilizers J. Agric. Food Chem. 44:3383- 3387.

Álvarez, J. M., and D. Gonzalez. 2006. Zn transformations in neutral soil and Zn efficiency in maize fertilization J. Agric. Food Chem. 54:9488- 9495.

Anderson, W.B. 1972. Zinc in soils and plant nutrition. Adv. Agron. 24:147−186.

Barber, S.A. 1995. Soil Nutrient Bioavailability: A Mechanistic Approach (second ed.), John Wiley & Sons, Inc, NY.

Boawn L.C.1973. Comparison of zinc sulphate and ZnEDTA as zinc fertilizer sources. Soil Sci. Soc. Am. Proc. 37:111-115.

Cakmak, I., H. Ekiz, A. Yilmaz, B. Torun, N. Köleli, I. Gültekin, and S. Eker. 1997a. Differential response of rye, triticale, bread and durum wheats to zinc deficiency in calcareous soils. Plant Soil. 188:1-10.

Cakmak, I., M. Kalayci, H. Ekiz, H.J. Braun, and A. Yilmaz. 1999. Zinc deficiency as an actual problem in plant and human nutrition in Turkey: A NATO-science for stability project. Field Crop Res. 60:175-188.

Cakmak, I. 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil. 302:1-17.

Dhillon, K.S., and S.K. Dhillon. 1983. Relative efficiency of different chelates in the supply of applied zinc to maize and wheat. J. Nucl. Agric. Biol. 12: 93−96.

Dou, H., and A.K. Alva. 1998. Nitrogen uptake and growth of two citrus rootstock seedlings in a sandy soil receiving different controlled-release fertilizer sources. Bio. Fert.Soils. 26:169-172.

EI-Gawhary S.M., W.L. Lindsay, and W.D. Kemper. 1970. Effect of EDTA on the self-diffusion of Zn in aqueous solution and in soil. Soil Sci. Soc. Am. Proc. 34:66-70 91.

Elsokkary, I.H., and L. Lag. 1978. Distribution of different fractions of Cd, Pb, Zn and Cu in industrially polluted and non-polluted soils of Odda region, Norway. Acta Agric. Scand. 28:262-268.

Gangloff, W.J., D.G. Westfall, and G.A. Peterson, J.J. Mortvedt. 2002. Relative Availability coefficients of organic and inorganic Zn fertilizers J. Plant Nutr. 25:259- 273.

Gangloff, W. J, D.G. Westfall, G.A. Peterson, and J. J. Mortvedt. 2006. Mobility of organic and inorganic Zn fertilizers in soils Commun. Soil Sci. Plant Anal. 37:199- 209.

Ge, J., Wu, R., X. Shi, H. Yu, M. Wang, and W. Li. 2002. Biodegradable polyurethane materials from bark and starch. II. Coating material for controlled-release fertilizer. J. Appl. Polym. Sci. 86:2948-2952.

Hodgson J.F. 1969. Contribution of metal-organic complexing agents to the transport of metals to roots. Soil Sci. Soc. Am. Proc. 33:68-75.

Hergert, G.W., G.W. Rehm, and R.A. Wiese. 1984. Field evaluation of zinc sources band applied in ammonium polyphosphate suspension. Soil Sci. Soc. Am. J. 48:1190−1193.

Kabata-Pendias, A., and H. Pendias. 1985. p. 99-108. In Trace Elements in Soils and Plants; CRC: Boca Raton, FL.

Kalayci, M., B. Torun, S. Eker, M. Aydin, L. Ozturk, I. Cakmak. 1999. Grain yield, zinc efficiency and zinc concentration of wheat genotypes grown in a zinc-deficient calcareous soil in field and green house. Field Crops Res. 63:87-98.

Khoshgoftarmanesh, A. H., H. Shariatmadari, N. Karimian, M. Kalbasi, and M.R. Khajehpour. 2004. Zn efficiency of wheat cultivars grown on saline calcareous soil J. Plant Nutr. 27:1953- 1962.

Lahav, N., and M. Hochberg. 1975. Kinetics of fixation of iron and zinc applied as metal chelates. Soil Sci. Soc. Am. Proc. 39:55−58.

Lebourg, T., H. Sterckeman, T. Ciesielski, and N. Proix. 1998. Trace metal speculation in three unbuffered salt solutions used to assess their bioavailability in soil J. Environ. Qual.27:584-590.

Lindsay, W. L. 1979. Chemical Equilibra in Soils; Wiley, NY.

Lucena, J.J. 2006. p. 103-128. Synthetic iron chelates to correct iron deficiency in plants. In Iron Nutrition in Plants and Rhizospheric Microorganisms; Barton, L.; Abadia, J., Eds.; Springer-Verlag Academic Publishers: Dordrecht, The Netherlands.

Loué, A. 1988. Los Microelementos en Agricultura; Mundi-Prensa: Madrid, Spain.

McBride, M.B. 1989. Reactions controlling heavy metals solubility in soils. Adv. Soil Sci. 10:1−56.

Modaihsh, A.S. .1990. Zn diffusion and extractability as affected by Zn carrier and soil chemical properties Fert. Res. 25: 85- 91.

Montalvo, D., F. Degryse, R.C. da Silva, R. Baird, and M.J. McLaughlin. 2016. Agronomic effectiveness of zinc sources as micronutrient fertilizer. Advances in Agronomy 139, 215-267.

Mortvedt J.J., and P.M. Giordano. 1969. Extractability of zinc granulated with macronutrient fertilizers in relation to its agronomic effectiveness J. Agric. Food Chem. 17:1272- 1275.

Mortvedt, J.J., and R.J. Gilkes. 1993. p. 33-44. Zinc fertilizers. In Zinc in Soils and Plants; Robson, A. D., Ed.; Developments in Plant and Soil Sciences 55; Kluwer Academic Publishers: Dordrecht, The Netherlands.

Nayyar, V.K., and P.N. Takkar. 1980. Evaluation of various Zn sources for rice grown on alkali soil Z. Pflanzenarnaehr. Bodenkd. 143:489- 493.

Rehm G.W., R.A. Wiese, and G.W. Hergert. 1980. Response of corn to zinc source and rate of Zn band applied with either orthophosphate or polyphosphate. Soil Sci 129:36-44.

Riandy, A.P., and D.F. Sofyan. 2015. Applying nano-carbon as coating material for slow released fertilizer to increase Indonesian rice yield and to reduce nitrogen loss in surface water of paddy soil. J. Inf. Technol. Manag. 18:34-40.

Rico, M. I., J.M. Álvarez, and J.I. Mingot. 1996. Efficiency of Zn ethylenediaminetetraacetate and Zn lignosulfonate soluble and coated fertilizers for maize in calcareous soil J. Agric. Food Chem. 44:3219- 3223.

Richardson, G.M., D.A. Bright, and M. Dodd. 2006. Do current standards of practice in canada measure what is relevant to human exposure at contaminated sites? II: oral bioaccessibility of contaminants in soil. Hum. Ecol. Risk Assess. 12(3):606-616.

Roohani, N., R. Hurrell, R. Kelishadi, and R. Schulin.013. Zinc and its importance for human health: An integrative review. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences, 18(2), 144-157.Sharpless, R. G., E.F. Wallihan, and F.F. Peterson. 1963. Retention of zinc by some arid zone soil materials treated with zinc sulfate. Soil Sci. Soc. Am. Proc. 33:901−904.

Shavit, U., M. Reiss, and A. Shaviv. 2003. Wetting mechanisms of gel-based controlled release fertilizers. J. Control Release. 88:71-83.

Singh, B., and G.S. Sekhon. 1977. Effect of soil properties on adsorption and desorption of zinc by alkaline soils. Soil Sci. 126:366−369.

Thind, S.S., P.N. Takkar, and R.L. Bansal. 1990. Chemical pools of zinc and the critical deficiency level for predicting response of corn to zinc application in alluvium derived alkaline soils. Acta. Agron. Hung. 39:219−226.

Udo, E.J., J.S. Bohn, and T.C. Tucker. 1970. Zinc adsorption by calcareous soils. Soil Sci. Soc. Am. Proc. 34:405−407.

Viets, F.G. 1962. Chemistry and availability of micronutrients in soils. J. Agric. Food Chem.10:174-178.

# Chapter 2 - Literature review

## 2.1 Behavior of Zinc in soil

Zinc is one of the essential trace elements which are necessary for the growth of crop plants. Zinc is also an essential element in animals along with other elements, such as copper, iron, and manganese. These are also called micronutrients because plants require them in very small quantities (5-100 mg $kg^{-1}$). Soils contain essential as well as non-essential trace elements, and concentrations may vary. Several factors, such as soil parent material, input additions via manures, fertilizers, agrochemicals, etc., affect the concentrations of essential and non-essential trace elements in soils. Not the total concentration in soil, the availability of trace elements determines their plant uptake. The availability of Zn in soil, its uptake by plants or its diffusion/movement in soil profile differs, not only based on Zn but also depending on soil properties. These soil properties include clay minerals, organic matter, soil pH, soil texture, $CaCO_3$ content, etc. This section will discuss total Zn concentrations in different soils, its mineral solubility in soils, its solution speciation, the reaction pathways zinc undergoes in soils, other reactions of Zn with clay minerals, organic matter, and metal oxides. All factors mentioned above determine Zn speciation (Zn forms) in soil, whether Zn presents as bound to organic or inorganic complexes or free ions. Due to this, even though Zn concentration might be adequate in soils, Zn might not be in available forms in which plant roots can uptake Zn. On the other hand, it can be present in labile form but not in the immediate vicinity of the plant roots. All these put a question mark on the factors affecting the bioavailability of Zn and the need to discuss it.

### 2.1.1. Total zinc concentrations in soils

The average concentration of Zn varies from 50-60 mg $kg^{-1}$. Zinc concentrations can have a range of 10-300 mg $kg^{-1}$ with an average of 50 mg $kg^{-1}$ (Kiekens, 1995). Old soils, such as soils in Australia, formed from weathering of rocks, total zinc concentrations are < 2-180 with an average of 34 mg $kg^{-1}$ (Tiller, 1983).

**Table 2.1 Total Zn concentrations in soils worldwide**

| Region | Texture | Total Zn (mg $kg^{-1}$) |
|---|---|---|
| Victoria and South Australia (Bertrand et al., 2002) | Alkaline non-calcareous soil<br>Calcareous soil | 4-41<br>5-36 |
| USA (Holmgren et al., 1993) | - | 56.5 |
| European (Angelone and Bini, 1992) | - | 68 |
| England and Wales (McGrath and Loveland, 1992) | - | 97 |
| France (Baize, 1997) | Sandy<br>Silty<br>Loam<br>Clayey<br>Very clayey | 17<br>40<br>63.5<br>98<br>132 |
| Poland (Kabata-Pendias and Pendias, 1992) | Sandy<br>Loess<br>Loam | 37<br>60<br>75 |
| Germany (Gorny et al., 1992) | Sandy<br>Loam<br>Clay | 27.3<br>59.2<br>76.4 |

All the above examples show how Zn concentrations are affected by soil texture. Sandy soils having lower concentrations of Zn as compared to clay soils having greater concentrations

of Zn. This can be explained by the presence of greater Zn content in clay and shale parent materials, as well as having high CEC enable clays to absorb and retain more Zn relative to sandy soils having lower CECs. All over the world, sandy soils are found to be the most Zn deficient soils. (Norvell and Lindsay, 1970)

### 2.1.2 Zinc minerals solubility in soil

**Table 2.2 Equilibrium constants for reactions involving zinc**

| Reaction No. | Equilibrium reactions | Log K° |
|---|---|---|
| 1. | $Zn^{2+} + 2e^- \leftrightarrows Zn$ | -25.80 |
| | Oxides and Hydroxides | |
| | $Zn(OH)_2(amorp) + 2H^+ \leftrightarrows Zn^{2+} + 2H_2O$ | |
| 2. | $\alpha$-$Zn(OH)_2 + 2H^+ \leftrightarrows Zn^{2+} + 2H_2O$ | 12.48 |
| 3. | $\beta$-$Zn(OH)_2 + 2H^+ \leftrightarrows Zn^{2+} + 2H_2O$ | 12.19 |
| 4. | $\gamma$-$Zn(OH)_2 + 2H^+ \leftrightarrows Zn^{2+} + 2H_2O$ | 11.78 |
| 5. | $\varepsilon$-$Zn(OH)_2 + 2H^+ \leftrightarrows Zn^{2+} + 2H_2O$ | 11.74 |
| 6. | $ZnO$ (Zincite) $+ 2H^+ \leftrightarrows 2Zn^{2+} + H_2O$ | 11.53 |
| 7. | | 11.16 |
| | Carbonates | |
| | $ZnCO_3$ (smithsonite)$+ 2H^+ \leftrightarrows Zn^{2+} + CO_2 + H_2O$ | |
| 8. | | 7.91 |
| | Soil Zn | |
| | Soil $Zn + 2H^+ \leftrightarrows Zn^{2+}$ | |
| 9. | | 5.80 |
| | Chlorides | |

| | | |
|---|---|---|
| 10. | $ZnCl_2 \leftrightarrows Zn^{2+} + 2Cl^-$ | 7.07 |
| | Sulphates | |
| 11. | $ZnSO_2 + 2H^+ \leftrightarrows Zn^{2+} + 2SO_4^{2-}$ | 3.41 |
| 12. | $ZnO.2ZnSO_2 + 2H^+ \leftrightarrows 3Zn^{2+} + 2SO_4^{2-} + H_2O$ | 19.12 |
| 13. | $Zn(OH)_2.ZnSO_2 + 2H^+ \leftrightarrows 2Zn^{2+} + SO_4^{2-} + H_2O$ | 7.50 |
| | Phosphates | |
| 14. | $Zn_3(PO_4)_2.4H_2O$ (hopeite) + $4H^+ \leftrightarrows 3Zn^{2+} + 2H_2PO_4^- + 4H_2O$ | 3.80 |
| | Hydrolysis species | |
| 15. | | -7.69 |
| 16. | $Zn^{2+} + H_2O \leftrightarrows ZnOH^+ + H^+$ | -16.80 |
| 17. | $Zn^{2+} + 2H_2O \leftrightarrows ZnOH_2^\circ + 2H^+$ | -27.68 |
| 18. | $Zn^{2+} + 3H_2O \leftrightarrows ZnOH_3^- + 3H^+$<br>$Zn^{2+} + 4H_2O \leftrightarrows ZnOH_4^{2-} + 4H^+$ | -38.29 |
| | | 2.33 |
| 19. | Other complexes<br>$Zn^{2+} + SO_4^{2-} \leftrightarrows ZnSO_4^\circ$ | |

Zinc present in soil solution is a tiny proportion of the total Zn content of the soil. For example, Kabata-Pendias and Pendias (1992) reported that the Zn concentration in soil solution range from 4-270 µg $L^{-1}$ (ppb, volume basis), considerably lower than average total Zn concentrations in soil, which is around 50-80 mg $kg^{-1}$(ppm, a mass basis). However, soils with very low pH, that is, acid soils, soluble Zn concentrations have been found to be 7130 µg L-1, indicating a strong, inversely proportional relationship to soil pH.

According to Kiekens (1995), the reaction

Soil-Zn + $2H^+ \leftrightarrows Zn^{2+}$ $\log K° = 5.80$

can be expressed as $\log Zn^{2+} = 5.8 - 2pH$, or $pZn = 2pH - 5.8$.

The above equation shows that the activity of $Zn^{2+}$ in soils is directly proportional to the square of the proton activity. Therefore, the solubility of zinc will decrease with increasing values of soil pH.

The solubility of several zinc minerals decreases in the following order:

$Zn(OH)_2$ (amorphous) > α−$Zn(OH)_2$ > β− $Zn(OH)_2$ > γ−$Zn(OH)_2$ > ε−$Zn(OH)_2$ > $ZnCO_3$ (smithsonite) > ZnO (zincite) > Zn $(PO_4)_2.4H_2O$) (hopeite) > soil Zn > Zn $Fe_2O_4$(franklinite).

These minerals indicate a range of $Zn^{2+}$ solubility of $10^{-8}$, while franklinite is the least soluble of these Zn minerals, depending upon the species of Fe controlling its solubility. The crystalline Zn hydroxides have comparable solubilities to ZnO (zincite) and Zn $(OH)_2$ (amorp).

Solubility of Zn minerals such as Zn $(OH)_2$, ZnO, and $ZnCO_3$ is about $10^5$ times greater than soil Zn (usually representative of Zn adsorbed to solid surfaces). These sources of Zn will therefore be excellent fertilizer Zn sources because of their high dissolution rate, help to maintain adequate levels of $Zn^{2+}$ in soils for plant uptake. (Lindsay, 1979)

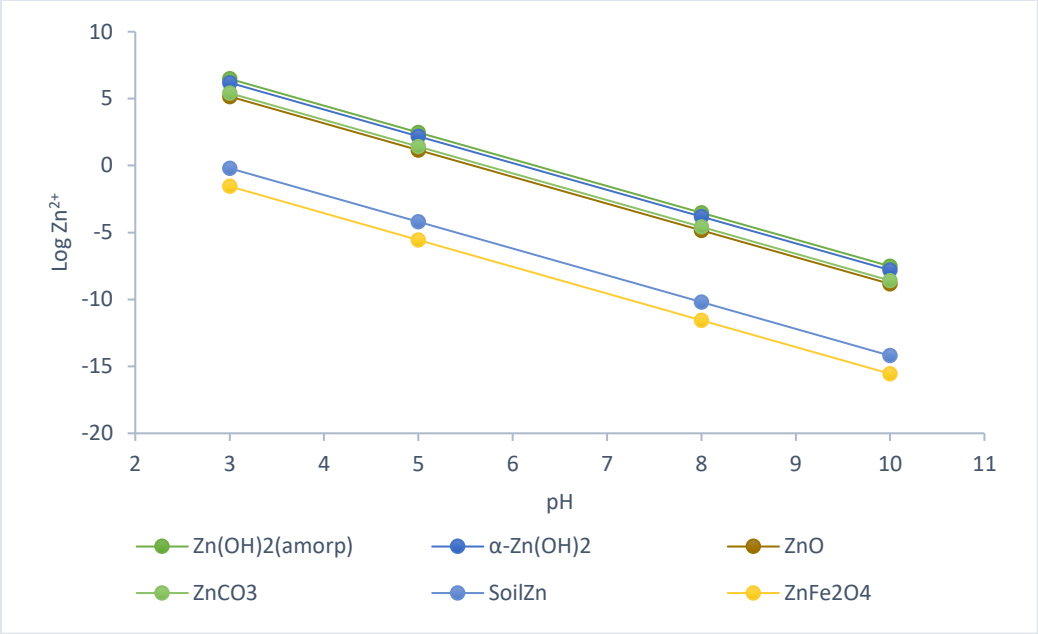


**Figure 2.1 The solubilities of several Zn minerals as compared to soil Zn (Redrawn from Lindsay, 1979)**

### 2.1.3 Zinc in solution

Micronutrients in soil solution exist as hydrated cations or oxyanions. Sometimes, these may also be present as inorganic or organic complexes. The free trace metal ions (for example, Zn, Cu, Ni) are surrounded by six water molecules, written as $Zn(H_2O)_6$, but commonly a simplified representation is used, i.e., $Zn^{2+}$, where the hydrated water is omitted (Barrow, 1999). Micronutrient uptake in plants has a poor correlation with soil total elemental concentration (Perez-Cid et al., 1998). This is because plants mostly take up trace elements such as Zn from the soil solution. Therefore, the availability of Zn for plant uptake is related to its soil solution concentration and the soil's capacity to replenish soil solution upon plant uptake. Hence, there is a need to understand the nature of reactions occurring, zinc species involved, and how it changes with change in prevalent conditions.

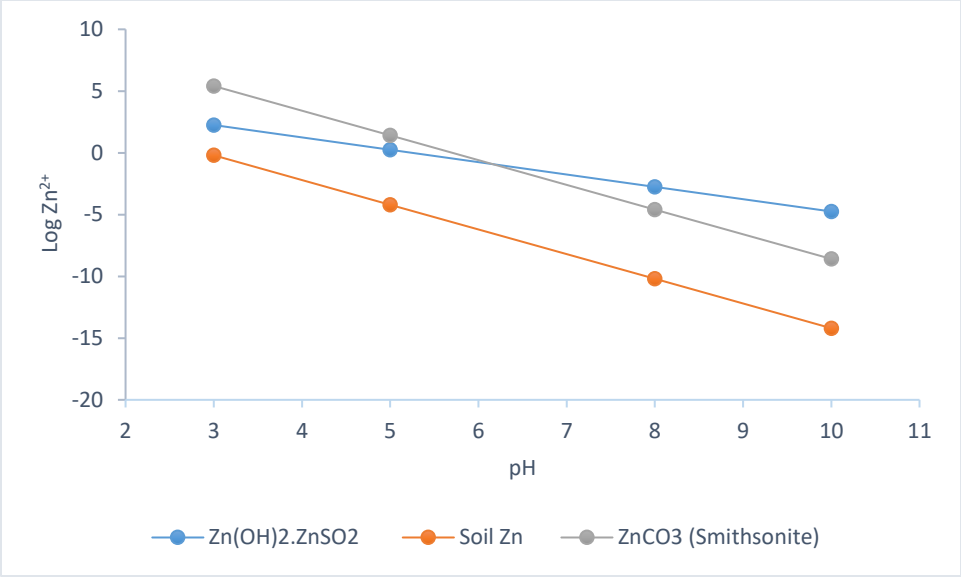


**Figure 2.2 Solubilities of Zn $(OH)_2$, $ZnSO_2$ and $ZnCO_3$ compared to soil-Zn (Redrawn from Lindsay, 1979)**

The zinc hydrolysis species in aqueous solutions are plotted in Fig 3, assuming it is in equilibrium with soil Zn. The solution speciation of Zn is also highly affected by soil pH. Soil pH governs the speciation of Zn in the solution.

Free Zn ion species ($Zn^{2+}$) predominates when soil pH is below 7.7. At soil pH above 7.7, $ZnOH^+$ is the main species, and the neutral species Zn $(OH)_2$ dominates for soil pH range above 9. The activity of $Zn^{2+}$ is $10^{-4}$ M at soil pH 5, but it decreases to $10^{-10}$ M at soil pH of 8 (Kiekens, 1995), which can also hinder the uptake of Zn in alkaline environments.

Other soluble complexes of Zn can be observed with phosphate, chloride, sulfate, and nitrate ions. The neutral sulfate ($ZnSO_4°$) and phosphate ($ZnHPO_4°$) species are the most predominant species and significantly contribute to the total concentration of Zn concentration in solution.

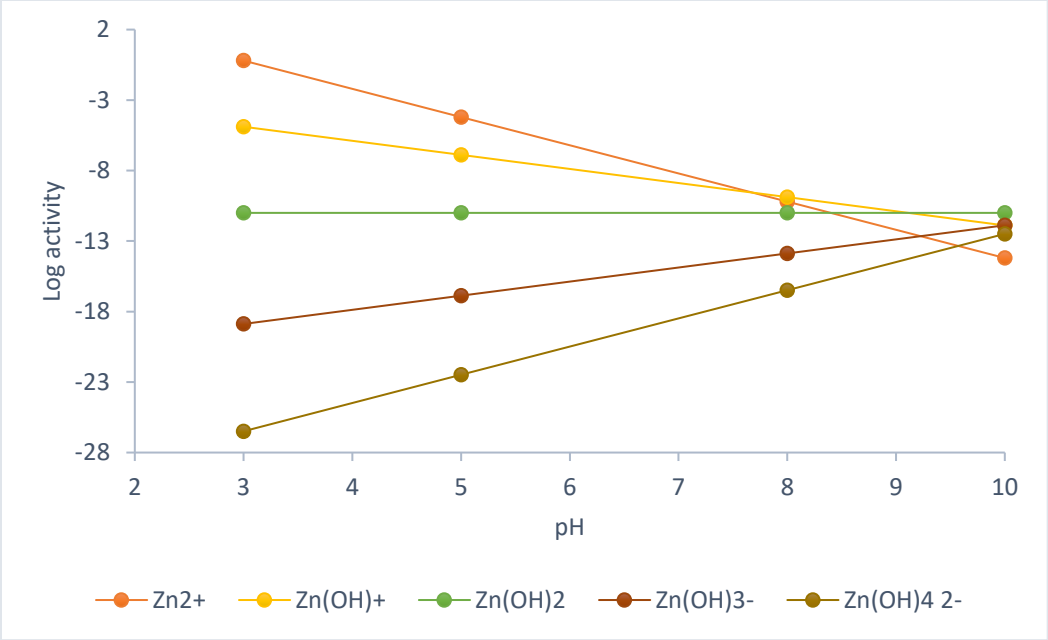


**Figure 2.3 The hydrolysis species of $Zn^{2+}$ in soil solution, in equilibrium with soil-Zn. (Redrawn from Lindsay, 1979)**

The activity of $ZnSO_4°$ species is similar to that of $Zn^{2+}$ when $SO_4^{2-}$ is $10^{-2.33}$ M. Zinc sulfate ($ZnSO_4°$) complex may increase the solubility and availability of $Zn^{2+}$ in soils when used with the fertilizers having acidulation effect upon their addition to the soil such as ammonium sulfate ($NH_4(SO_4)_2$). Therefore, the inclusion of sulfate in zinc fertilizer is beneficial as it would increase the mobility and solubility of $Zn^{2+}$ in soils. (Lindsay, 1979)

Zinc also forms complexes with low molecular weight organic acids that can contribute to the soluble Zn concentration in soil. Available Zn status can be improved with heavy manure applications, which helps increase soluble and organically complexed zinc (Alloway, 2004).

Barrow (1993) observed that organic ligands such as humic acids could reduce Zn adsorption onto oxisol soil, which strongly complexed Zn. Organically complex forms of Zn are much more soluble, mobile, and plant available in soils. Complexation of Zn with organic ligands will also reduce adsorption onto mineral surfaces (Harter, 1991).

Kiekens and Cammerlynck (1992) reported an even distribution of organically complexed forms of Zn in soil and inorganic or $Zn^{2+}$ in solution. Stevenson (1991) observed that the organic complexes in soils are formed in a particular order of preference, which is: $Fe^{3+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Fe^{2+} > Mn^{2+}$.

### 2.1.4 Fate of Zn in soils:

The focus is shifting from determining total elemental contents to identifying the different chemical forms of the elements in the soils (Kalnicky and Singhvi, 2001). Zinc in soil can undergo various mechanisms responsible for Zn losses in the environment. Zinc can be present in both soil solution and solid phase. Talking about the solid phase, 'sorption' is a term that means the loss of a solute from an aqueous solution (Brun et al., 2001). It consists of two processes. First is adsorption, in which solute sticks to a solid surface, and the second is absorption in which the solute creeps inside the solid and clings to interior surfaces (Weitje et al., 1998; Brun et al., 2001). Adsorption mechanisms play a crucial role in plant Zn uptake. The mechanisms mentioned above control zinc concentrations in the soil solution, and the labile forms of Zn can be desorbed and become available to plants. Apart from these reactions, other mechanism that Zn can follow is precipitation. Precipitation, in contrast to sorption causes the development of a new solid phase. This occurs due to the fixation of excess amounts of zinc, maybe due to sorption of zinc in a hydrolyzed form and precipitation of $Zn(OH)_2$, or discrete precipitation of new solids from solution These mechanisms are discussed in detail in the following sections.

#### 2.1.4.1 Adsorption of Zinc

Adsorption mechanisms play a crucial role in the Zn soil-plant relationships. Zinc concentrations in the soil solution are controlled by these mechanisms, and therefore its

availability and uptake by plants as well. These mechanisms also control the labile forms of Zn, which can become available to plants after desorption. The adsorption mechanisms involve the adsorption of ions on solid surfaces (of clay minerals, metal oxides such as iron and manganese, and organic matter), include cation exchange (outer-sphere or diffuse swarm), binding to organic matter, specific adsorption (or chemisorption), and precipitation. Cation exchange is the reversible and nonspecific adsorption electrostatic attraction on negatively charged sites of colloidal surfaces of ions that are positively charged. This negative charge on soil colloids is of two types: it can be a structural charge imbalance (i.e., permanent charge) and pH-dependent charge, e.g., on organic matter or hydrous oxides of metals and permanent charge, because of isomorphic substitution in the crystal lattice. In the case of variable charges, surfaces bear net positive charge at pH lower than the point of zero charges (PZC), and at pH above or greater than PZC, the surfaces bear net negative charges.

Exchangeable adsorption of Zn cation in soils can be expressed as:

$Zn^{2+}$ + M-Soil ⇆ Zn-Soil + $M^{2+}$

where M is a divalent cation.

The relative replaceability of exchangeable cations is described by a lyotropic series. Beginning with the most easily removed cation, the lyotropic series for divalent exchangeable cations are (hydrated radii in nanometers):

$Mg^{2+}$ (0.428) > $Zn^{2+}$ (0.430) > $Co^{2+}$ (0.423) > $Cu^{2+}$ (0.419) > $Cd^{2+}$ (0.426) > $Ni^{2+}$ (0.404) > $Ca^{2+}$ (0.412) > $Sr^{2+}$ (0.412) > $Pb^{2+}$ (0.401) > $Ba^{2+}$ (0.404) (Essington, 2005).

This suggests that $Zn^{2+}$ can be easily replaceable by other divalent metal cations, depending on their identity and concentration.

#### *2.1.4.1.1 Isomorphic substitution*

Pickering (1980) summarized the reactions with clay minerals, emphasizing the differences between clay minerals. The illites and montmorillonites have a 2:1 structure. These are a sandwich of silica tetrahedron- alumina octahedron - silica tetrahedron layer. For these clay minerals, Isomorphic substitution is much more considerable. The permanent charge is much greater than kaolinite, which has a 1:1 layer, Si-tetra hedra, and Al-octa hedra structure. This can be because of the involvement of interlayer spaces in cation exchange. Exchange is slower for these minerals than for kaolinite and slower in illites than in montmorillonites. Relatively less isomorphic substitution and a large proportion of charge in Kaolinites can be due to disruption of the structure at the edges of particles.

#### *2.1.4.1.2 Variable charge*

Organic soils are usually Zn deficient, and therefore, reactions between zinc and organic matter have also been widely studied. Shuman (1980) indicated that weak acids are the functional groups that hold zinc and concluded that extensive research needed mechanisms specification and group characterization. Kiekens (1995) stated that adsorption of Zn to clay surfaces or organic matter seems to follow two different mechanisms based on soil pH conditions. In acidic conditions, it is related to cation exchange, and therefore, relatively easy to replace. In alkaline conditions, it involves complexation of Zn with organic ligands and chemisorption, suggesting strong retention.

Cation exchange capacity (CEC) increases with increased pH because the variable charge colloidal surfaces bear more negative charges at high pH. There is a decrease in CEC upon acidification of soils due to a smaller number of negatively charged sites. Cations attracted to negatively charged solid surfaces follow an order of preference. An example for the iron oxide

goethite is Cu > Pb > Zn > Co > Cd; for the clay mineral illite, the order is: Pb > Cu > Zn > Ca > Cd > Mg, and for peat the order is: Pb > Cu > Cd = Zn > Ca (Robinson and Clothier, 2004). These examples show that the preference for Zn to get adsorb onto oxides or clay minerals is less than Cu and Pb (Alloway, 2004).

Cations adsorbed by cation exchange are beneficial in avoiding leaching and can be immediately replaced to become available for plant root uptake. Harter (1991) reported that phosphate ions could increase Zn adsorption on hydrous oxide surfaces. This could be explained by phosphate reducing the point of zero charges (PZC) through chemisorption onto soil minerals. Hence, increasing negative surface charge, resulting in greater Zn adsorption on cation sites or binding to phosphorous or other complexation sites.

2.1.4.2. Precipitation and dissolution

Under neutral to alkaline pH conditions, metals such as Al, Zn, Fe, Cu, and Cd can be precipitated as hydroxides (Evans, 1989). For example, the dissolution of α-Zn $(OH)_2$ is described by (Lindsay, 1979; Smith et al., 1997):

$$\alpha\text{-Zn}(OH)_2 + 2H^+ \leftrightarrows Zn^{2+} + 2H_2O \qquad \text{Log } K° = 12.19$$

The equilibrium constant can be written as:

$$\text{Log}(Zn^{2+}) = 12.19 - 2pH$$

From the above equation, if the activity of $Zn^{2+}$ in solution is controlled by α-Zn $(OH)_2$ (s), it will increase by a factor of 100 with each one-unit decrease in pH. At pH 7, the $Zn^{2+}$ activity will be 0.03 mol $L^{-1}$. At pH 8, the $Zn^{2+}$ activity would be 100 times less, at about 20 mg $L^{-1}$. However, because of the formation of soluble metal complexes, the solubility might be greater than the value indicated by the free ion activity. Fig 2.3 shows the activity of the free $Zn^{2+}$ and several Zn hydroxides complexes, which are in equilibrium with α-Zn $(OH)_2$(s) as a

function of pH. Above soil pH of 11, greater hydroxy complexes can be formed, causing an increase in the total Zn activity (Fig 2.3) (Lindsay, 1979).

Mineral phases in soils contain many mixed solids and a very small proportion of the trace element precipitate. Precipitation occurs when the solubility product has been exceeded a specific limit (McBride, 1991). Other mineral surfaces can control precipitation as these surfaces will control or reduce the supersaturation. A solid mixture can also be formed either by inclusion or by co-precipitation.

Current research has shown that more than 50% of the dissolved zinc occurs as the free cation, and the concentration of free cation depends on the free cation activity of calcium and magnesium (Kadir, 1993). This relationship may reflect both adsorption and precipitation processes, and interactions between these elements. Calcium and magnesium can displace zinc from solution complexes and from adsorption sites on the soil solids which has lower solubility. Bloomfield (1981) observed that Zn was mobilized from the basic carbonates and from the oxides which afterwards transformed to other species, mainly carbonates.

### 2.1.5 Zinc availability and bioavailability

Diethylene-triamine penta acetic (DTPA) acid is used as an extractant to characterize the available zinc in soils. Such extraction methods merely characterize the "labile" form of Zn in soils that consist of water soluble, adsorbed, exchangeable, chelated, or occluded zinc (Fig. 4). Most of the Zn in soils occurs on surfaces of clays or bound to organic matter, and hydrous oxides of metals and a very small portion are present in solution form (Armour et al., 1990). As primary root uptake of Zn is from the soil solution, plant availability depends upon the concentration of Zn in soil solution and the capacity of soil to replenish it (Fig. 4).

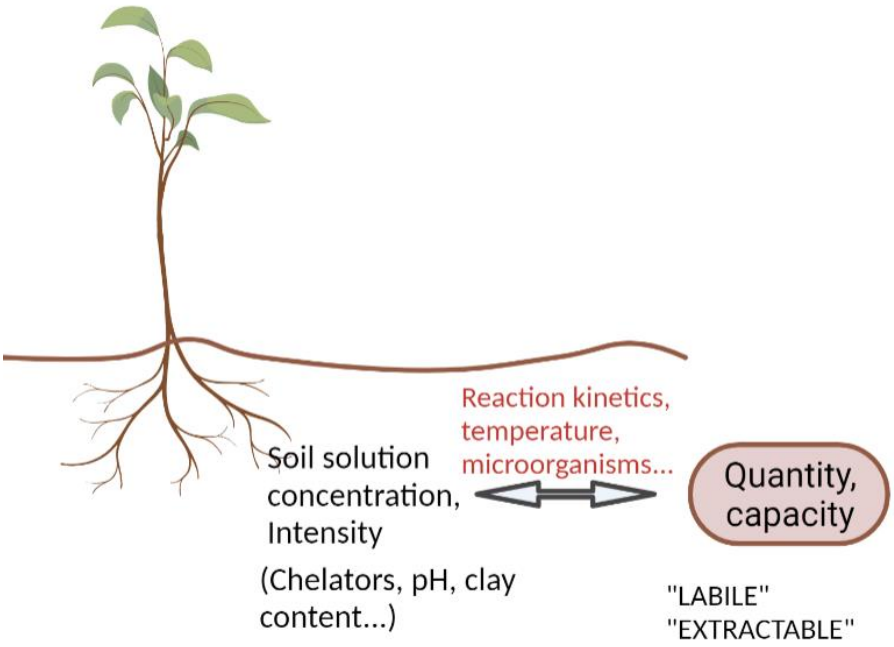


**Figure 2.4 Quantity/intensity ratio for the availability of the nutrients and factors determining the "bioavailability" of Zn. (Redrawn from A.D. Robson, 1993)**

#### 2.1.5.1 Supply by mass flow and diffusion

The DTPA extractable zinc can, therefore, be useful in estimating the capacity of soils to provide the adequate Zn amount required by the plant roots.

For a given pool size in the soil, the portion that is easily accessible to the plants for the uptake is called the "bioavailability" (Barber, 1984).

Bioavailability of Zn is limited mainly due to the low mobility of Zn in soil solution and, therefore less availability for plants. Several factors are known to influence the correlations between extractable Zn in soil and plant uptake (Lindsay and Cox, 1985). More specifically, root growth and surface area, and mass flow and diffusion are the crucial processes influencing Zn bioavailability. Therefore, a deeper understanding of these factors and processes is necessary to understand this concept.

In addition to elemental concentration and quantity in soil solution, transport to the roots is of considerable importance for Zn plant uptake. Other important factors are- root growth and surface area as these determine the distance between the quantity (exchangeable fraction) and root surface (Fig. 4). Zinc soil solution concentration is usually very low as most of it is bound to the soil matrix. Thus, the zinc supply is mainly controlled by diffusion to the roots. Zinc soil solution concentration is highly dependent on soil pH, decreases with an increase in soil pH (Jeffery and Uren, 1983; Brummer et al., 1986). Soil solution Zn present as a labile complex and as a free metal (Jeffery and Uren, 1983; McGrath et al., 1988). Wilkinson et al. (1968) reported that diffusion is critical for Zn supply. He studied the depletion of Zn around roots using $^{65}Zn$ and autoradiographic methods in wheat plants. Asher (1987) used different plant species and explored flowing nutrient solutions. Based on these studies, the adequate Zn concentrations in rhizosphere solution ranges between $6 \times 10^{-8}$M and $8 \times 10^{-6}$ M. These concentrations are much greater than expected in the bulk soil solutions, and high pH soils. In the case of chelate-buffered, free $Zn^{2+}$ activities in the solution range between $10^{-10}$ and $10^{-11}$ M, adequate for the growth of tomato (Parker et al.,1992). The bioavailability of Zn is mainly limited by its low mobility in the soil solution and therefore, low spatial availability. As discussed earlier as well, root growth and surface area play an important role in Zn bioavailability. The spatial accessibility of soil containing 0.5 mg $kg^{-1}$ DTPA Zn can increase two-fold by doubling the root surface area. Several environmental and soil factors particularly influence the extractable soil Zn and therefore, its plant uptake (Lindsay and Cox, 1985) are discussed below. In a nutshell, we can say that root growth and surface area are the crucial parameters that can affect Zn bioavailability.

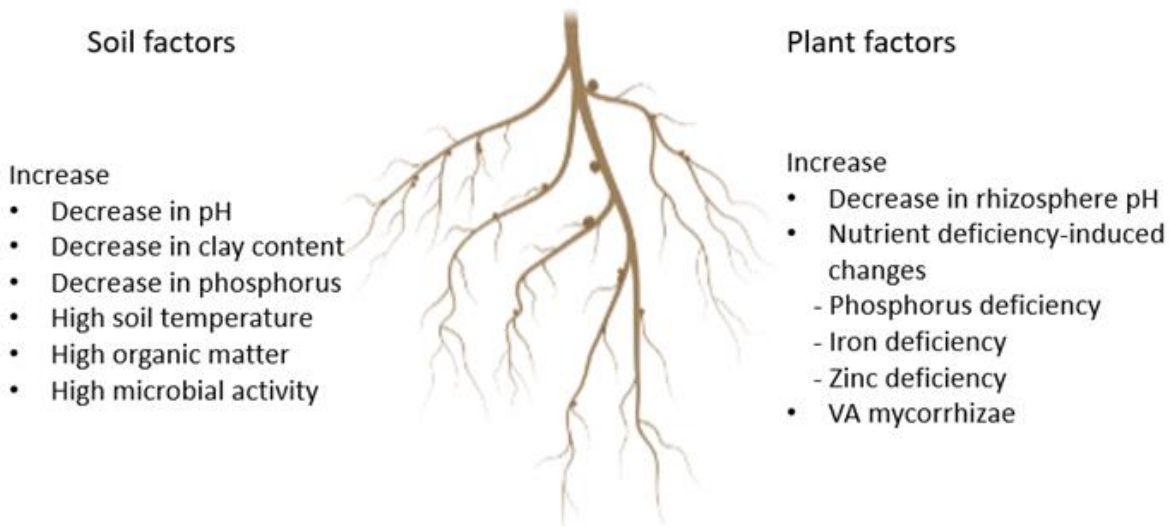


**Figure 2.5 Soil and plant factors decreasing or increasing Zinc availability and uptake by soil grown plants (redrawn from A.D. Robson, 1993).**

### 2.1.5.2 Soil factors affecting zinc bioavailability

Soil pH is the most important of all the soil factors affecting Zn plant availability (Fig. 5). Zinc concentration may decrease with increasing pH (Barber, 1984; Moraghan and Mascagni, 1991). Therefore, Zn diffusion coefficients in alkaline calcareous soils may be about 50 times lower than in acid soils (Moraghan and Mascagni, 1991).

The addition of lime to acid soils decreases plant Zn content (Grove and Sumner, 1985; Parker and Walker, 1986). Despite this decrease, there may be no or very little change in DTPA extractable zinc (Lins and Cox, 1988), suggesting that zinc 'intensity' and 'rate' control its supply to the roots (Fig. 5).

On the contrary, liming can be done to avoid Zn toxicity in crops grown on Zn polluted soils (Lee and Craddock, 1969; EI-Kherbawy et al., 1989).

As discussed earlier, soil extraction methods may have no or a very little response to changes in soil pH as compared to Zn concentration in soil solution and thus plant uptake. Therefore, monitoring pH changes can help to improve the correlation between extractable Zn

and plant uptake (Lins and Cox, 1988; Brennan and Gartrell, 1990). Besides soil pH, soil organic matter is the other soil property that can be considered to improve correlations (Brennan, 1992a). Zinc diffusion rate in soils may decrease with high soil organic matter (Sharma and Deb, 1988). Zn desorption and the formation of soluble complexes can explain this, leading to an increased release and, therefore, increased Zn supply to plant roots (Fig. 5). Likewise with microbial activity in the bulk soil. Zinc concentration in soil solution may be increased with increased soil temperature due to the presence of biologically produced chelators mobilizing Zn from insoluble forms (Linehan et al., 1989). Other soil factors such as phosphorous and iron concentration in soil is discussed below in different sections of this literature review.

## 2.2 Zinc interactions with other nutrients

This section examines the interactions of other nutrients present in the soil with Zn. To study how this interaction will affect the reactions, uptake in plants, and plant growth. There is a need to identify the factors responsible for any Zn response to adding another nutrient compound/s. The major nutrients required for plant growth are nitrogen and phosphorus. Nitrogen and phosphorus are being added as fertilizers all over the world. It is important to study their relationship with Zn and how the reactions can limit the supply of Zn or vice versa. Similar interaction of Zn with other essential nutrients is also important to study specifically for soils with a low supply of both nutrients.

### 2.2.1 Phosphorus and zinc interactions

An extensive literature is present concerning P-Zn interactions. Phosphorous and Zn interaction studies are confusing because of the discrepancy in the conditions used in the research done. The missing critical evaluations of the experimental conditions that are relevant to Zn-deficiency and acceptance of the irrelevant conclusions have led to the extensive use of the

available data on Zn-deficiency caused by P (Robson, 1993). So, proper conditions that are crucial for Zn-deficiency to occur should be studied. Then the conclusion should be made based on whether increased P application should or should not decrease Zn concentrations in plant shoots. One of the situations in which P salts reduce plant Zn concentrations is where Zn and P contents are marginal or limiting, and when P is added, it promotes growth which leads to the dilution of Zn concentration to the levels of Zn-deficiency (Boawn et al., 1954; Loneragan et al., 1979; Singh et al., 1988; Rogers and Wu, 1948; Loneragan 1951; Bingham et al., 1958; Sharma et al., 1968). In this case, it is hard to say whether P have reduced Zn absorption by roots or Zn translocation from roots to shoots.

There is good evidence for P salts to reduce the absorption of Zn from soils. Phosphorous can suppress root infection by vesicular-arbuscular mycorrhizae, and therefore, it has been shown to induce Zn-deficiency in plants (Lambert et al., 1979). Another way P can affect Zn absorption is by enhancing the sorption of Zn to soil components. This has been demonstrated in experiments with hydrous oxides of Fe and Al (Stanton and Burger, 1967, 1970; Bolland et al., 1977). has Addition of P fertilizers to soil can influence other soil factors, such as soil pH and Zn concentrations, and this can result invaried effects on Zn retention (Saeed and Fox, 1979; Friesen et al., 1980; Barrow, 1987). Barrow (1987) reported that changing pH and changing surface charge in P sorption on soils are two major mechanisms that affect Zn retention. In support of this, the formation Zn links to the surface through P molecules was proposed by Stanton and Burger (1967). Barrow (1987) suggested that P ions inhibit Zn absorption directly, however, some studies (Stukenholtz et al., 1966; Edwards and Kamprath, 1974; Safaya, 1976; Cogliatti et al., 1991) are not helpful to prove because of the irrelevant experiment conditions chosen and then conclusions were made based on those conditions (Robson, 1993). These studied conditions

included plants showing no response to Zn, Zn precipitation by P, and in consideration of other factors which varied with P treatments, for example, cation concentration and soil pH. Phosphorous had minimal effect on Zn adsorption by wheat seedlings when the concentration of nutrient anions was varied in the solutions (Chaudhry and Loneragan, 1972a). There is no direct evidence that P can induce Zn-deficiency by inhibiting Zn transport from roots to shoots (Chaudhry and Loneragan, 1970). However, under certain conditions such as high Zn supply, immobilization of Zn in the shoots can be exacerbated by P because of the formation of Zn phytate, which is seen in a wide range of crop plants (Van Steveninck et al., 1993). Some sources reported that the P supply does not change Zn concentration. Increased P supply in case of low Zn treatments exacerbates Zn deficiencies while not affecting Zn concentrations in plant shoots. Moreover, application of Zn, in this case, can remediate Zn-deficiency and even increase plant growth (Millikan, 1940, 1951; Boawn and Leggett, 1964; Boawn and Brown, 1968; Millikan et al., 1968; Loneragan et al., 1979, 1982; Christensen and Jackson, 1981).

### 2.2.2 Nitrogen and zinc interactions

There are several ways through which Zn deficiencies can be exacerbated by nitrogen. The first primary and predominant interaction is N promotes plant growth, which changes the soil pH of the root environment. If the soil lacks adequate amounts of both Zn and N (i.e., in the absence of N fertilizers), wheat grain yield did not respond to the Zn deficiency. Still, it showed signs of Zn-deficiency when N fertilizer was added to the soil (Chaudhry and Loneragan, 1970). This was explained by the dilution effect resulted in greater biomass of wheat plant upon addition of N fertilizer. The second type of interaction between N and Zn can be due to changes in soil pH caused by N fertilizer addition. Nitrogen can ameliorate Zn absorption by plants due to

acidifying effects of ammonium-based N fertilizers. Ammonium fertilizers were reported to have a pronounced impact on $ZnSO_4$ when applied concurrently with N dressings (Viets et al., 1953).

Two other effects of N salts on Zn nutrition have been observed. Ammonium ($NH_4^+$) salts inhibited Zn absorption in wheat seedlings from low $Zn^{2+}$ concentrations, and this was stronger than the Zn inhibition caused by alkali metals (Chaudhry and Loneragan, 1972a, c). Also, nitrogen can induce Zn-deficiency by restricting Zn transport to the shoots (Ozanne, 1955). However, these were not supported by other studies (Chaudhry and Loneragan, 1970) in which nitrogen fertilizer addition improved Zn concentration in plant shoots.

### 2.2.3 Zinc interactions with other macronutrients

Macronutrient cations such as Ca, K, Mg, and other cations inhibit plant Zn absorption from soil solution. Soil pH is the most important factor for these cations to affect Zn concentrations in solution. For six legumes grown in solution at constant pH, plant shoot Zn concentrations were greatest at the lowest Ca concentrations and decreased gradually with increasing Ca concentrations in solution (Bell et al., 1989). These findings complement the results reported by a short-term study with wheat seedlings; Zn absorption inhibition was attributed to Ca concentrations (Chaudhry and Loneragan, 1972a, c). Other macronutrient cations K, $NH_4$, and Mg inhibited Zn absorption at low Ca concentration, with increasing Ca concentrations, the inhibitory effects weakened and eventually disappeared, pointing that they operate through the same mechanism as Ca (Chaudhry and Loneragan, 1972a, c). The effect of Ca on Zn concentrations is complex depending upon the impact of salts on soil pH and hence Zn availability and concentration in soil solution. Zinc concentrations increased with a supply of $CaSO_4$, which decreased soil pH from 5.6 to 4.8 whereas decreased when treated with an equivalent amount of $CaCO_3$, which increased soil pH from 5.7 to 6.6 (Wear, 1956).

### 2.2.4 Zinc interactions with other micronutrients

#### 2.2.4.1 Zinc and copper interactions

Interaction between Cu and Zn can be observed for wheat grain yield in soils deficient in both Cu and Zn (Toms, 1958; Chaudhry and Loneragan, 1970; Kausar et al., 1976). Competition between absorption of Zn and Cu may reduce Cu uptake in short-term excised leaf studies (Bowen, 1969). It has also been reported by other studies performed in water culture with excised tissues (Hawf and Schmid, 1967; Schmid et al., 1965; Bowen, 1969) and seedlings (Giordano et al., 1974; Chaudhry and Loneragan, 1972b). Copper uptake was depressed by increased Zn absorption seen in wheat plants, but no effect of Zn uptake depression was observed in the same experiment. When Cu and Zn are present in sufficient quantities, mainly in chelated forms, Zn concentration can be affected by increasing $Cu^{2+}$ activity in roots and shoots of maize (Bell et al., 1991). Copper concentrations can be affected by increasing $Zn^{2+}$ activity in roots and shoots of barley (Norvell and Welch, 1993). The effect of Cu on Zn is thought to be indirect through its impact on senescence. Copper in plants has been observed to affect Zn redistribution from wheat leaves. In wheat plants with Cu deficiency, the senescence and export of Cu and Zn was delayed compared to the plants with an adequate supply of Copper (Hill et al., 1979).

#### 2.2.4.2 Zinc and Iron interactions

Iron and Zn interactions are complex to study. Increasing Zn supply to plant roots has been observed to decrease (Safaya, 1976; Jolley and Brown, 1991), increase (Watanabe et al., 1965; Jolley and Brown, 1991), or have some effect on (Norvell and Welch, 1993) Fe concentration in shoots. On the other hand, increasing Fe, generally depresses Zn concentration in plant tissues (Watanabe et al., 1965; Zhang et al., 1991b). But in some situations, it has been

reported to increase (Giordano et al., 1974), have no effect on (Chaudhry and Loneragan, 1972b) or decrease (Giordano et al., 1974; Rashid et al., 1976; Zhang et al., 1991b) rates of plant roots Zn absorption. The interaction of Fe and Zn depends on the experimental details- plant species, background concentrations and concentration of the elements added, and complexation of Fe. High concentrations of $Fe^{2+}$ in flooded soils suppress Zn absorption and enhance Zn-deficiency in rice, but it can also be because several soil reactions occurring due to flooding that can alter Zn concentrations in soil solution (Forno et al., 1975; Beckwith et al., 1975).

## 2.3 Zinc fertilizers

Several inorganic and organic Zn fertilizers are being used to correct Zn deficiencies. These sources differ based on their physical characteristics, chemical reactivity, cost, and effectiveness. Zinc fertilizer properties affect fertilizer behavior in soil. Physical form and water solubility are the two important fertilizer properties that add to the source effect. Also, the chemical reactions that occur between Zn and the macronutrient carriers affect the diffusion and speciation of Zn. Therefore, there is a need to carefully select the most effective and economical Zn fertilizer specific to the conditions available. This part discusses fertilizer Zn sources, their methods of application, and their reactivity in soil.

### 2.3.1 Zinc sources

The four major Zn sources are inorganic, synthetic chelates, inorganic and organic complexes. These sources vary with respect to the Zn content, cost, and effectiveness for crops. The first type of Zn source used is inorganic Zn sources, including ZnO, $ZnSO_4$, $ZnCO_3$, Zn $(NO_3)_2$, and $ZnCl_2$. Zinc sulfate ($ZnSO_4$) is the most common inorganic sources available in both crystalline and granular forms, whereas ZnO is available as a fine powder or in granular form. Zinc oxide has very low solubility in water, and because of this reason, it is not

immediately available to the plants after its application (Mortvedt, 1991). Second type of Zn source is synthetic chelates are complex forms of micronutrients that are generally formed by complexing a chelating agent with a metal ion through coordinate bonding. Chelated fertilizers are being used because of their greater mobility than other Zn sources. The availability of the metal chelate to the plants is affected by its stability.

The effectiveness of a metal chelate depends on its ability to keep the metal in the chelated form, depending upon its low rate of substitution of metal cations for other cations present in the soil. Zinc-ethylenediamine tetra-acetate (ZnEDTA) is the most common synthetic chelate in use, having a stability constant of 17.5 greater than that of Ca-EDTA (11.6) (Norvell, 1991). Therefore, even in soils with high free $Ca^{2+}$ ions, Zn can preferably be bound to EDTA. In this case, ZnEDTA remains effective as Zn is chelated and not available for substitution or adsorption to negatively charged soil colloids as ZnEDTA itself is negatively charged. The only limitation with using synthetic chelates is their cost. The third type of Zn source used is inorganic complex of Zn, including ammoniated $ZnSO_4$ solution, and ammoniated $ZnCl_2$ solution. Four $NH_3$ are complexed with Zn, but Zn does not remain intact with these molecules after its introduction into the soil. The fourth type of Zn source is natural organic complexes, and these are manufactured by reacting Zn salts with organic by-products of the wood pulp industry. The organic complexes include lignosulfonates, polyflavonoids, and phenols. The cost of the organic Zn complexes is lower than synthetic chelates but is also less effective than ZnEDTA (Mortvedt, 1979). Most of the Zn fertilizers have residual effects on the soil. Several studies have shown that crop response to Zn application can be seen even after five years of Zn application. Zinc concentrations will increase with the annual application of Zn due to its residual effect in soil.

**Table 2.3 Commonly used zinc fertilizers:**

| Inorganic compounds | | |
|---|---|---|
| Compound | Formula | Zn content (%) |
| Zinc sulphate monohydrate | $ZnSO_4.H_2O$ | 36 |
| Zinc sulphate heptahydrate | $ZnSO_4.7H_2O$ | 22 |
| Zinc oxysulphate | $xZnSO_4.xZnO$ | 20-50 |
| Basic zinc sulphate | $ZnSO_4.4Zn(OH)_2$ | 55 |
| Zinc oxide | $ZnO$ | 50-80 |
| Zinc carbonate | $ZnCO_3$ | 50-56 |
| Zinc chloride | $ZnCl_2$ | 50 |
| Zinc nitrate | $Zn\ (NO_3)_2.3H_2O$ | 23 |
| Zinc phosphate | $Zn_3(PO_4)_2$ | 50 |
| Ammoniated zinc sulphate solution | $Zn\ (NH_3)_4SO_4$ | 10 |
| Organic compounds | | |
| Compound | Formula | Zn content (%) |
| Disodium zinc EDTA | $Na_2ZnEDTA$ | 8-14 |
| Sodium zinc HEDTA | NaZnHEDTA | 6-10 |
| Sodium zinc EDTA | NaZnEDTA | 9-13 |
| Zinc polyflavonoid | | 5-10 |
| Zinc lignosulphonate | | 5-8 |

Mortvedt and Gilkes (1993), Martens and Westermann (1991), and Srivastava and Gupta (1996)

### 2.3.2 Application methods

There are several different ways of applying Zn into the soil. The most common method is soil application. It is challenging to apply Zn fertilizers separately in the field as it is both labor and cost intensive. Therefore, both granular and fluid NPK fertilizers are used as carriers of Zn. It helps increase uniformity and reduce cost in the application of Zn while using conventional approaches to apply. One main advantage of bulk blending Zn fertilizers with granular NPK is that recommended rates of Zn, N, P, and K can be provided by producing fertilizer grades. This method is becoming very commonly used, and its popularity is significantly increasing all over the USA, resulting in the addition of Zn and other micronutrients in the plants (Mortvedt and Cox, 1985). However, the main disadvantage is that blending operation and Processing of the Zn blended fertilizers can cause segregation of Zn, resulting in the non-uniform application of Zn. Gilkes (1975) reported a significant reduction in Zn concentration of granules in ordinary superphosphate (OSP) fertilizer where the Zn had been added as ZnO powder before granulation.

Another popular method is incorporating Zn into macronutrients at the time of manufacture. This can reduce the cost of separate applications but can also reduce the Zn availability to plants because when mixed with macronutrient fertilizers under conditions of high temperature and moisture causes several reactions to occur. For instance, ZnEDTA, when mixed with phosphoric acid, acid decomposition of the chelate molecules reduces Zn availability (Mortvedt and Cox, 1985). The level of water-soluble Zn incorporated into ammoniated phosphate fertilizers decreases the Zn's immediate effectiveness and immediate plant availability (Mortvedt, 1968).

Coating Zn fertilizers onto granular NPK fertilizers eliminates the possibility of segregation. The agronomic effectiveness of Zn coated onto soluble granular NPK fertilizers should be similar to that of Zn incorporated during manufacture. The ground Zn fertilizer (<0.25 mm) used to adhere to the NPK granules. When grown on a Zn-deficient soil, Bean (Phaseolus vulgaris L.) yields were similar with $ZnSO_4$ or ZnO coated onto a granular NPK fertilizer or incorporated during manufacturing. However, Zn uptake was greater with $ZnSO_4$ than with ZnO, irrespective of the method of application.

### 2.3.3 Reactions of Zn fertilizers

Zinc availability to plants is determined by two factors- soil chemical properties and the physical state of Zn application. The physical state, i.e., granular or fluid, controls the reactions zinc undergo upon its addition to soil and thus its availability for plant uptake. For granular fertilizers, wetting of the granule is the first step that would happen upon its introduction in soils. The wetting could occur via two processes- by mass flow and capillary action from the moist soil and by water vapor transfer to the granule from the soil or the atmosphere (Lawton and Vomocil, 1954; Williams, 1969). Water movement towards the granule could restrict Zn movement from the granule to the soil. Limited phosphorous diffusion in calcareous soil was due to a similar mechanism (Hettiarachchi et al., 2006). On the other hand, the mass flow of moisture from soil towards the application point was not observed when fluid fertilizers were injected into the soil (Hettiarachchi et al., 2006). Therefore, the physical form of fertilizer significantly affects fertilizer bioavailability (Hettiarachchi et al., 2010). Zinc movement in the soil can be observed by sampling soil sections at increasing distances from the Zn fertilizer application point (Ghosh, 1990; Hettiarachchi et al., 2010). From these experiments, two different zones have been identified based on their different zinc concentrations. The zone adjacent to the fertilizer

application is Zn saturated zone, whereas the zone is farther away from the point of application has low Zn concentrations (Ghosh, 1990). Due to different Zn concentrations, various chemical reactions may occur. Zinc precipitation may be observed in the Zn saturated zone, whereas adsorption reactions might occur in the low Zn concentration zone. Direct identification methods such as synchrotron techniques can be used to investigate solid-phase reaction products. Most of the available information regarding the chemical reactivity of Zn and the formation of Zn reaction products is mostly obtained from laboratory experiments that investigated the chemical reactions between Zn and macronutrient carriers. Ghosh (1990) also demonstrated that the rate of Zn dissolution from granulated NP (nitrogen or phosphorus) fertilizers is affected by soil pH, soil texture, soil water content, and incubation time. In a yellow earth soil with moisture content maintained at field capacity, the Zn percentages that had diffused from granules in 21 days were: $ZnSO_4$ - 95%, MAP (monoammonium phosphate) - 90%, DAP (diammonium phosphate) - 51 %, OSP (ordinary superphosphate) - 98%. These variations occurred because of water-insoluble Zn compounds in ammonium phosphate (AP) fertilizers and the alkaline reaction of DAP. Another major factor that affected Zn concentration in the soil was soil water content. The percentage of Zn lost from the granules was linearly correlated with soil water content. Fertilizers such as MAP and DAP do not produce an acidulation effect during dissolution; therefore, the strong effect of soil pH was seen on the dissolution of Zn from these fertilizer granules. Whereas a very minimal effect of soil pH was observed on Zn dissolution from acidic fertilizer granules, for example, OSP. The above results showed that Zn solubility and hence Zn availability to plants decreased significantly in Zn incorporated in AP fertilizers. The inefficiency of Zn fertilizers is supposed to be greatest in sandy and alkaline soils. Solid-phase reaction products were investigated using X-ray diffraction (XRD) analysis for Zn applied as $ZnSO_4$, ZnS, and ZnEDTA in four soils

(Kalbasi et al., 1978). Incubation time for soils was for 2-32 weeks and the soils were sampled from the zone adjacent to the fertilizer placement site were used for analysis. No crystalline products were detected in soils treated with ZnEDTA whereas Sphalerite was traced in soils treated with ZnS. In two calcareous soils, Zn carbonates were identified in soils treated with $ZnSO_4$. In alkaline noncalcareous soil, Zn $(OH)_2$ was detected shortly after $ZnSO_4$ application, but it was not detected after 4 weeks. This study highlights the limitations of XRD for studying speciation of Zn in soils, as Zn adsorbed onto soil minerals cannot be detected by these techniques. Several studies have reported that Zn solubility and mobility depend on soil pH and cation exchange capacity. Degryse et al. (2015) observed that Zn diffusion for spot applied $ZnSO_4$ (0.35 mg Zn) increased with decreasing soil pH in three soils used. Mortvedt and Giordano (1967b) also reported greater Zn diffusion for spot applied $ZnSO_4$ (0.38 mg Zn) in very acid soil (pH 3.4) as compared to in alkaline soil (pH 7.7). Findings regarding Zinc diffusion when applied as ZnO are limited in the literature. Available data suggest that ZnO when is applied as a fine powder mixed with soil, its dissolution happens within days in acid soils and months in alkaline soils (Smolders and Degryse, 2002; Voegelin et al., 2005). However, when ZnO is in granular form, the dissolution is expected to be much slower because of reduced surface area in contact with the soil (Mortvedt, 1992). Zinc, when applied as coated onto granular NPK fertilizers, undergoes a little different pathway. Zinc can also react with the N component of fertilizers, whereas as compared to Zn reactions with phosphorus, Zn and nitrogen reactions products are water-soluble compounds (Lehr, 1972). Milani et al. (2012) observed the speciation and dissolution behavior of ZnO coated onto urea granules. Results showed that coating did not change Zn speciation as ZnO was the dominant mineral detected on the surface of the granule. Results from a column dissolution technique showed that the cumulative Zn

release from the ZnO-coated urea granules was comparable to that of Zn release from ZnO alone. This suggested that the initial high pH (7.5) because of urea hydrolysis did not restrict ZnO dissolution. From solubility diagrams, hydrozincite is predicted to control the solubility of ZnO from the Zn-coated urea granules.

## 2.4 Conclusions and knowledge gaps

Zinc deficiency is a significant problem faced by the whole world. Although inorganic or organic Zn sources are being used to remediate these deficiencies, no single source available or yet to develop will solve the problem. The performance of a Zn fertilizer is known to be specific to soil type. There is a need to develop cost-effective and efficient Zn source which can remain effective in diversified unwanted conditions. Zinc management in agricultural soils is a complex, therefore a better understanding of Zn reactions and pathways is necessary to develop an effective Zn source. There is a lack of literature comparing mixtures of inorganic and organic sources of Zn under similar experimental conditions. A new granular Zn mixture: a mixture of zinc oxide (ZnO), zinc sulfate ($ZnSO_4$), and zinc-ethylenediamine tetra acetic acid (ZnEDTA) fertilizer with 62% Zn has recently been introduced in the central part of the United States, hypothesizing that Zinc mixture (Zn mix) should inherit the good characteristics from the components used in its formation, e.g., greater solubility from $ZnSO_4$, slow-release properties from ZnO, and greater mobility and availability from ZnEDTA. We carried out three major studies consist of incubation and greenhouse studies. Incubation studies were focused on understanding basic research for applied Zn source in an only soil environment, whereas greenhouse studies focused on learning Zn behavior and effectiveness for wheat crop. The major factors influencing the efficiency of Zn fertilizers are Zn source, application method, application rate, and soil type. To cover these aspects, our studies consist of (i) different inorganic Zn

sources compared to chelated Zn source when applied as sole Zn fertilizers, and Zn-coated urea fertilizers (ii) Compared sole Zn fertilizers in neutral pH soil (iii) Understanding the fact that behavior of these sources differ with soil type, we used four soils having different physicochemical properties. However, the first prerequisite to managing trace elements like Zn in soils is a good understanding of the various processes and mechanisms that govern their behavior. Therefore, to know the reaction mechanisms better, chemical speciation for Zn fertilizers and Zn-coated urea fertilizers was studied using Synchrotron X-ray absorption (XAS).

## 2.5 References

Alloway, B.J. 2004. Zinc in Soils and Crop Nutrition. International fertilizer Industry Association and International Zinc Association, Brussels, Belgium and Paris, 135.

Angelone, M. and C. Bini. 1992. Trace elements concentrations in soils and plant of western Europe. p. 19-60. In D.C. Adriano (ed.) Biogeochemistry of Trace Metals. Lewis Publishers, Boca Raton, FL.

Armour, J.D., G.S.P. Ritchie and A.D. Robson. 1990. Extractable zinc in particle size fractions of soils from Western Australia and Queensland. Aust. J. Soil Sci. 28:387-397.

Asher, C.J. (ed.) 1987. Effects of nutrient concentration in the rhizosphere on plant growth. Proc. XIII$^{th}$ Congress Int. Soc. Soil Sci. (ISSS), Symp., 5$^{th}$, Hamburg. August. 1986. Congress Int. Soil Sci. (ISSS), Hamburg.

Baize, D. 1997. Total content of metallic trace elements in soil (in French), INRA Editions, Paris. 409 pp.

Barrow N.J. 1987. The effects of phosphate on zinc sorption by a soil. 1. Soil Sci. 38:453-459.

Barrow, N.J. 1993. Mechanisms of Reaction of Zinc with Soil and Soil Components. p. 15-32. In A.D., Robson (ed.) 1993. Zinc in Soils and Plants, Kluwer Academic Publishers, Dordrecht.

Barber, S.A. 1984. Soil Nutrient Bioavailability. A Mechanistic Approach. John Wiley and Sons, NY.

Beckwith R.S., K.G. Tiller, and E. Suwadji. 1975. The effects of flooding on the availability of trace metals to rice in soils of differing organic matter status. p. 135-149. *In* D.J.D. Nicholas and A.R. Egan (ed.). Academic Press, NY.

Bell P.F., R.L. Chaney, and J.S. Angle. 1991. Determination of the copper$^{2+}$ activity required by maize using chelator-buffered nutrient solutions. Soil Sci. Soc. Amer. J. 55:1366-1374.

Bell R.W., D.G. Edwards and C.J. Asher. 1989. Effects of calcium supply on uptake of calcium and selected mineral nutrients by tropical food legumes in solution culture. Aust. J. Agric. Res. 40:1003-1014.

Bingham F.T., J.P. Martin and J.A. Chastain. 1958. Effects of phosphorus fertilization of California soils on minor elements nutrition of citrus. Soil Sci. 86:24-31.

Bloomfield, C. 1981. The translocation of metals in soils. *In* D.J. Greenland, M.H.B. Hayes (ed.) The Chemistry of Soil Processes, John Wiley & Sons Ltd., Chichester, UK.

Bolland M.D.A., A.M. Posner, and J.P. Quirk. 1977. Zinc adsorption by goethite in the absence and presence of phosphate. Aust. J. Soil Res. 15:279-286.

Boawn L.C., F.G. Viets, and C.L. Crawford. 1954. Effect of phosphate fertilizers on zinc nutrition of field beans. Soil Sci. 78:1-7.

Boawn L.C. and J.C. Brown. 1968. Further evidence for a P-Zn imbalance in plants. Soil Sci. Soc. Amer. Proc. 32:94-97.

Boawn L.C. and G.E. Legget. 1964. Phosphorus and zinc concentrations in Russet Burbank potato tissue in relation to development of zinc deficiency symptoms. Soil Sci. Soc. Amer. Proc. 28:229-232.

Bowen J.E. 1969. Absorption of copper, zinc and manganese by sugar cane tissue. Plant Physiology 44:255-261.

Brennan, R.F. 1992a. The relationship between critical concentrations of DTPA-extractable zinc from the soil for wheat production and properties of south-western Australian soils responsive to applied zinc. Commun. Soil Sci. Plant Anal. 23:747-759.

Brennan, R.F. and J.W. Gartrell. 1990. Reaction of zinc with soil affecting its availability to subterranean clover. I. The relationship between critical concentrations of extractable zinc and properties of Australian soils responsive to applied zinc. Aust. J. Soil Res. 28:293-302.

Bertrand, L.J., R.E. Holloway, R.D. Armstrong and M.J. McLaughlin. 2002. The rapid assessment of concentrations and solid phase associations of macro and micronutrients in alkaline soils by mid-infrared diffuse reflectance spectroscopy. Australian Journal of Soil Research, 41:61-76.

Bruemmer, G.W., J. Gerth and U. Herms. 1986. Heavy metal species, mobility and availability in soils. Z. Pflanzenernlihr. Bodenk. 149:382-398.

Brun, L.A., J. Maillet, P. Hinsinger, and M. Pepin. 2001.Evaluation of copper availability to plants in copper-contaminated vineyard soils; Environ. Pollut. 111:293-302.

Chaudhry F.M. and J.F. Loneragan. 1970. Effects of nitrogen, copper, and zinc fertilizers on the copper and zinc nutrition of wheat plants. Aust. J. Agric. Res. 21:865-879.

Chaudhry F.M. and J.F. Loneragan. 1972a. Zinc absorption by wheat seedlings: I. Inhibition by macronutrient ions in short-term experiments and its relevance to long-term zinc nutrition. Soil Sci. Soc. Amer. Proc. 36, 323- 327.

Chaudhry F.M. and J.F. Loneragan. 1972b. Zinc absorption by wheat seedlings: II. Inhibition by hydrogen ions and by micronutrient cations. Soil Sci. Soc. Amer. Proc. 36:327-331.

Chaudhry F.M. and J.F. Loneragan. 1972c. Zinc absorption by wheat seedlings and the nature of its inhibition by alkaline earth cations. J. Exp. Bot. 23:552-560.

Christensen N.W. and T.L. Jackson. 1981. Potential for phosphorus toxicity in zinc-stressed com and potato. Soil Sci. Soc. Amer. J. 45:904-909.

Cogliatti D.H., N. Alcocer and G.E. Santa Maria. 1991. Effect of phosphorus concentration on zinc-65 uptake in Gaudiniajragilis. J. Plant Nutr. 14:443-452.

Degryse, F., R. Baird, and M. McLaughlin. 2015. Diffusion and solubility control of fertilizer- applied zinc: chemical assessment and visualization. Plant Soil 386:195-204.

Edwards J.H. and E.J. Kamprath. 1974. Zinc accumulation by com seedlings as influenced by phosphorus, temperature, and light intensity. Agron. J. 66:479-482.

EI-Kherbawy, M., J.S. Angle, A. Heggo and R.L. Chaney. 1989. Soil pH, rhizobia, and vesicular-arbuscular mycorrhizae inoculation effects on growth and heavy metal uptake of alfalfa (Medicago sativa L.). J. Biology and Fertility of Soils. 8:61-65.

Evans, L.J. 1989. Chemistry of metal retention by soils; J. Environ. Sci. Technol. 23:1046-1056.

Forno D.A., S. Yoshida and C.J. Asher. 1975. Zinc deficiency in rice. I. Soil factors associated with the deficiency. Plant Soil 42:537-550.

Friesen D.K., A.S.R. Juo and M.H. Miller. 1980. Liming and lime-phosphorus-zinc interaction in two Nigenan ultisols: I. Interactions in the soil. J. Soil Sci. Soc. Amer. 44:1221-1226.

Ghosh, A.K. 1990. Chemistry and agronomic effectiveness of Zn-enriched fertilizer. Ph.D. diss., University of Western Australia, Nedlands, W.A.

Giordano M., J.C. Noggle and J.J. Mortvedt. 1974. Zinc uptake by rice, as affected by metabolic inhibitors and competing cations. Plant Soil 41:637-646.

Gilkes R.J. 1975. Factors influencing the release of Cu and Zn additives from granulated superphosphate. J. Soil Sci. 28:105-111.

Gorny, A., J. Uterman, and W. Eckelmann. 1992. Germany: in Heavy Metal (Trace Element) and Organic Matter Contents of European Soils. European Comission, CEN Soil Team N 30, Secretariat, Nederlands Normalisatie-institute (NEN) Delft, The Netherlands.

Grove, J.H. and M.E. Sumner. 1985. Lime induced magnesium stress in corn: Impact of magnesium on phosphorus availability. Soil Sci. Soc. Am. J. 49:1192-1196.

Hawf, L.R. and W.E. Schmid. 1967. Uptake and translocation of zinc by intact plants. Plant Soil 27:249-260.

Hettiarachchi G.M., E. Lombi, M.J. McLaughlin, M.J., D.J. Chittleborough, and P. Self. 2006. Density changes around phosphorus granules and fluid bands in a calcareous soil. Soil Sci. Soc. Am. J. 70:960-966.

Hettiarachchi G.M., E. Lombi, M.J. McLaughlin, M.J., D.J. Chittleborough, and C. Johnston. 2010. Chemical behavior of fluid and granular Mn and Zn fertilisers in alkaline soils. Aust. J. Agric. Res. 48:238-247.

Hill, J., A.D. Robson, and J.F. Loneragan.1979. The effect of copper supply on the senescence and the retranslocation of nutrients of the oldest leaf of wheat. Ann. Bot. 44:279-287.

Holmgren, C.G.S., M.W. Meyer, R.L. Chaney, and R.B. Daniels. 1993. Cadmium, lead, zinc, copper and nickel in agricultural soils in the United States. Journal of Environmental Quality, 22: 335-348.

Harter, R.D. (1991). Micronutrient Adsorption-Desorption Reactions in Soils. *In* J.J. Mortvedt (ed.) Micronutrients in Agriculture.

Jeffery, J.J. and N.E. Uren. 1983. Copper and zinc species in the soil solution and effects of soil pH. Aust. J. Soil Res. 21:479-488.

Jolley V.D. and J.C. Brown. 1991. Factors in iron-stress response mechanism enhanced by Zn-deficiency stress in Sanilac, but not Saginaw navy beans. J. Plant Nutr. 14:257-265.

Kalbasi M., G.J. Racz, and L.A. Lewen-Rudgers. 1978. Reaction products and solubility of applied zinc compounds in some Manitoba soils. Soil Sci. 125:55.

Kauser M.A., F.M. Chaudhry, A. Rashid, A. Latif and S.M. Alam. 1976. Micronutrient availability to cereals from calcareous soils. I. Comparative Zn and Cu deficiency and their mutual interaction in rice and wheat. Plant Soil 45:397-410.

Kabata-Pendias, A. and H. Pendias. 1992. Trace Elements in Soils and Plants (2nd ed.) p. 365. CRC Press, Boca Raton.

Kalnicky, D.J. and R. Singhvi. 2001. Field portable XRF analysis of environmental samples; J. Hazard. Mater. 83:93-122.

Kiekens, L and R. Cammerlynck. 1992. Landwirtsch. Forsch. **39**: 255.

Kiekens, L. 1995. Zinc. p. 284-305. *In* Alloway, B.J. (ed.) Heavy Metals in Soils (2nd edn.). Blackie Academic and Professional, London.

Lambert D.H., D.E. Baker and H. Cole. 1979. The role of mycorrhizae in the interaction of phosphorus with zinc, copper, and other elements. Soil Sci. Soc. Amer. J. 43:976-980.

Lawton K. and J.A. Vomocil. 1954. The dissolution and migration of phosphorus from granular superphosphate in some Michigan soils. Soil Sci. Soc. Am. Proc. 18:26-32.

Lee, C.R. and G.R. Craddock. 1969. Factors affecting plant growth in high-zinc medium. II. Influence of soil treatments on growth of soybeans on strongly acid soil containing zinc from peach sprays. Agron. J. 61:565- 567.

Lehr, J. 1972. Chemical reactions of micronutrients in fertilizers. p. 459-503. *In* J. Mortvedt, P. Giordano and W. Lindsay (ed.) Micronutrients in Agriculture, Soil Science Society of America, Madison, WI.

Lindsay, W.L. 1979. Chemical equilibria in soils. Wiley, NY.

Linehan, D.J., A.H. Sinclair and M.E. Mitchell. 1989. Seasonal changes in Cu, Mn, Zn and Co concentrations in soil in the root-zone of barley (Hordeum vulgare L.). J. Soil Sci. 40:103-115.

Lindsay, W.L. and F.R. Cox. 1985. Micronutrient soil testing for the tropics. Fert. Res. 7:169-200.

Lins, I.D.G. and F.R. Cox. 1988. Effect of soil pH and clay content on the zinc soil test interpretation for corn. Soil Sci. Soc. Am. J. 52:1681-1685.

Loneragan J.F., T.S. Grove, A.D. Robson and K. Snowball. 1979. Phosphorus toxicity as a factor in zinc phosphorus interaction in plants. Soil Sci. Soc. Amer. J. 43:966-972.

Loneragan J.F., D.L. Grunes, R.M. Welch, E.A. Aduayi, A. Tengah, V.A. Lazar and E.E. Cary. 1982. Phosphorus accumulation and toxicity in leaves in relation to zinc supply. Soil Sci. Soc. Amer. J. 46:345-352.

Loneragan J.F. 1951. Effect of applied phosphate on the uptake of zinc by flax. Aust. J. Sci. Res. B4:108-114.

Martens, D.C. and D.T. Westermann (1991) Fertilizer applications for correcting micronutrient deficiencies. p. 549-592. *In* J.J. Mortvedt, F.R. Cox, L.M. Shuman, and R.M. Welch (ed.) Micronutrients in Agriculture (2nd ed.) Soil Science Society of America, Madison, Wisc.

McGrath, S.P., J.R. Sanders and M.H. Shalaby. 1988. The effect of soil organic matter levels on soil solution concentrations and extractabilities of manganese, zinc and copper. Geoderma 42:177-188.

McBride, M. 1991. Processes of heavy metal and transition metal sorption by soil minerals. p. 149-176. *In* G. Bolt, M. De Boodt, M. Hayes, M. McBride, and E. De Strooper (ed.) Interactions at the Soil Colloid-Soil Solution Interface; Kluwer Academic Publishers, Dordrecht, The Netherlands.

McGrath, S.P. and P.J. Loveland. 1992. Soil Geochemical Atlas of England and Wales, Blackie Academic and Professional, Glasgow.

Milani, N., M.J. McLaughlin, S.P. Stacey, J.K. Kirby, G.M. Hettiarachchi, D.G. Beak, G. Cornelis. 2012. Dissolution kinetics of macronutrient fertilizers coated with manufactured zinc oxide nanoparticles. J. Agric. Food Chem. 60, 3991-3998.

Millikan C.R. 1940. Zinc Requirement of wheat. The effect of superphosphate. J. Dept. Agric., Victoria. 38:135- 136.

Millikan C.R. 1951. Diseases of flax and linseed. Zinc deficiency. Dept. Agric. Vict., Aust. Tech. Bull. No.9:90-108.

Millikan C.R., B.C. Hanger and E.N. Bjarnason. 1968. Effect of phosphorus and zinc levels in the substrate on Zn distribution in subterranean clover and flax. Aust. J. bioI. Sci. 21:619-640.

Moraghan, J.T. and H.J. Mascagni. 1991. Environmental and soil factors affecting micronutrient deficiencies and toxities. p. 371-425. *In* J.J. Mortvedt, F.R. Cox, L.M. Shuman, and R.M. Welch (ed.) Micronutrients in Agriculture. Madison, WI.

Mortvedt, J.J. 1992. Crop response to level of water-soluble zinc in granular zinc fertilizers. Fertil. Res. 33:249-255.

Mortvedt, J.J. 1968. Crop response to applied zinc in ammoniated phosphate fertilizers. J. Agr. Food Chern. 16:241-245.

Mortvedt, J.J. 1979. Crop response to zinc sources-applied alone or with suspensions. Fert. Solutions 23:64-79.

Mortvedt, J.J. 1991. Micronutrient fertilizer technology. p. 523-548. *In* J.J. Mortvedt, F.R. Cox, L.M. Shuman, and R.M. Welch (ed.) Micronutrients in Agriculture. Madison, WI.

Mortvedt, J.J. and F.R. Cox. 1985. Production, marketing, and use of calcium, magnesium, and micronutrient fertilizers. p. 455-481. *In* Fertilizer Technology and Use. Ed. OP Englestad. Soil Sci. Soc. Am., Madison, WI, USA.

Mortvedt, J.J. and R.J. Gilkes. 1993. Zinc fertilisers. p. 33-44. *In* A.D. Robson (ed.) Zinc in Soils and Plants. Kluwer Academic Publishers, Dordrecht.

Norvell, W.A. and W.L. Lindsay. 1970. Lack of evidence for $ZnSiO_3$ in soils. Soil Sci Soc Am Proc 34:360-361.

Norvell, W.A. 1991. Reactions of metal chelates in soils and nutrient solutions. p. 187-227. *In* Fertilizer Technology and Use. Ed. OP Englestad. Soil Sci. Soc. Am., Madison, WI, USA.

Norvell, W.A. and R.M. Welch. 1993. Growth and nutrient uptake by barley *(Hordeum vulgare* L. cv Herta). Studies using an N-(2-Hydroxyethyl) ethylenedinitrilotriacetic acid buffered nutrient solution technique. I. Zinc ion requirements. Plant Physiol. 101:619-625.

Ozanne, P.G., T.C. Shaw and D.J. Kirton. 1965. Pasture responses to traces of zinc in phosphate fertilizers. Aust.1. Exp. Agric. Anim. Husb. 5:29-33.

Parker, M.B. and M.E. Walker. 1986. Soil pH and manganese effects on manganese nutrition of peanut. Agron. J. 78:614-620.

Parker, D.R., J.J. Aguilera and D.N. Thomason. 1992. Zinc-phosphorus interactions in two cultivars of tomato (Lycopersicon esculentum L.) grown in chelator-buffered nutrient solutions. Plant and Soil 143:163-177.

Pickering W.F. 1980. Zinc interaction with soil and sediment components. p. 71-112. *In* J.O. Nriagu (ed.) Zinc in the Environment. John Wiley and Son, NY.

Rashid A., F.M. Chaudhry and M. Sharif. 1976. Micronutrient availability to cereals from calcareous soils. Zinc absorption by rice and its inhibition by important ions of submerged soils. Plant Soil 45:613-623.

Rogers L.H. and C. Wu. 1948. Zinc uptake by oats as influenced by application of lime and phosphate. J. Arner. Soc. Agron. 40:563-566.

Saeed M. and R.L. Fox. 1979. Influence of phosphate fertilization on zinc adsorption by tropical soils. Soil Sci. Soc. Arner. J. 43:683-686.

Safaya N.M. 1976. Phosphorus-zinc interaction in relation to absorption rates of phosphorus, zinc, copper, manganese, and Iron in corn. Soil Sci. Soc. Am. J. 40:719-722.

Schmid W.E., H.P. Haag, E. Epstein. 1965. Absorption of zinc by excised barley roots. Physiol. Planta. 18:860-869.

Sharma, K.N. and D.L. Deb. 1988. Effect of organic manuring on zinc diffusion in soils of varying texture. J. Indian Soc. Soil Sci. 36:219-224.

Sharma K.C., B.A. Krantz, A.L. Brown and J. Quick. 1968. Interaction of Zn and P in top and root of com and tomato. Agron. J. 60:453-456.

Shuman L.M. 1980. Zinc in soils. In Zinc in the Environment. p. 39-69. In J.O. Nriagu (ed.) Zinc in the Environment. John Wiley and Son, NY.

Singh J.P., R.E. Karamanos and J.W.B. Stewart. 1988. The mechanism of phosphorus-induced zinc deficiency in bean (Phaseolus vulgaris L.). Can J. Soil Sci. 68:345-358.

Smith, R.M., A.E. Martell, R.J. Motekaitis. 1997. NIST critically selected stability constants of metal complexes database Version 3.0; U.S. Department of Commerce, Gaithersburg, MD.

Smolders, E., F. Degryse. 2002. Fate and effect of zinc from tire debris in soil. Environ. Sci. Technol. 36:3706-3710.

Srivastava, P.C. and U.C. Gupta. 1996. *Trace Elements in Crop Production*. Science Publishers, Lebanon, New Hampshire, 356pp.

Stanton, D.A. and R.D. Burger. 1967. Availability to plants of zinc sorbed by soil and hydrous iron oxides. *Geoderma, 1*:13-17.

Stevenson, F.J. 1991. Organic Matter-Micronutrient Reactions in Soil. *In* J.J. Mortvedt, F.R. Cox, L.M. Shuman, and R.M. Welch (ed.) Micronutrients in Agriculture. Madison, WI.

Tiller, K.G. 1983. Micronutrients. p. 365-387. In Division of Soils, CSIRO. Soils an Australian Viewpoint. CSIRO/Academic Press, Melbourne.

Toms, J. 1958. The use of copper and zinc in the cereal-growing districts of Western Australia. I. Dept. Agric. Western Australia, series 3, 7:197-203.

Van Steveninck, R.F.M., A. Babare, D.R. Fernando, M.E. Van Steveninck. 1993. The binding 618 of zinc in root cells of crop plants by phytic acid. In: Plant nutrition - from genetic 619 engineering to field practice, Proceedings of the Twelfth International Plant Nutrition 620 Colloquium, 21-26 September 1993, 775-778, Perth, Western Australia. Kluwer 621 Academic Publishers, Dordrecht, Netherlands

Voegelin, A., S. Pfister, A.C. Scheinost, M.A. Marcus, R. Kretzschmar. 2005. Changes in zinc speciation in field soil after contamination with zinc oxide. Environ. Sci. Technol. 39:6616-6623.

Watanabe, F.S., W.L. Lindsay, S.R. Olsen. 1965. Nutrient balance involving phosphorus, iron, and zinc. Soil Sci. Soc. Amer. Proc. 29:562-565.

Wear, J.I. 1956. Effect of soil pH and calcium on uptake of zinc by plants. Soil Sci. 81:311-315.

Weitje, L. 1998. Mixture toxicity and tissue interactions of Cd, Cu, Pb and Zn in earthworms (oligochaeta) in laboratory and field soils: a critical evaluation of data; Chemosphere, 36:2643-2660.

Williams C.H. 1969. Moisture uptake by surface-applied superphosphate and movement of the phosphate and sulphate into the soil. Aust. J. Soil Res. 7:307-316.

Wilkinson, H.F., J.F. Loneragan and J.P. Quirk. 1968a. The movement of zinc to plant roots. Soil Sci. Soc. Amer. Proc. 32:831-833.

Zhang, F., V. Romheld, and H. Marsclmer. 1991b. Diurnal rhythm of release of phytosiderophores and uptake rate of zinc in iron-deficient wheat. Soil Sci. Plant Nutr. 37:671-678.

# Chapter 3 - Fate and plant uptake of different zinc fertilizer sources upon their application to an alkaline calcareous soil.

## Abstract

Fertilizer Zn behaves differently in different soils depending on soil properties. Calcareous soils having high pH and high $CaCO_3$ are sensitive to Zn deficiency. Various sorption-desorption reactions control the Zn concentration in soil solution and Zn availability to crops. Understanding the interactions and speciation of Zn is very important to gain more insights into the fate of added Zn in calcareous soil and its efficient management in soil for optimum crop production. Spatial evaluation of Zn fate, transport and extractability was measured using incubation, visualization, and greenhouse studies, and Zn plant uptake was measured in the greenhouse study. The objectives were to compare the efficiency and investigate the reaction products and pathways of five Zn only sources (ZnO, $ZnSO_4.7H_2O$, Zn mix (mixture of ZnO, $ZnSO_4$, and EDTA), ZnP, and ZnEDTA) compared to one control (No-Zn) and five Zn-coated urea sources (ZnO-U, $ZnSO_4.7H_2O$-U, Zn mix-U, ZnP-U, and ZnEDTA-U compared to urea control (No-Zn+ urea). Zinc was applied in the greenhouse study at a rate of 10 kg $ha^{-1}$ (Zn only sources) and 5 kg $ha^{-1}$ (Zn-coated urea sources). In comparison, 2.42 mg of Zn/petri dish (Zn only sources) and 0.12 mg Zn/ petri dish (Zn-coated urea sources) were used for the incubation-visualization studies. Plant biomass and Zn plant uptake were greater for ZnO, Zn mix (60% ZnO), and ZnEDTA. Both Zn diffusion and extractability were greatest for ZnEDTA. Zinc oxide and Zn mix were slow and fast/slow mix-release fertilizers, respectively, they appeared to be efficient Zn treatments due to their slow-release behavior when applied to calcareous soil. Additionally, Zn-coated urea sources provided more soluble and plant available Zn due to the enhanced solubility facilitated by its co-additive urea. Synchrotron-based X-ray

absorption near-edge structure (XANES) spectroscopy results were in agreement and confirmed different reaction products for Zn-coated urea products. Our results showed that high solubility might not be the only indicator to test Zn fertilizer use efficiency; less soluble sources behave as efficiently as highly soluble sources due to the reduced sorption reactions limiting Zn availability, especially in calcareous soils.

## 3.1 Introduction

Zinc fertilizers are typically blended with, incorporated into, or coated onto macronutrient fertilizer. Blending helps to provide cost-effective delivery of the fertilizer to the soil and blending helps to achieve uniform distribution of Zn in the field. One of the most important factors controlling the effectiveness of Zn fertilizer is its solubility, which depends on the Zn source and properties of soil to which it is applied and therefore affects plant growth and development (Amrani et al., 1999; Shaver et al., 2007; Westfall et al., 2005). Mortvedt and Giordano (1969) reported a significant correlation between Zn solubility and its Zn availability to crops when applied as zinc oxide (ZnO) or zinc sulfate incorporated into macronutrients.

Besides the water solubility of the Zn source, other soil factors affect the behavior of different Zn sources in soil. Different Zn fertilizers undergo different reactions when added to the soil depending upon the reaction mechanisms and pathways occurring in the soil. Interaction of soil components with added Zn is inevitable, affecting the spatial distribution and speciation of added Zn. Although, Zn fertilizer behavior can be studied by exploring the correlation between total elemental composition (total zinc), soil pH, diethylenetriamine pentaacetatic acid (DTPA) extractable Zn, and water-soluble Zn. The reactions occurring in soil determine the fate and behavior of applied Zn fertilizer, thus, its effectiveness. Although the chemical and physical behavior of Zn sources in soils has been widely investigated (Jacquat et al., 2008; Voegelin et al.,

2005; Degryse et al., 2011), there is limited understanding of the fate and transformations of different Zn fertilizer mixtures (mixtures of readily soluble and slowly soluble Zn sources) upon their introduction to the soil. The information on the reactions and solid phase speciation of Zn when applied as different mixtures of inorganic and organic sources to soils is of vital importance. This lack of knowledge limits the refinement of the appropriate fertilizer sources and the development of more effective formulations for different soils.

Identification of Zn species in the soil solid phase is complex but has been possible using synchrotron X-ray absorption spectroscopic techniques (XAS). These techniques have provided an outstanding research tool for in situ investigations of the solid phase speciation of trace elements such as Zn in soil environments at molecular levels (Hettiarachchi et al., 2008; Lombi and Susini, 2009; McNear et al., 2005, Milani et al., 2015). Speciation of trace elements by XAS is not limited by their low concentrations or lack of crystallinity in soils. However, XAS speciation is relying on linear combination fitting to produce speciation and quantitation results (Scheckel and Ryan, 2004). Thus, integration of traditional and advanced analytical techniques, such as XAS, will help us study the behavior of different sources in selected alkaline calcareous soil, compare their efficiencies, and choose the best Zn fertilizer specific to soil type.

Few studies have directly compared an inorganic Zn source with Zn chelates. A new granular Zn mixture (mixture of zinc oxide, zinc sulfate, and zinc-ethylenediamine tetra acetic acid) fertilizer with 62% Zn has recently been introduced in the central part of the United States. Zinc mix should inherit the good characteristics from the components used in its formation, e.g., greater solubility- $ZnSO_4$, slow-release-ZnO, and greater mobility-ZnEDTA. To help differentiate between physical and chemical factors in the availability of different Zn fertilizers, these were added directly to soil for comparison.

This study aimed to compare five different Zn treatments (zinc oxide (ZnO), zinc sulfate (ZnSO4), zinc mixture of ZnO, $ZnSO_4$, and ZnEDTA (Zn mix), zinc phosphate, $Zn_3(PO_4)_2.4H_2O$ (ZnP), and zinc-ethylenediamine tetra acetic acid (ZnEDTA)) and five different Zn-coated urea treatments (zinc oxide coated urea (ZnO-U), zinc sulfate coated urea ($ZnSO_4$-U), zinc mix coated urea (Zn mix-U), zinc phosphate coated urea (ZnP-U), and zinc-ethylenediamine tetra acetic acid coated urea (ZnEDTA-U)) when applied to alkaline calcareous soil in plant-less, soil incubation experiments and with plants, using Zn responsive spring wheat line in a greenhouse experiment.

We hypothesize that Zn mix treatment provides greater plant acquisition. Being a mixture of ZnO, $ZnSO_4$, and ZnEDTA, Zn mix is expected to have inherent beneficial characteristics from its components. For example, Zn mix should exhibit greater solubility as $ZnSO_4$, slow release as ZnO, and greater mobility as ZnEDTA.

## 3.2 Materials and Methods

### 3.2.1 Soil collection

Calcareous soil (Ulysses silt loam) from Oakley City, KS was used for this study. The surface soil (0 to 15 cm depth) was collected, air-dried, and sieved to < 4 mm by passing through stainless steel mesh/sieve.

After carefully breaking the large aggregates, soil was sieved through a 2-mm sieve to be used for initial analysis and incubation studies. The soil was analyzed for soil texture, pH, total organic carbon (TOC), and cation exchange capacity (CEC) and other properties. The pH was measured in a 1:10 soil: Milli Q water extract (Watson and Brown, 1998); extractable P was determined by Olsen method (Olsen et al., 1954); electrical conductivity (EC) was determined using the method described by Whitney (1998). The maximum water holding capacity (MWHC) was determined using Jenkinson and Powlson method (1976). Total Zn concentration in soil was

determined using aqua regia (Zarcinas, 1996). Cation exchange capacity was determined by displacement method (Soil Survey Staff, 2011); soil carbonates were determined by Allison and Moodie (1965); and TOC content of the soil was measured by dry combustion method (Wright and Bailey, 2001). The maximum water holding capacity (MWHC) was determined using Jenkinson and Powlson method (1976). Cation exchange capacity, soil carbonates, TOC content determinations were done by the Soil testing laboratory, Kansas State University

### 3.2.2 Experiment set up

The study consisted of three experiments two incubation and one greenhouse experiment.

#### 3.2.2.1 Incubation study- I (Zn only treatments)

This study was composed of 42 Petri dishes (53mm diameter and 13.9mm height). The soil was packed to achieve a uniform bulk density of 1.05 Mg m-3. Packing was done using soil prewetted to 15% of MWHC. After packing, the soils were brought to 55% MWHC, the covers were placed on the dishes, the edges were wrapped in Parafilm, and the dishes were inverted and allowed to equilibrate at room temperature (≈24°C) for at least 24 hours. Treatments were then carefully introduced at the exact center of the dish. Treatments were added at a rate of 2.43 mg Zn/Petri dish. The seven treatments replicated three times consisted of no-Zn control, no-Zn and EDTA control, ZnO, $ZnSO_4$, Zn mix (mixture containing 60% ZnO, 25% $ZnSO_4$, and EDTA to $ZnSO_4$ 1:8 ratio), ZnP, ZnEDTA.

**Table 3.1 Initial chemical and physical properties of calcareous soil**

| Parameter | Value |
|---|---|
| pH (1:10 soil: water) | 9.0±0.1 |
| CEC*, cmolc/kg | 21.3 |
| $CaCO_3$, % | 7.6 |
| Total Zn, mg kg-1 | 38.7±0.36 |
| DTPA Zn, mg kg-1 | 0.36±0.05 |
| Total P, mg kg-1 | 634 |
| Available P (Olson) P, mg kg-1 | 31 |
| Total Organic Carbon, % | 0.64 |
| Total- N % | 0.09 |
| Sand, Silt, and Clay, % | 12.7, 65.5, 21.8 |
| MWHC** | 58.6±0.04 |
| Soil texture | Silt loam |

*Cation Exchange Capacity; **Maximum Water Holding Capacity

Following treatment introduction, Petri dishes were covered, and Parafilm was employed to seal Petri dishes to mitigate moisture loss. The dishes were wrapped in aluminum foil to prevent light exposure and incubated for five weeks in the dark at 25°C. Following incubation, the soils in dishes were separated into four concentric circular sections with radii of 0-4 mm, 4-8 mm, 8-17 mm, and 17 mm- 26.5 mm, starting from the center. The soils were then dried at 40°C (Fisher Scientific Drying Oven, Waltham, MA), weighed, and finely ground with a mortar and pestle.

### 3.2.2.2 Incubation study- II (Zn-coated urea treatments)

The experiment setup was carried out as discussed earlier, besides a few changes. This experiment was comprised of 36 large Petri dishes (85 mm diameter and 11.5 mm height) packed with a uniform bulk density of 1.1 Mg $m^{-3}$. Packing was done using soil prewetted to 14% of MWHC. Treatments were added at a rate of 0.12 mg Zn/Petri dish. The six treatments replicated three times consisted of Control (Urea+ no-Zn), ZnO-U, $ZnSO_4$-U, Zn mix-U, ZnP-U, and ZnEDTA-U. After five weeks, the dishes were sectioned into four concentric circular sections with radii of 0-8 mm, 8-15.5 mm, 15.5-27 mm, and 27 mm-dish edges. The sections were then dried at 40°C, weighed, and finely ground with a mortar and pestle.

For ongoing study analyses for both incubation studies, visualization was done for days 1, 7, 21, and 35. Visualization was done using Degryse et al. (2015) method. By this method, Zn movement was investigated using calcium carbonate ($CaCO_3$). Calcium carbonate sorbs Zn through surface complexation and precipitation (Comans and Middelburg, 1987). Calcium impregnated papers were prepared using Whatman filter paper no. 1. Filter papers were cut to the exact diameter of the Petri dish. Papers were soaked in 1 M $CaCl_2$ for at least 30 minutes (min) and then transferred to 0.4 M $Na_2CO_3$ for about 10 min, resulting in precipitation of $CaCO_3$ in the paper. The papers are rinsed with DI water, dried, and stored for later use. The deployment of papers was done for 2-hour for contact times between soil and fertilizer up to 1 day, 3-hours deployment time for contact times up to 1 week, 4-hours deployment time when the fertilizer has been in contact with the soil for more than one week (day- 21, and 35 for present study). For color development, dithizone dye was used. The stock solution was prepared by dissolving 50 mg of dithizone in 0.5 mL of ethanol + 0.1 mL of concentrated ammonia and diluting to 10 mL with deionized water. A pink color appeared in the high-Zn zone; full-color development took

about 20 min. In the end, filter papers were scanned, and the size of the pink zone was quantified using imaging software (GIMP, 2.10.20).

Soil samples from both incubation studies were analyzed for pH (1:10 soil: water), water-extractable Zn (1:10 soil: water), and the total Zn concentration in the soil using the aqua regia. Soil (0.5 g) was digested using 5 mL of aqua regia solution (1:3 (v/v) $HNO_3$: HCl) as follows. Briefly, samples pretreated with aqua regia solution were allowed to react overnight at room temperature. The soils were digested at the temperature of 75°C for 30 minutes (min), 100°C for 30 min, 110°C for 30 min, and 140°C until the acid volume decreased to ≈1.0 mL. The digested samples were cooled, diluted to 25 mL of 0.1% $HNO_3$, and filtered through a no. 42 Whatman filter paper (Zarcinas, 1996). In each batch of digestion, one blank and one sample of standard reference soil material (NIST 2711a - Montana soil) was included as QA/QC controls. The analysis was done using the inductively coupled plasma optical emission spectrometry (ICP-OES, Varian 720-ES, Santa Clara, CA)

Synchrotron analysis

Composite samples from the first and second sections of Zn-coated urea treatments (Incubation study- 2) were chosen for this study. Soils were prepared by mixing equivalent quantities of soil from each replicate of the treatments. Then, to ensure maximum homogenization, the triplicate sample mixtures were ground to a fine powder with an agate mortar and pestle. Composite samples were packed in sample holders with 2 mm thick Plexiglas slits (Figure 3.1, 3.2). Both sides of the sample holders were sealed with Kapton® tape (Bertech, Torrance, CA). Zinc K-edge X-ray absorption near-edge spectroscopy (XANES) spectra were collected at the beamline 5 BM-D (DuPont-Northwestern-Dow Collaborative Access Team, DND-CAT) at the Advanced Photon Source (APS), Argonne National Laboratory, in Argonne,

IL. Data were collected at ambient temperature at Zn (≈9458 to 10210 eV) in fluorescence mode. Two Aluminum foil layers were installed on the detector to suppress the soil Fe signal to minimize any interference from Fe. Concerning the sample surface, the angle of the incident X-ray beam was at 45°. The energy was calibrated using standard Zn-metal foil. With each sample, we attempted to collect the Zn-metal foil spectra. The number of spectra collected per sample was 3 to 10 (mostly 6). Data processing and analysis were done using Athena software version 0.9.26 (Ravel, 2018). This software was used to perform background correction, normalization, and linear combination fitting of the experimental spectra using previously collected Zn standards, according to the concepts set forth by Werner and Prietzel (2015). Briefly, three to six scans per sample were merged to reduce noise. Then the background was corrected, and the spectra were normalized. Using carefully chosen Zn reference spectra, linear combination fitting was done to reconstruct the experimental (soil) spectra. Twelve normalized reference spectra were used for linear combination fitting: franklinite ($ZnFe^{3+}2O_4$), $ZnSO_4$, ZnEDTA, hydrozincite ($Zn_5(CO_3)_2(OH)_6$), zincite (ZnO), hopeite ($Zn_3(PO_4)_2.4H_2O$), willemite ($Zn_2SiO_4$), smithsonite ($ZnCO_3$), hemimorphite ($H_6O_9Si_2Zn_4$), scholzite ($CaZn_2(PO_4)_2.2(H_2O)$), Zn adsorbed calcium carbonate, gahnite ($ZnAl_2O_4$).

#### 3.2.2.3 Greenhouse study

The experiment was carried out in February 2021, in the Throckmorton Plant Sciences Greenhouse Complex at Kansas State University, Manhattan, KS (39°12'N, 96°35'W, 325 m above sea level) by using acrylic containers (23.9 cm length and 11.9 cm height) (Figure S3.3). The acrylic containers contained two slits to mount a 400-mesh screen to restrict the plant roots from moving into the Zn treatment compartment. Wheat (*Triticum aesitvum* L.) seeds were a high-Zn spring wheat line (BW 53856, BW 53856 GRIN-Global (cimmyt.org), developed under

the Harvest Plus initiative by Dr. Velu Govindan (CIMMYT). Seeds were provided by Dr. Mary Guttieri (USDA-ARS) from a Yuma, AZ seed increase, with permission of Dr. Govindan. Seeds were planted into germination trays containing a mixture of Oakley city soil and sand (1:2) and kept in a growth chamber for three weeks at 4°C).

Before soil packing, the basal dose of nitrogen (N), phosphorous (P), and potassium (K) were mixed in the soil. Nitrogen was added as urea at a rate of 120 kg $ha^{-1}$, in only Zn fertilizers and control (with Urea and No-Zn). For all Zn-coated urea fertilizers, urea amount added with Zn-coated urea fertilizers was calculated, and the additional urea addition for basal N dose was done accordingly, to balance N amount in all the treatments. Phosphorus was added as $KH_2PO_4$ at a rate of 40 kg $ha^{-1}$. Potassium was added as $KH_2PO_4$ fertilizer based on the P rate.

Acrylic containers were packed with pre-wetted soil to achieve a uniform bulk density of 1.02 Mg $m^{-3}$. The soil was prewetted, bringing it to 10% of MWHC for packing. After packing, the soils were brought to 60% MWHC, and the containers were weighed. The containers were covered with polythene sheets and were allowed to equilibrate for at least 24 hours. The next day, three weeks old uniform seedlings were selected and transplanted in the middle of the container (one seedling/container) on one side of the screen (opposite to the treatment addition) parallel to the treatment introduction point. The next day after transplantation of the seedlings, treatments were introduced on one side of the screen (1 cm away from the screen and at 1.25 cm depth).

The following treatments were used: (1) Control (No-Zn + No Urea); (2) ZnO; (3) $ZnSO_4$; (4) Znmix; (5) ZnP; (6) ZnEDTA; (7) Urea; (8) ZnO coated urea; (9) $ZnSO_4$ coated urea; (10) Zn mix coated urea; and (11) ZnP coated urea; (12) ZnEDTA coated urea. The experimental design was a randomized complete block design with three replications. Zinc rate was10 kg $ha^{-1}$

for Zn only treatments (through treatments 2 to 6) while it was 5 kg $ha^{-1}$ for Zn-coated urea treatments (through treatments 8 to 12). Lower Zn rate used for Zn-coated urea treatments was because of the Zn coating on urea granules, addition of small rate of Zn led to addition of greater rates of urea addition. Even though we decided to add Zn at a rate of 5 kg $ha^{-1}$, it led to application of four times greater N rate (480 kg $ha^{-1}$) than the recommended rate.

To ensure good growth, containers were weighed, and their weight was subtracted from the weight taken on the first day of the study after bringing them to moisture content of 60% of MWHC. The difference in weight is weight lost and plants were watered with DI water every day (amount of water= amount of water lost) to all containers throughout the experiment period. The greenhouse temperature was maintained within the range of 20°C to 23°C throughout the experiment. A 12 h daylight and 12 h darkness photoperiod regime was maintained with overhead sodium lights for all five weeks until harvesting. The pots were rotated weekly to ensure that all pots received equal amounts of light.

Rhizon samplers (Rhizon MOM soil moisture sampler, 19.21.22, Rhizosphere Research Products, Wageningen, Netherlands) were used to collect the soil water samples at the end of the third and fifth week of the study, inserting Rhizon samplers closer to the plant roots. Soil water was collected in vacutainers and then transferred to 15 mL centrifuge tubes. Samples were acidified by adding one drop of concentrated nitric acid immediately upon collection and stored in a refrigerator at 4°C until analysis. Soil water samples were analyzed for Zn using an inductively coupled plasma-mass spectrometry (ICP-MS, 7500 CX, Agilent, Santa Clara, CA, USA). Standard water reference (Trace Elements in Natural Water, SRM1643f) from the National Institute of Science and Technology (NIST, Gaithersburg, MD) was used as part of the quality assurance quality control (QA/QC) protocol (recoveries were within 100 ± 20%). Since

plant uptake is directly proportional to Zn present in solution, soil water data will give us actual representation of available Zn for different treatments at two different times- end of third and end of fifth week.

Processing and chemical analysis of plant samples: Aboveground biomass was harvested at the end of the fifth week of the study using stainless steel knife. The plant samples were washed with tap water, followed by deionized water (DI) water, followed by 5 g $kg^{-1}$ sodium lauryl sulfate [$CH_3(CH_2)_{10}CH_2OSO_3Na$] solution to remove any adhered soil particles (Attanayake et al., 2015). Finally, plant samples were washed thoroughly with Milli Q water. This method removed all the adhering soil particles. Then samples were placed in pre-weighed paper bags. Fresh sample weight was recorded. Plant samples were dried at 68°C for about four days until they reached a constant weight. Dried sample weights were recorded. Dried plant materials were ground using a Willey Mini Mill-Arthur Thomas-type grinder (Thomas Scientific), sieve size of 250 mm (60 mesh), and stored doubling the small zip lock bags.

A sub-sample (0.5 g) of ground aboveground biomass was digested with 10 mL of trace metal grade nitric acid ($HNO_3$) in Teflon tubes by microwave (CEM, Matthews, NC). The digestion unit was ramped up to 200° C and held for 15 minutes (1600 W at 100%, 15-minute ramp time, 15-minute cooling time). Standard plant reference material (wheat flour, 1567b) from the National Institute of Science and Technology (NIST, Gaithersburg, MD) was also digested and analyzed alongside the plant materials as part of the quality assurance quality control (QA/QC) protocol (recoveries were within 100 ± 20%). Reagent blanks, internal standards, and spiked digestions were also included for QA/QC. Sample digests were filtered with Whatman filter paper No.42 under the fume hood in 15 mL centrifuge tubes and stored at 4° C until

analysis. Samples were analyzed for Zn, P, and Fe using an ICP-MS or ICP-OES based on their concentrations.

Zinc uptake was calculated as follows:

Zn uptake (g/pot) = (Zn concentration of plant tissue * dry weight of biomass)

Processing and chemical analysis of soil samples:

After harvesting plants, the soil in the container was sectioned into five sections, considering the screen as 0 cm. Soil sectioning was done as- 7.25-2 cm (section 1), 2- 0 cm (section 2), 0-3 cm (section 3), 3-6 cm (section 4), 6-14.52 cm (section 5) (Figure S3.4), air-dried, roots were removed, and soils were sieved through 2-mm sieve. Samples were analyzed for pH (1:10 soil: water), the total elemental concentration in the soil using aqua regia as discussed before (Zarcinas, 1996). Extractable Zn was determined using DTPA as follows. Ten grams of ground, air-dried, passed through 1.0 mm stainless steel sieve soil sample was taken in 125 mL conical flask, 20 mL of DTPA- triethanolamine (DTPA-TEA) extraction solution was added, each flask was covered with Parafilm, and was shaken using a horizontal shaker with 8.0 cm of stroke length and 120 cycles $min^{-1}$ for 2 hours (Lindsay and Norvell, 1978). Suspensions were filtered through Whatman filter paper no. 42, and samples were analyzed using the ICP-OES.

### 3.2.3 Statistical Analysis

Statistical analysis of all data was done using SAS version 9.4 (SAS Institute Inc., Cary, NC, 2020) One-way ANOVA using PROC GLM was used to analyze soil data. The data analysis was performed as a split-plot completely randomized design. The main plot was Zn fertilizer treatment, and the sub plot factor was soil section. Pairwise comparison was done using

LSD at $\alpha \leq 0.05$. One-way ANOVA using PROC GLIMMIX was used to analyze plant data and pairwise comparison was done at $\alpha \leq 0.05$ using LS means.

ANOVA using PROC MIXED was performed to evaluate Type III tests of fixed effects (treatment and time) on visualization and soil water Zn data. The data analysis was performed as a split-plot completely randomized or completely randomized block design with repeated measures, respectively, for visualization and soil water data. The main plot factor was the treatment, and the subplot factor was time. Pairwise comparison was done at $\alpha \leq 0.05$ using Tukey test.

## 3.3 Results and Discussion

### 3.3.1 Incubation studies

#### 3.3.1.1 Visualization

*Zinc only incubation study*

The Zn diffusion zones for four visualization times- for days 1, 14, 21, and 35 for Zn only incubation experiment (Figure S3.5) and for Zn-coated urea experiment (Figure S3.6). The quantified area for pink zone using GIMP 2.10 for Zn only incubation study and Zn-coated urea incubation study showed in Figure 3.1, and Figure 3.2, respectively. Data analyzed using GIMP 2.10.20 showed that Zn in the case of ZnEDTA diffused farther from the point of application than other treatments. Zinc diffusion was in order ZnEDTA > $ZnSO_4$ > Zn mix ≈ ZnO ≈ ZnP (Figure 3.1). Even though $ZnSO_4$ is very soluble, it still did not diffuse as ZnEDTA did because of the various adsorption and precipitation reactions occurring in soil (Rico et al., 1996). Chelated Zn, ZnEDTA can avoid most reactions that leads to Zn fixation and therefore is mobile and diffuse farther.

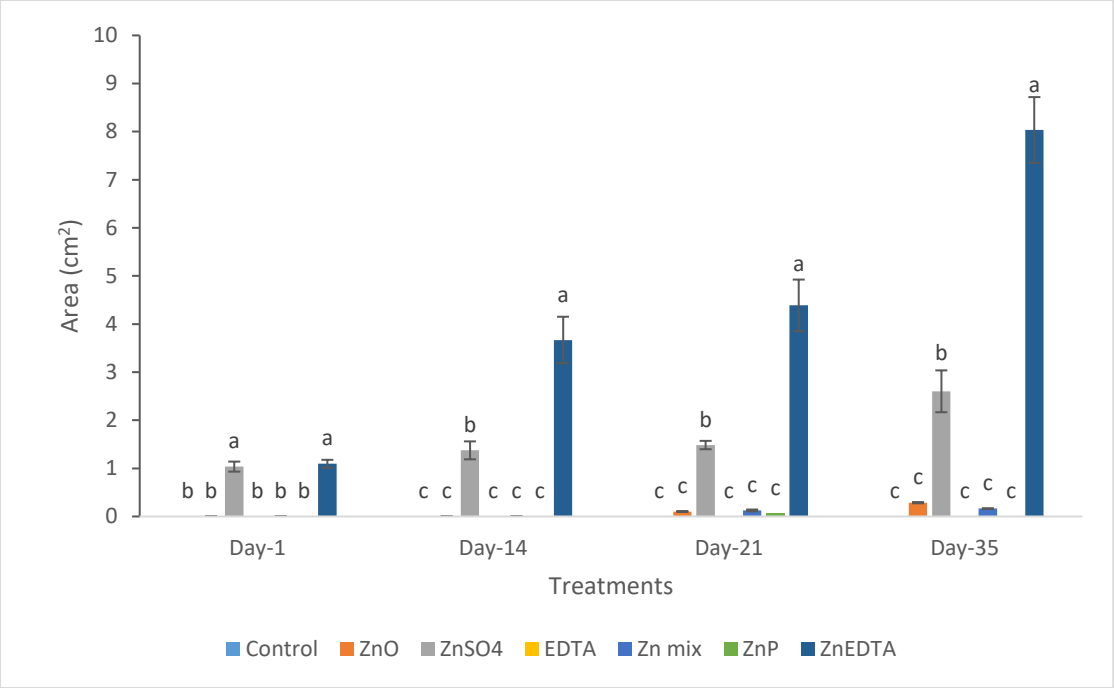


**Figure 3.1 Calculated area of the pink zone using GIMP software for Zn only incubation study at day 1, 7, 21, and 35. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; EDTA= ethylenediamine tetra acetic acid with no-Zn; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a time period containing the same letter are not significantly different at $P \leq 0.05$ according to Tukey pairwise method. Error bars represent the standard error of three replicates.**

The diffusion of Zn in the case of ZnO, Zn mix, and ZnP was minimal. Zinc diffusion seemed to depend upon solubility and pH. Inherent low solubility most likely contributed to their slow dissolution and increased sorption (Alloway, 2009; Rashid and Ryan, 2004).

*Zinc coated urea incubation study*

For zinc coated urea treatments, diffused Zn observed at day 35, was in order ZnEDTA-U≈ ZnP-U > $ZnSO_4$-U≈ Zn mix-U > ZnO-U (Figure 3.2). High Zn diffusion for $ZnSO_4$ and ZnEDTA coated urea can be explained by their solubility and mobility as mentioned above. Zinc diffused to the second section of the petri dish irrespective of the source.

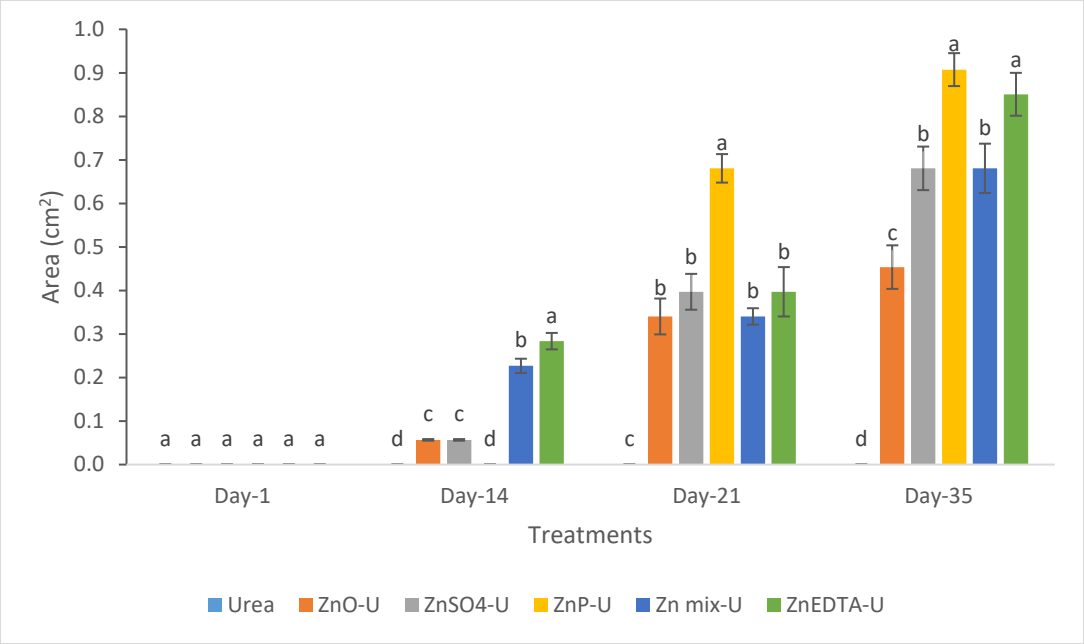


**Figure 3.2 Calculated area of the pink zone for Zn-coated urea incubation study at day 1, 7, 21, and 35. Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a time period containing the same letter are not significantly different at $P \leq 0.05$ according to Tukey pairwise method. Error bars represent the standard error of three replicates.**

When added as Zn-coated urea, Zn availability and mobility seemed to be comparable for ZnP-U and ZnEDTA-U and Zn mix-U, and ZnO-U and $ZnSO_4$-U. As mentioned above, pH and ability to avoid sorption being major factors responsible for availability and diffusion. Sorption reactions are the major reasons behind Zn losses and its transformations to unavailable forms. Urea hydrolysis increased soil pH (section 3.3.1.2 and Figure 3.8), and that might have also affected the degree of sorption of Zn-coated urea treatments as discussed in section 3.3.1.3 and Figure 3.10. These effects are discussed in sections 3.3.1.2 and 3.3.1.3 in more detail.

### 3.3.1.2 Soil pH

*Zinc only incubation study*

Soil pH was significantly lower for $ZnSO_4$ and EDTA treatments than the other treatments and control in the first and second or the first section, respectively (Figure 3.2). The reason for this response is that when $ZnSO_4$ comes in contact with water, being highly soluble, it is hydrolyzed to form sulfuric acid and zinc hydroxide. Sulfuric acid is a strong acid, while zinc hydroxide is a weak base; hence, the resulting solution is acidic. The reason behind EDTA addition reducing the pH of POA is its low pKa values (0, 1.5, 2, 2.66), because of the deprotonation of four carboxyl groups as well as due to deprotonation of the two amino groups (pKa values 6.16 and 10.24) (Coleman, 2008). The addition of EDTA to alkaline soil leads to the deprotonation of all four carboxyl groups and one amine group, releasing 5 protons, thereby decreasing pH.

Soil pH was significantly greater for ZnP for POA, and the reason behind the increase in pH is that ZnP dissolution consumes $H^+$ or releases $OH^-$ ions which increase soil pH (Su et al., 2019)

$$Zn_3(PO_4)_2.4H_2O \rightarrow 3Zn^{2+} + 2HPO_4^{2-} + 2OH^-$$

*Zinc coated urea incubation study*

For Zn-coated urea experiment, soil pH was lower than the initial soil pH for all the treatments (Figure 3.3). This pH reduction is due to urea hydrolysis and release of protons upon nitrification:

$$CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2$$

$$2NH_3 + 2H^+ \rightarrow 2NH_4^+$$

$$2NH_4^+ + 4O_2 \rightarrow 2NO_3^- + 2H_2O + 4H^+$$

Therefore, two moles of protons were produced per one mole of urea added.

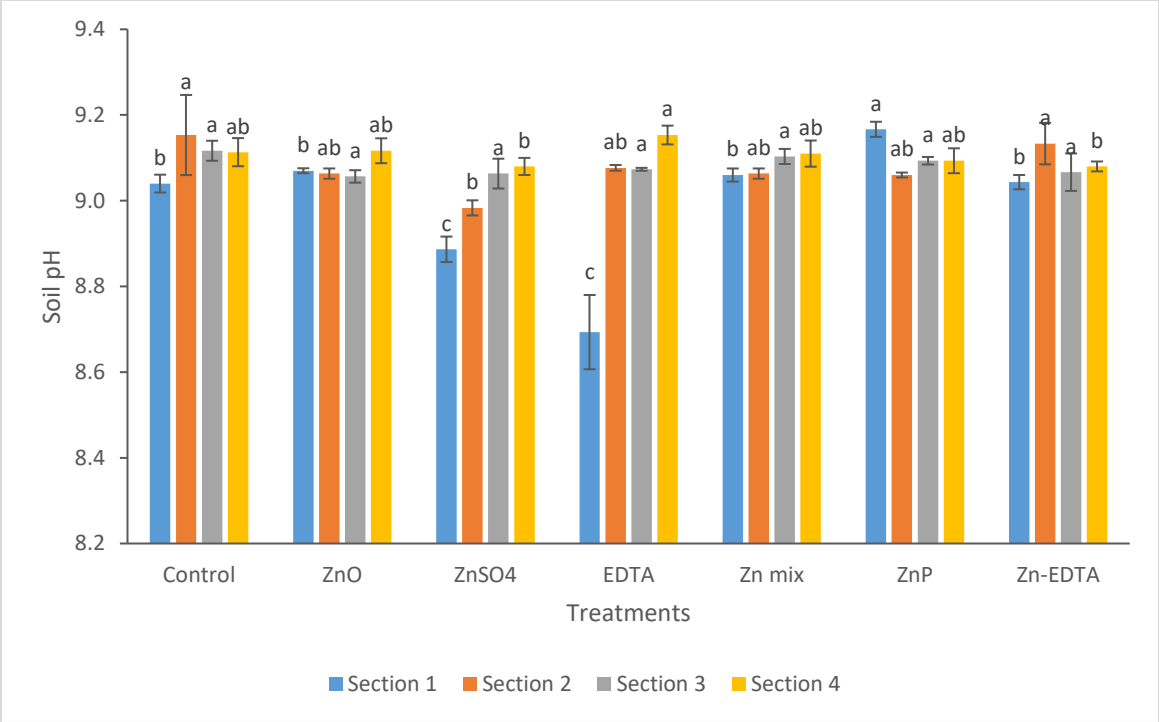


**Figure 3.3 Soil pH for Zn only treatments at the end of the incubation study 1. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; EDTA= ethylenediamine tetra acetic acid with no-Zn; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

All the Zn-coated urea treatments showed significantly lower pH than the urea treatment in section 2. This decrease can be explained by Zn diffusion to second section and therefore, reduction in soil pH, However, because free $CaCO_3$ in calcareous soil tends to neutralize the acidulated pH, soil pH would return to a value close to the original with time. Greater pH observed for sections 3 and 4 can be due to both the soil trying to buffer pH change and the lack of Zn migration to these sections.

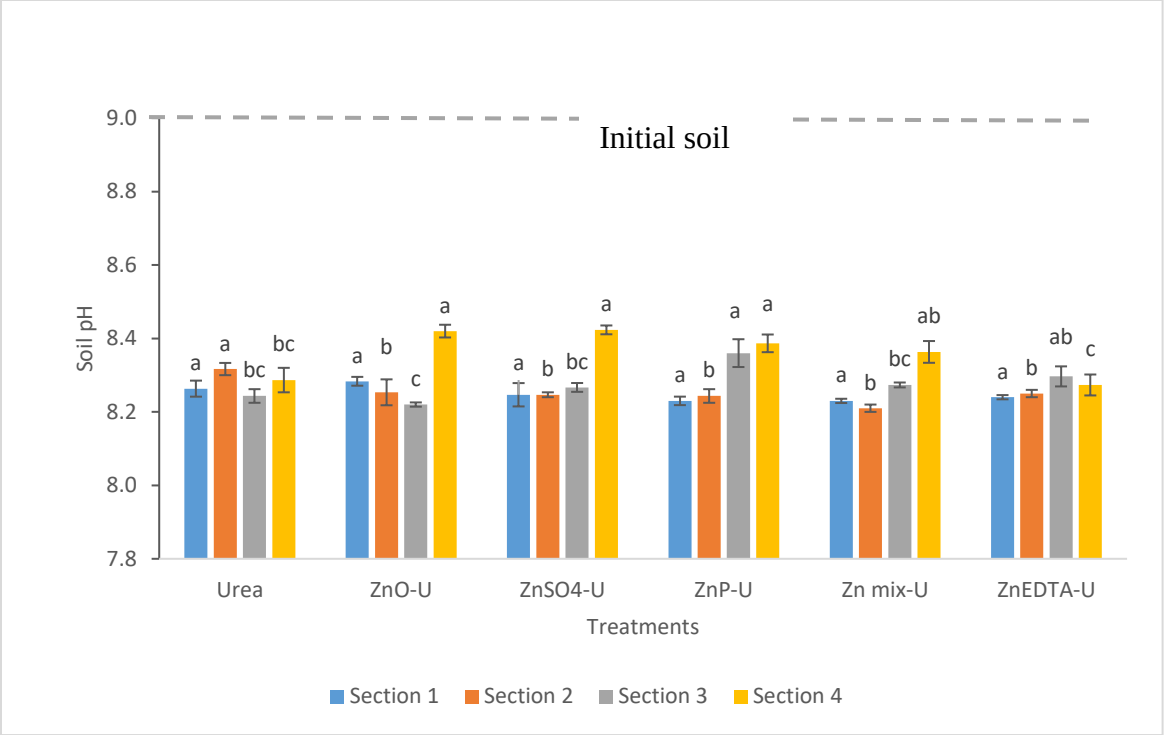


**Figure 3.4 Soil pH for Zn-coated urea experiment at the end of the incubation study 2. Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

### 3.3.1.3 Diffusion of added zinc

*Zinc only incubation study*

The precipitation and sorption reactions occurring in calcareous soil with Soil-Ca, free $CaCO_3$, and Ca in soil solution hinders Zn and other nutrients, such as P, movement (Hettiarachchi et al., 2006 and 2008; Lombi et al., 2004). Zinc adsorption onto reactive sites restricts Zn diffusion. Calcareous soils with high clay content have many reactive sites, which increases channel tortuosity that restricts lateral Zn movement via mass flow and diffusion. Comparing different treatments, ZnEDTA treatment permeated out to a larger soil volume (Figure 3.4).

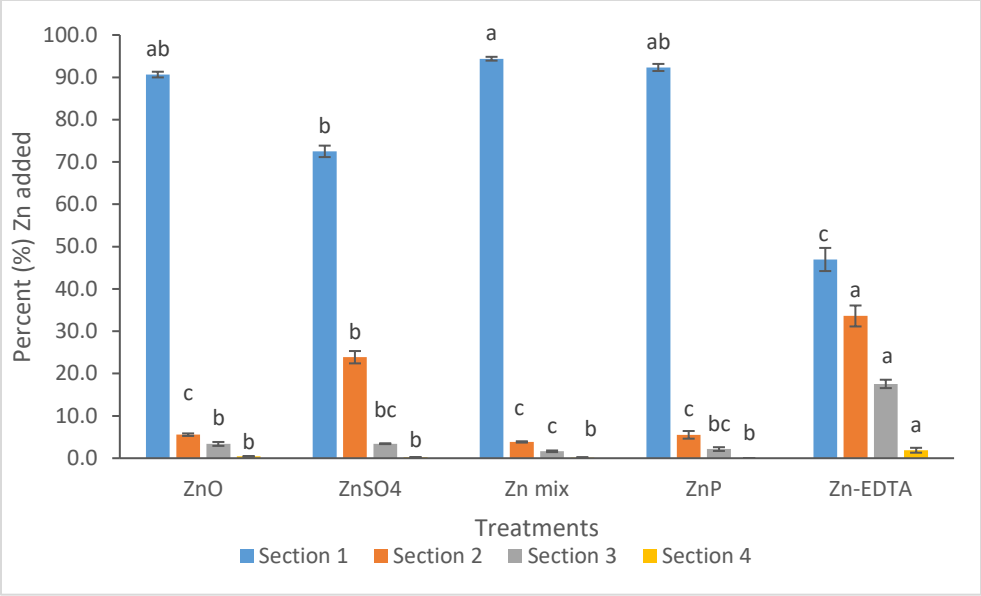


**Figure 3.5 Percentage of Zn added recovered in each section for Zn only incubation study. ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; EDTA= ethylenediamine tetra acetic acid with no-Zn; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

Therefore, the percent Zn added recovered from section 1 was significantly lower for ZnEDTA. The percent Zn recovered from sections 2, 3, and 4 was significantly greater for ZnEDTA treatment than all other Zn treatments proving its superior diffusion to other sections of the soil. A significantly greater amount of Zn was recovered from $ZnSO_4$ in the second section than ZnO, Zn mix, and ZnP. High Zn diffusion in $ZnSO_4$ is due to its greater water solubility compared to sparingly soluble ZnO and ZnP. All other treatments ZnO, Zn mix, and ZnP, were relatively immobile. More than 80% of the added Zn recovered from the section of application (section 1). The lack of migration to the second and other sections observed in these treatments may be related to the resultant greater soil pH after adding these treatments (in addition to inherently high soil pH) and, therefore, low solubility and/or more Zn sorption (Figure 3.2).

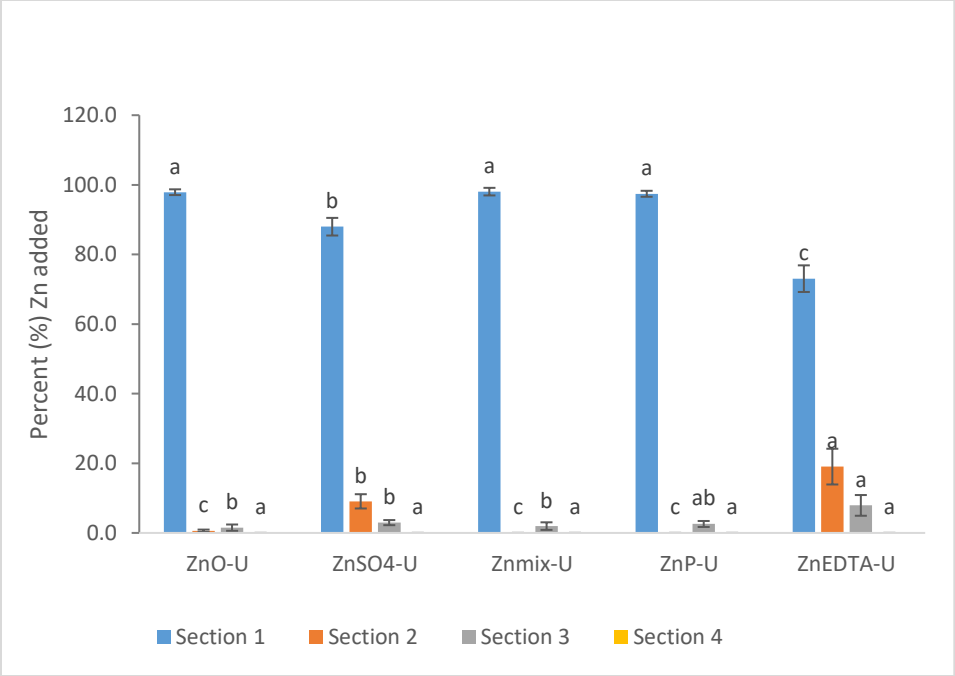


**Figure 3.6 Percentage of Zn added recovered in each section for Zn-coated urea incubation study. ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

*Zinc coated urea incubation study*

Zinc, when applied as coated onto granular NPK fertilizers, undergoes somewhat different pathway. Zinc can also react with the N component of fertilizers, whereas as compared to Zn reactions with phosphorus, Zn and nitrogen reactions products are water-soluble compounds (Lehr, 1972). Milani et al. (2012) observed the speciation and dissolution behavior of ZnO coated onto urea granules. Results showed that coating did not change Zn speciation as ZnO was the dominant mineral detected on the surface of the granule, also confirmed in the present study using XANES (section 3.3.1.5).

The results from the present study revealed that the fertilizer Zn mobility is highly dependent on the solubility and stability of the Zn source used in calcareous soil (Figure 3.5).

Most of the Zn in the present incubation study remained in the first section (more than 95%) for ZnO-U, Zn mix-U, and ZnP-U, and 88±2.5% for $ZnSO_4$, and 73±3.8% in ZnEDTA-U. High Zinc diffusion observed in ZnEDTA-U for section-3, and section- 4 was due to the tendency of this complex to stay in solution form a discussed in section 3.3.1.4.

#### 3.3.1.4 Water extractable zinc

*Zinc only incubation study*

Zinc diffusion in soil could occur in either as solution or solid phase (Oliveira et al., 2010; Metwally et al., 1993; Rico et al., 1996). Prasad et al. (1975) reported that Zn diffuses predominantly in the solid phase in Ca-dominated soils. Available Zn or cationic Zn gets adsorbed onto free $CaCO_3$ present in the soil, and then its desorption farther from the application point, helping Zn to diffuse. Thus, Zn diffusion is controlled by the kinetics of labile Zn desorption from the adsorbed phase (Oliveira et al., 2010). Comparing all the treatments, ZnEDTA showed a significantly greater water extractability than other treatments for the second, third, and fourth sections (Figure 3.6). Therefore, in calcareous soil, no matter what mechanism Zn diffusion follows, through soil solution or in the solid phase, ZnEDTA can increase soil Zn concentrations in solution by converting solid-phase labile Zn into soluble Zn or by preventing irreversible fixation of Zn into the solid phase (Hamza and Sadnandan, 2005; Gupta and Deb, 1984; Parsad et al., 1975). Compared to ZnSO4, EDTA is known to form strong Zn complexes (Parsad et al., 1975). Although no-Zn was added in EDTA treatment, the observed water-extractable Zn is the reflectance for EDTA complexing with soil Zn due to the high stability of ZnEDTA complex (i.e., enhanced solubility via complexation) and making soil Zn labile and available. There were no significant differences observed for water-extractable Zn for EDTA treatment and $ZnSO_4$ treatment.

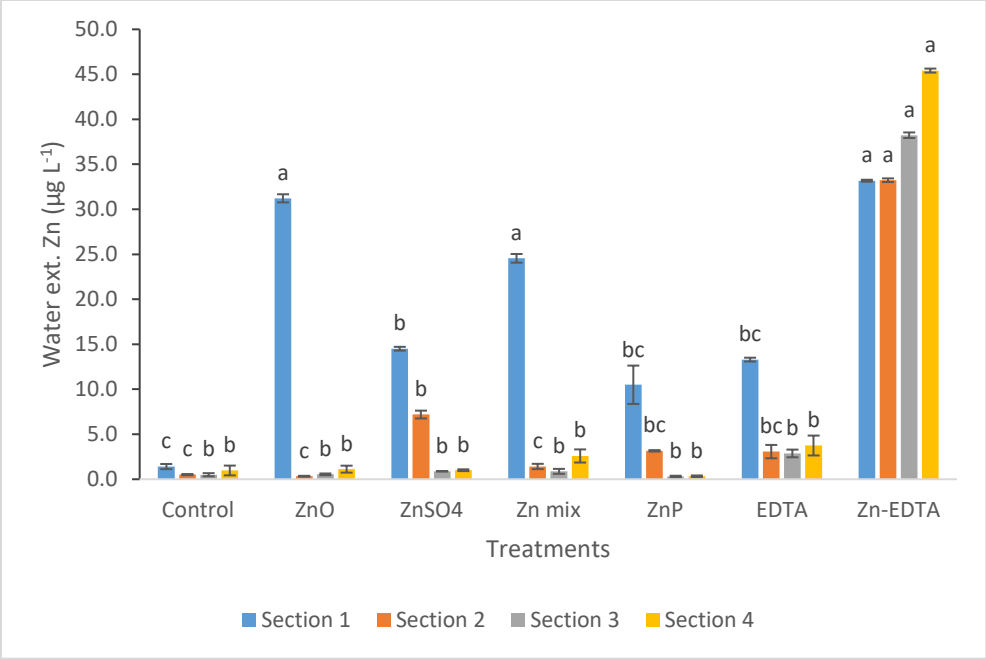


**Figure 3.7 Water extractable Zn for Zn only experiment in the incubation study 1. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; EDTA= ethylenediamine tetra acetic acid with no-Zn; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

It appears that EDTA complexed with soil Zn and increased its extractability. Zinc oxide, and zinc mix treatments behaved similarly, water extractable Zn for the first section (application section) was significantly greater than $ZnSO_4$. Although, ZnO and Zn mix showed a significantly less/no movement of Zn to other sections of the Petri dish, some applied Zn remained water extractable. The available literature has suggested that water-soluble Zn is a good indicator of availability for granular Zn fertilizers (Mortvedt and Giordano, 1969; Gangloff et al., 2002).

*Zinc coated urea incubation study*

Water extractable Zn was significantly greater for sections 1, 2, and 3 for ZnEDTA-U than all other treatments (Figure 3.7). To our knowledge, it is due to the same reasons ZnEDTA showed high water extractability as mentioned above.

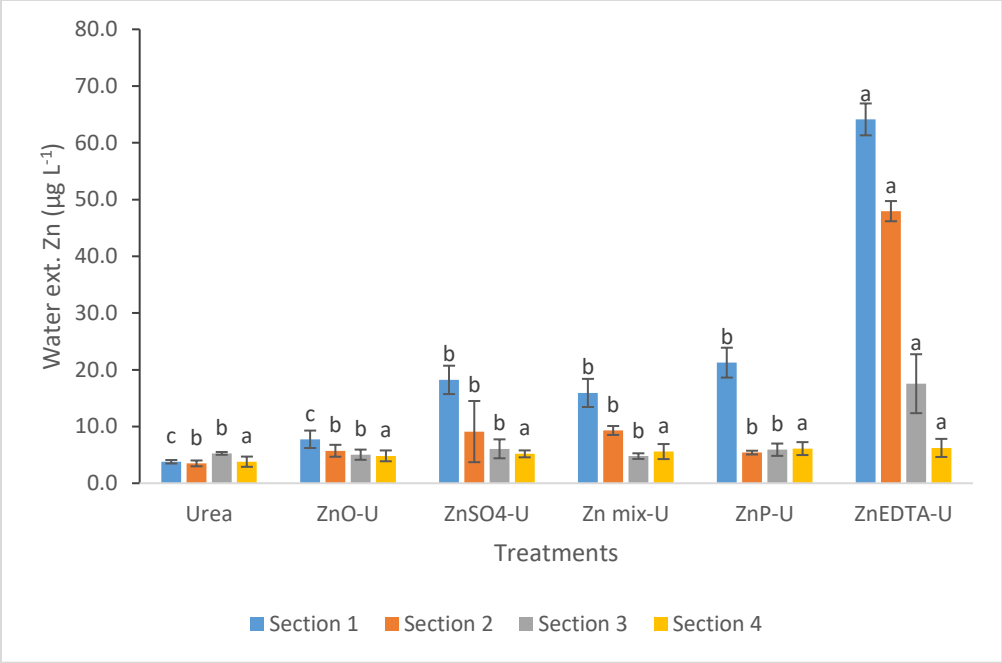


**Figure 3.8 Water extractable Zn in each section for Zn-coated urea incubation study. ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

Water extractable Zn for Zinc mix-U, and ZnP-U was not significantly different from $ZnSO_4$-U referring to the increased solubility of Zn in Zn mix-U and ZnP-U due to acidulation effect caused urea hydrolysis followed by nitrification (Figure 3.4). The water extractable Zn for section- 1 for ZnO-U treatment was significantly lower than all other Zn-coated urea treatments, indicating that the presence of urea is helping it to be transformed into a less soluble form but enough to persist in the environment. In the water extracts, zincite was predicted to control the solubility of the urea+ ZnO fertilizer (Degryse et al., 2020), also confirmed by XANES results in the present study (Figure 3.9). Zinc is known to move away from the application site by the process of diffusion. The diffusion rate is directly proportional to the concentration of the solution at soil: granule interface (Degryse et al., 2020). The solution concentration depends on

Zn solubility under favorable conditions and conditions affecting Zn solubility are soil pH and composition of the Zn fertilizer. The localized effect if the Zn fertilizer composition on Zn diffusion when applied as granular fertilizer has been shown through soil sampling (e.g., Gilkes et al., 1975; Hettiarachchi et al., 2010), synchrotron-based techniques (Hettiarachchi et al., 2008), and colorimetric visualization (Degryse et al., 2015). The present study supported this information, Zn diffusion was less for ZnO-U because of the low water extractability for this treatment.

#### 3.3.1.5 Synchrotron results

*Zinc coated urea incubation study*

X-ray absorption near-edge structure spectroscopy combined with analysis of XANES data by linear combination fitting (LCF), using relevant reference spectra (i.e., standards), provide speciation and quantitation results. The results can be used to understand the fate and behavior of Zn in soil. The soil samples from Zn-coated urea incubation study from sections 1 and 2 were analyzed for XANES. The XANES spectra for Zn and their fits from LCF (dashed lines) for first and second soil sections and Zn-coated urea granules are shown in Figure 3.8. Zinc in urea (urea-control), ZnO-U, ZnP-U, and the second section of Zn mix-U treatments dominated by zinc carbonate. This observation can be partly due to the native Zn present as $ZnCO_3$. In addition, Zn may also precipitate as Zn carbonate in high pH (Alloway, 1995; Adriano, 2001) carbonate-rich soils. Several authors have postulated that in neutral and calcareous soils, Zn solubility is mainly controlled by Zn hydroxide ($Zn(OH)_2$), smithsonite ($ZnCO_3$) or hydrozincite ($Zn_5(OH)_6(CO_3)_2$) (Sharpless et al., 1969; Saeed and Fox, 1977). Zinc sorption by $CaCO_3$ and precipitation of Zn hydroxide or Zn hydroxyl carbonates are mechanisms controlling Zn solubility in calcareous soils (Papadopoulos et al., 1989). Several studies observed

that with increasing soil pH, specific Zn adsorption to soil organic matter, clay minerals, oxides, and carbonates becomes more relevant.

Calcareous soil used for this experiment had a pH of 9.0, therefore, when Zn was applied as ZnO treatment, there was no expectation of observing any other Zn species because low solubility of ZnO under neutral and alkaline soil conditions. Voegelin et al. (2011) observed that ZnO addition did not create any artifacts in the soil, except increasing soil pH in the immediate surrounding due to the dissolution of ZnO particles. However, when Zn was added as ZnO coated urea granules, the XANES spectrum for ZnO coated urea treatments was best fitted with a mixture of zinc carbonate (82.7% in section 1 and 78% in section 2, indicating dissolved Zn precipitating out as $ZnCO_3$) and zincite (17.3 % in section 1 and 22.0% in section 2) (Figure 3.9). This can be due to the resulted acidulation by the Zn addition as ZnO-coated urea. When Zn is applied as ZnO coated urea granules, it significantly reduces soil pH as observed in the incubation experiment (Figure 3.3) and greenhouse experiment (Figure 3.7).

The spectrum for section-1, and 2 for $ZnSO_4$-U treatment was best fitted with a mixture of zinc carbonate (26.1% for section 1, 14.7% for section 2), zinc sulfate (50.6% for section 1, 67.9% for section 2), and gahnite (23.4% for section 1, 17.5% for section 2). The greater percentage of Zn present as $ZnSO_4$ for section 2 explains Zn diffusion to the second section and its greater water extractability as observed in the present study (Figure 3.7). The Zn added as $ZnSO_4$ to the soil is supposed to undergo great adsorption and fixation of Zn (Giordano and Morvedt, 1973). The presence of $ZnCO_3$ can be explained by dissolved Zn precipitation as $ZnCO_3$. Zinc in this treatment was also observed to be present as gahnite, which is zinc aluminum oxide.

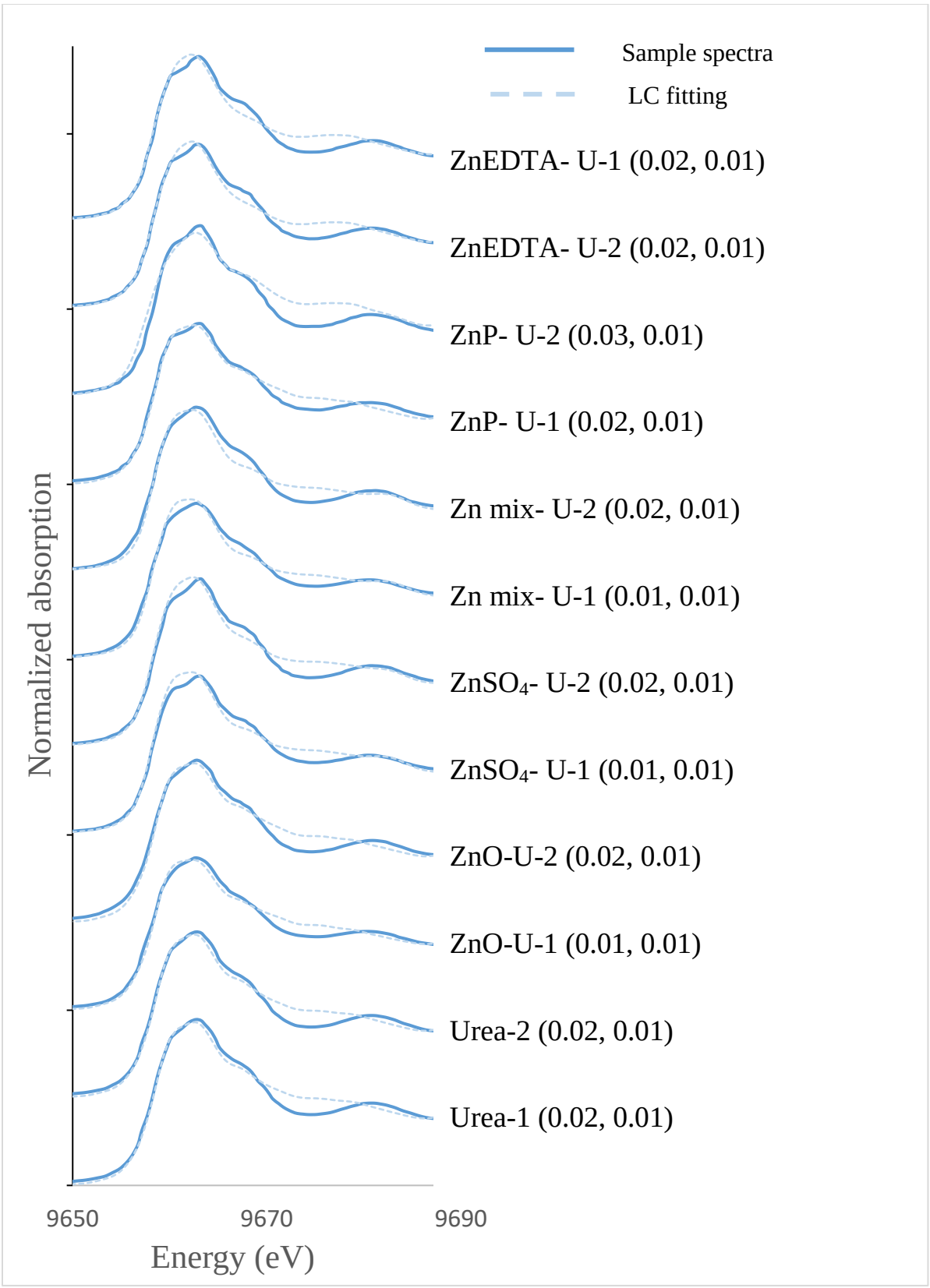
Sample spectra
LC fitting
ZnEDTA- U-1 (0.02, 0.01)
ZnEDTA- U-2 (0.02, 0.01)
ZnP- U-2 (0.03, 0.01)
ZnP- U-1 (0.02, 0.01)
Zn mix- U-2 (0.02, 0.01)
Zn mix- U-1 (0.01, 0.01)
ZnSO4- U-2 (0.02, 0.01)
ZnSO4- U-1 (0.01, 0.01)
ZnO-U-2 (0.02, 0.01)
ZnO-U-1 (0.01, 0.01)
Urea-2 (0.02, 0.01)
Urea-1 (0.02, 0.01)
Normalized absorption
9650
9670
9690
Energy (eV)

**Figure 3.9 Normalized X-ray absorption fine structure (XANES) Zn absorbance spectra of the selected rate of treatments after 35 days. Treatment (x, y) = Treatment (x=R factor, y= reduced chi square). Urea-1 = urea control section 1; Urea-2 = urea control section 2; ZnO-U-1= zinc oxide coated urea section 1; ZnO-U-2= zinc oxide coated urea section 2; $ZnSO_4$-U-1= zinc sulfate coated urea section 1; $ZnSO_4$-U-2= zinc sulfate coated urea section 2; Zn mix-U-1= zinc mix coated urea section 1; Zn mix-U-2= zinc mix coated urea section 2; ZnP-U-1= zinc phosphate coated urea section 1; ZnP-U-2= zinc sulfate coated urea section 2; ZnEDTA-U-1= zinc-ethylenediamine tetra acetic acid coated urea section 1; ZnEDTA-U-2= zinc-ethylenediamine tetra acetic acid coated urea section 2.**

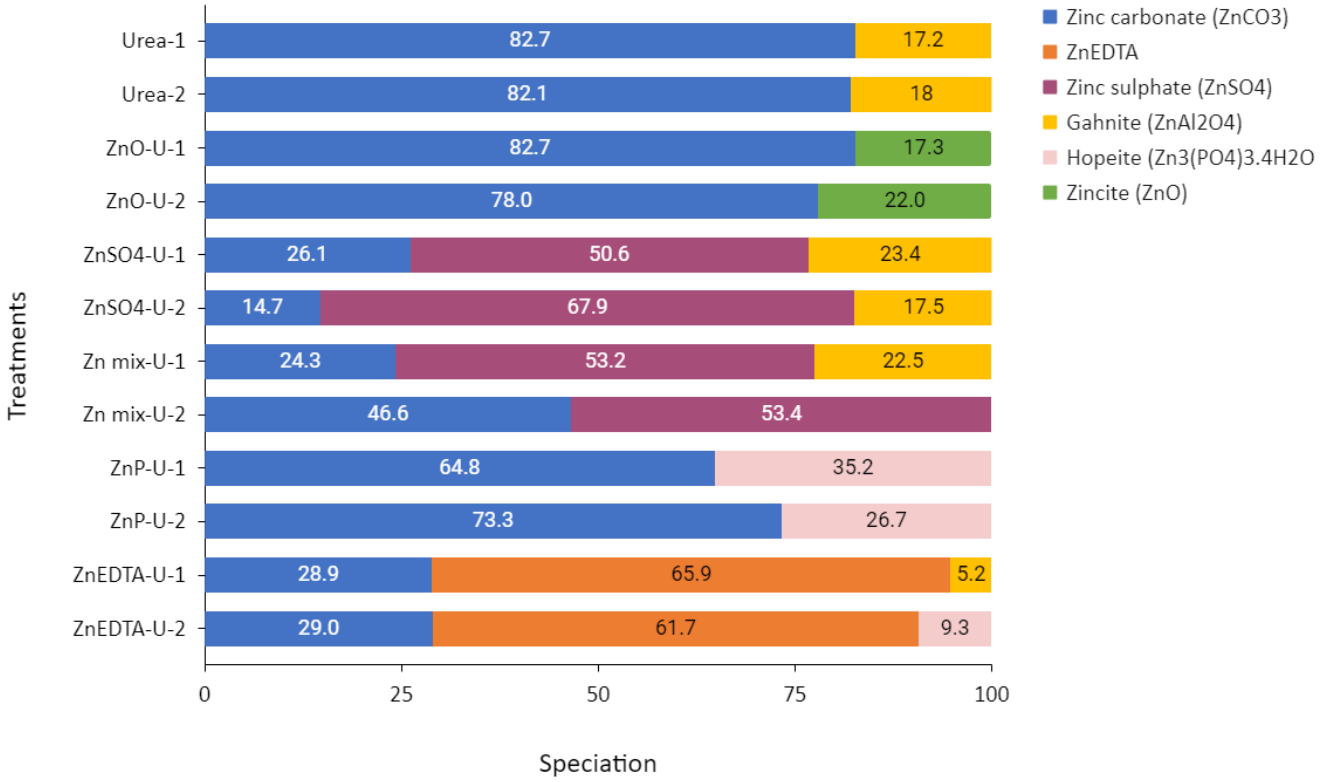


**Figure 3.10 Relative proportions of Zn species as estimated by linear combination fittings on XANES spectra. Urea-1 = urea control section 1; Urea-2 = urea control section 2; ZnO-U-1= zinc oxide coated urea section 1; ZnO-U-2= zinc oxide coated urea section 2; $ZnSO_4$-U-1= zinc sulfate coated urea section 1; $ZnSO_4$-U-2= zinc sulfate coated urea section 2; Zn mix-U-1= zinc mix coated urea section 1; Zn mix-U-2= zinc mix coated urea section 2; ZnP-U-1= zinc phosphate coated urea section 1; ZnP-U-2= zinc sulfate coated urea section 2; ZnEDTA-U-1= zinc-ethylenediamine tetra acetic acid coated urea section 1; ZnEDTA-U-2= zinc-ethylenediamine tetra acetic acid coated urea section 2.**

It is well known that several inorganic surface functional groups are present at the surfaces of secondary minerals such as hydrous oxides, oxides, and oxyhydroxides of Fe and Mn; hydrous oxides of Al and Si. Zinc sulfate dissociates to give $Zn^{2+}$ ions. Jacquat et al. (2009) reported that translocation of the Zn cation can result in the formation of three secondary Zn species in the subsurface soils: Zn inner-sphere adsorption complexes with Mn oxides, Si oxides, and as a Zn-Al-hydroxy interlayer material (HIM) soil precipitate. The recently identified Zn species are layered minerals containing Zn in octahedral sheets (Jacquat et al., 2009). Therefore,

to the authors' knowledge the presence of gahnite could be an indication of Zn present as Zn-Al association. Gahnite was observed as being the closest Zn spectra available that could match the Zn-Al species in our samples. Similar results to $ZnSO_4$-U treatment were observed for Zn mix-U. When Zn mix was applied as coated onto urea, $ZnSO_4$ acted as its dominant component despite of greater percentage of ZnO. This can be due to the acidulation effects of urea hydrolysis and the subsequent dissolution of ZnO. Zinc percentage present as $ZnCO_3$ increased as we moved to the second section implying more of Zn mix getting adsorbed and fixed rather than interacting with aluminum oxides and transforming to gahnite-like solid species. The spectra for soil sections 1, and 2 for ZnP-U treatment were best fitted with a mixture of $ZnCO_3$ (64.8% for section 1 and 73.3% for section 2), and hopeite (35.2% for section 1, and 26.7% for section 2). This was most likely due to unreacted Zn phosphates in the granule or the point of application of the soil treated with ZnP-U treatment. A significant portion of the Zn, applied as ZnP-U precipitated as ZnCO3 species. The presence of Zn as hopeite and $ZnCO_3$, both species have very low solubility, helps to understand its low mobility and water extractability observed in the incubation experiment (Figure 3.5 and Figure 3.7). As expected, a greater portion of added ZnEDTA-U remained as ZnEDTA form, which can be explained by the stability of the complex. This explains the observed high water extractable Zn in the incubation study (Figure 3.7). As mentioned before, the XANES technique provided us with comprehensive information on the interaction between the metal and soil components. Further, coupling the application of conventional chemical methods and highly advanced methods, such as XANES, widened the understanding of the mechanisms of chemical transformations of Zn in soils.

### 3.3.2 Greenhouse experiment

#### 3.3.2.1 Plant biomass and Zn uptake

*Zinc only*

Plant biomass increased with the addition of some Zn treatments (Figure 3.10). Plant biomass was significantly greater for ZnO, and Zn mix than control and other Zn treatments ($ZnSO_4$ and ZnP). No significant differences were observed between ZnO, Zn mix, and ZnEDTA indicating that ZnO and Zn mix behaved similar to ZnEDTA with respect to biomass production.

Plant Zn uptake was significantly greater for ZnEDTA treatment (Figure 3.10). Combined effect of high concentration of plant Zn (Figure 3.10) and biomass resulted in high Zn uptake for ZnEDTA. This can be because of the greater amount of Zn in soil solution (Figure 3.12) as well as the high mobility (3.16) of Zn in this treatment. Although Ca was abundant in this calcareous soil, it appears that the effect of Ca was not strong enough to mask the benefit of ZnEDTA, and thus ZnEDTA remained as the most effective Zn source for the plants. Norvell (1991) reported that the stability constant of ZnEDTA (17.5) greater than that of Ca-EDTA (11.6). Goos et al. (2000) reported that ZnEDTA was a prime source for maize crop. Similarly, Rico et al. (1996) found that ZnEDTA proved to be the most efficient source of Zn. Ortiz and Garcia (1998) also reported that fixation for chelated-Zn was much less than $ZnSO_4$. Mehdi et al. (1990) reported that the relative effectiveness was in order of ZnEDTA > $Zn(NO_3)_2$ > $ZnSO_4$ > $ZnCl_2$. Our study was in agreement with the studies mentioned above.

Zinc oxide and Zn mix (60% ZnO) treatments are sparingly soluble. ZnO and Zn mix were therefore not readily available to the plants. Water solubility of zinc sources is considered as one of the important criteria for zinc availability to plants (Slaton et al., 2005). Westfall and Gangloff (2001) observed the effectiveness of different Zn fertilizers decreased with respect to the percent water-soluble Zn. Lindsay (1991) reported that ZnO is reasonably soluble which is enough for its persistence in soil. Although the Zn movement for these treatments was low, still

these managed to provide required amount of Zn to plants resulting in plant Zn conc. and uptake non significantly different from highly soluble $ZnSO_4$ treatment. This can be due to ZnO and Zn mix acting as slow-release Zn fertilizers and therefore minimizing cationic Zn losses due to adsorption and precipitation. This slow-release effect can be because of the lower solubility of ZnO, and Zn mix treatments as observed in soil water Zn (Figure 3.12 and DTPA extractable results (Figure 3.13)

Plant Zn uptake for $ZnSO_4$ treatment was significantly greater than control due to added Zn but was significantly lower than ZnEDTA and no significant differences were observed from ZnO, Zn mix and ZnP. When $ZnSO_4$ is added to calcareous soil, the presence of active $CaCO_3$ results in Zn adsorption, leading to Zn precipitation as zinc hydroxide or carbonate or the formation of insoluble calcium zincate (Rico et al., 1996). Zinc hydroxides ($Zn(OH)_2$) and zinc carbonates ($ZnCO_3$) have less solubility than ZnO (Shivay et al., 2008) which makes $ZnSO_4$ effectiveness similar to less soluble Zn fertilizers despite of being of its highly soluble nature.

*Zinc coated urea*

The plant biomass was significantly lower for control (N as urea was mixed in whole soil) urea treatment (added as a pot treatment, similar to urea-Zn treatments) compared to all Zn-coated urea treatments (Figure 3.11). Adding Zn as Zn-coated urea significantly increased plant biomass and plant Zn uptake irrespective of the source of Zn used for coating. The greater biomass of Zn-coated urea treatments compared to control or urea treatments could be due to two reasons. First, the addition of Zn as Zn-coated urea fertilizers. Second could be due to Zn coating delaying N transformations in soil. To the authors' knowledge, Zn coating can help urea to stay longer in the soil, lowering the reaction rate and thus, reduced urea losses which, in turn, increase plant biomass.

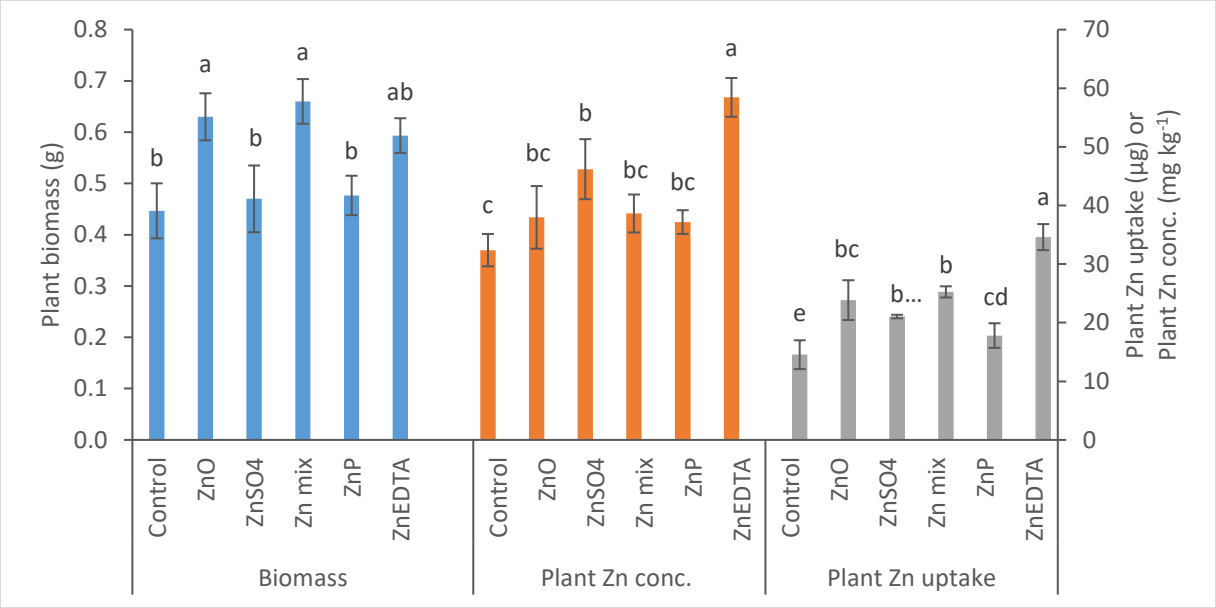


**Figure 3.11 Plant Zn uptake, plant Zn concentration, and biomass for Zn only treatments in the greenhouse study. Plant biomass is plotted against the primary axis, and plant Zn uptake and concentration are plotted against the secondary axis. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means within response variable containing the same letter are not significantly different at $P \leq 0.05$ according to LS-means. Error bars represent the standard error of three replicates.**

Nano-ZnO coatings on urea have been reported to reduce $NO_3$-N leaching by 35.1% (Jadon et al., 2018). The enhanced biomass in the control treatment compared to the urea treatment was most likely because of the difference in application methods. Although nitrogen levels were balanced in all the treatments, the basal amount of nitrogen for control was added by mixing it with soil. In contrast, the urea treatment was introduced at one point of application. This might have resulted in more losses of added urea via processes such as $NH_3$ volatilization than when it was mixed in well with soil. It should be noted that even though the Zn rate used for Zn-coated urea treatments was half the Zn rates used for Zn only treatment, the Zn uptake for both studies was quite comparable (Figure 3.10 and 3.11). It can be because of the increased availability of Zn because of the acidulation effect caused by urea addition (Figure 3.8).

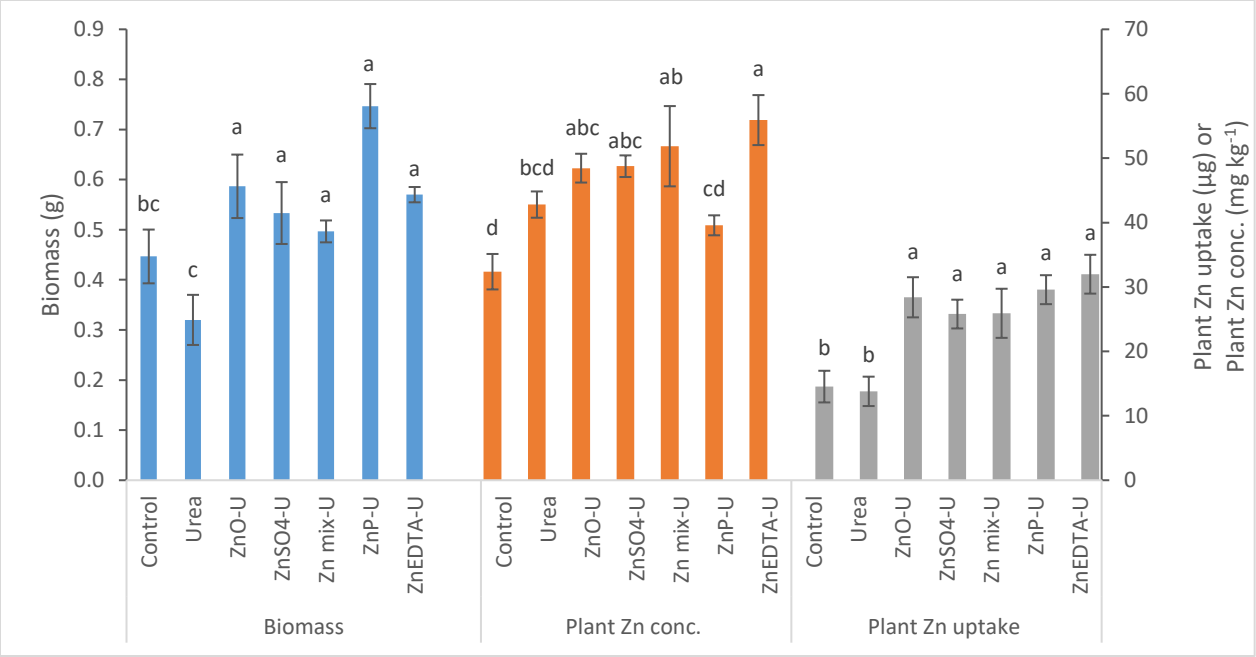


**Figure 3.12 Plant Zn uptake, plant Zn concentration, and biomass for Zn only treatments in the greenhouse study. Plant biomass is plotted against the primary axis, and plant Zn uptake and concentration are plotted against the secondary axis. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means within response variable containing the same letter are not significantly different at $P \leq 0.05$ according to LS-means. Error bars represent the standard error of three replicates.**

The acidic effect increases the availability of Zn (Jiang et al., 2012). The concentrations of exchangeable Zn correlated negatively with pH, and the result was consistent with that reported by Cakmak (2009). Another reason could be increased plant biomass due to Zn coating decreasing urea losses. The results also revealed that nitrogen and Zn had a synergistic effect on each other concerning plant uptake. Various authors reported that nitrogen application increased Zn plant uptake and concentrations (Cakmak et al., 2010a; Kutman et al., 2011; Xue et al., 2019). Increased input levels of N imparted greater vegetative growth and enhanced assimilation and metabolic rates (Oscarson, 2000), which resulted in better crop productivity in wheat (Yadav et al., 2012). Increased plant biomass is due to the role of Zn in the stimulation and catalysis of

various metabolic processes. As co-factor of enzymes Zn is involved in growth and development of plants, eventually leading to increased biomass and crop yield (Imran et al., 2015). Improved nutrient contents can be referred to as increased nutrient demands of plants due to biomass of the plant (Brown et al., 2005). Thus, application of nitrogen improved Zn uptake due to the improved enzymes activities and enhanced metabolic processes (Imran et al., 2015). Viets et al. (1953) reported that ammonium fertilizers have a pronounced impact on $ZnSO_4$ when applied concurrently with N dressings.

#### 3.3.2.2 Soil water zinc

*Zinc only*

In the calcareous soil, background water-soluble Zn concentrations were fairly low and ranged from 2.06- 2.7 µg $L^{-1}$ (Figure 3.12). Although the addition of Zn fertilizers increased soil water Zn, it did not differ significantly except for ZnEDTA treatment. Soil water Zn was significantly greater for ZnEDTA treatment than all other Zn treatments as well as the control (Figure 3.12). Soil water Zn for ZnEDTA treatment collected at the end of the third (ranging from 49.13 to 79.62 µg $L^{-1}$) and fifth week (52.25 to 93.4 µg $L^{-1}$) of the study was on an average 13 times greater than all Zn treatments and control for both third and fifth week of the study. The incubation study results complement the results from the greenhouse study (Figure 3.9). The greater soil water Zn observed was most likely due to ZnEDTA being 100% water-soluble and the tendency of ZnEDTA to prevent the irreversible fixation of Zn into the solid phase, and these two processes increased Zn in soil solution as observed by other researchers previously (Hamza and Sadnandan, 2005; Gupta and Deb, 1984; Parsad et al., 1975).

*Zinc coated urea*

Similar trends were observed in Zn-coated urea treatments (Figure 3.13). Zinc rate applied for Zn-coated urea was half the rate of Zn used for Zn only treatments, which explains the lower soil water Zn concentrations in Zn-coated urea treatments than Zn only treatments. Soil water Zn was significantly greater for ZnEDTA-U treatment collected at the end of the third (ranging from 27.02 to 40.64 μg $L^{-1}$) and fifth week (21.42 to 35.19 μg $L^{-1}$) than all other Zn treatments and urea (average of 4.82 μg $L^{-1}$ for third week and 4.48 μg $L^{-1}$ for fifth week). As mentioned above, this can be due to the greater solubility and stability of ZnEDTA complex. Ellis et al. (1965) also reported that ZnEDTA remained water soluble when coated onto the N-P-K fertilizer. Soil water Zn was observed to be significantly greater for urea and Zn-coated urea treatments than control. Hydrogen ions are consumed, and pH increases upon urea hydrolysis, whereas during the follow-up process of nitrification, the pH decreases (Jiang et al., 2012). Thus, the net effect of urea addition is acidification (Figure 3.8). The variation of soil pH may cause changes in the availability of Zn. The exchangeable fraction of Zn initially decreased as the pH value increased and then increased with decreasing pH. The concentrations of exchangeable Zn correlated negatively with pH, and the result was consistent with that reported by Cakmak (2009). Prasad (2005) and Neue et al. (1998) reported that combined application of nitrogen and Zn fertilizers increased Zn availability and solubility in the soil, which agrees with our results. This synergistic effect between Zn when coated onto urea treatment could be seen. Nitrogen and Zn could be due to rhizosphere-induced acidification, resulting from an excess of cations (especially $NH4^{+}$) over anions and thus inducing release of $H^{+}$ from the roots. Paterson et al. (2006) and Aciksoz et al. (2011b) reported that Zn uptake could be affected by an increase in the nitrogen supply due to the stimulation of soil microbial activity and increased root exudation.

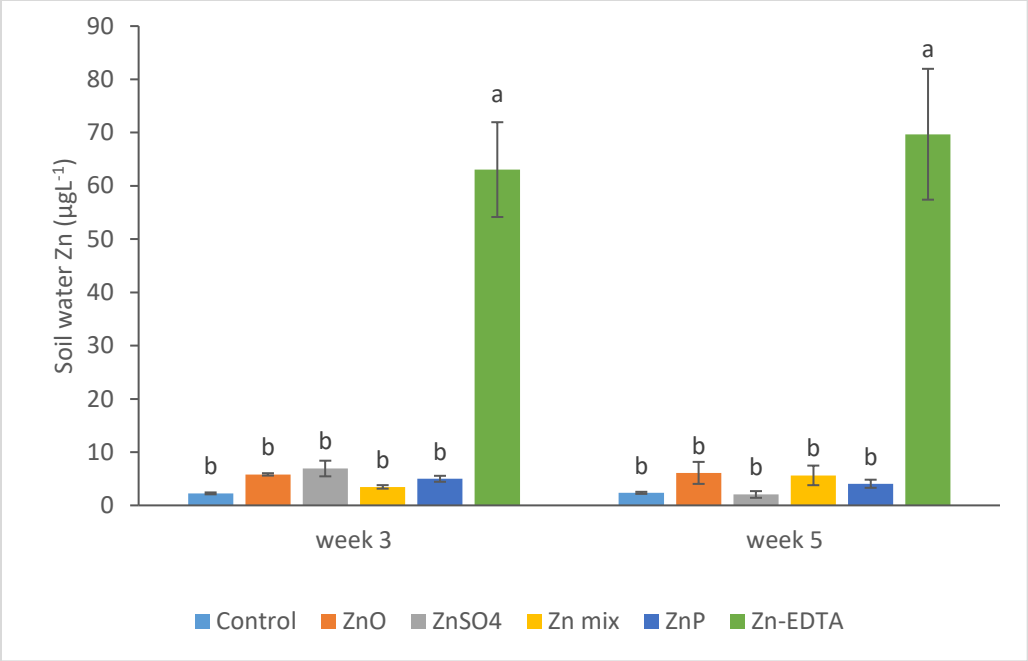


**Figure 3.13 Soil water Zn data collected at the end of third and fifth week of the study for Zn only treatments in the greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a time period containing the same letter are not significantly different $P \leq 0.05$ according to Tukey pairwise method. Error bars represent the standard error of three replicates.**

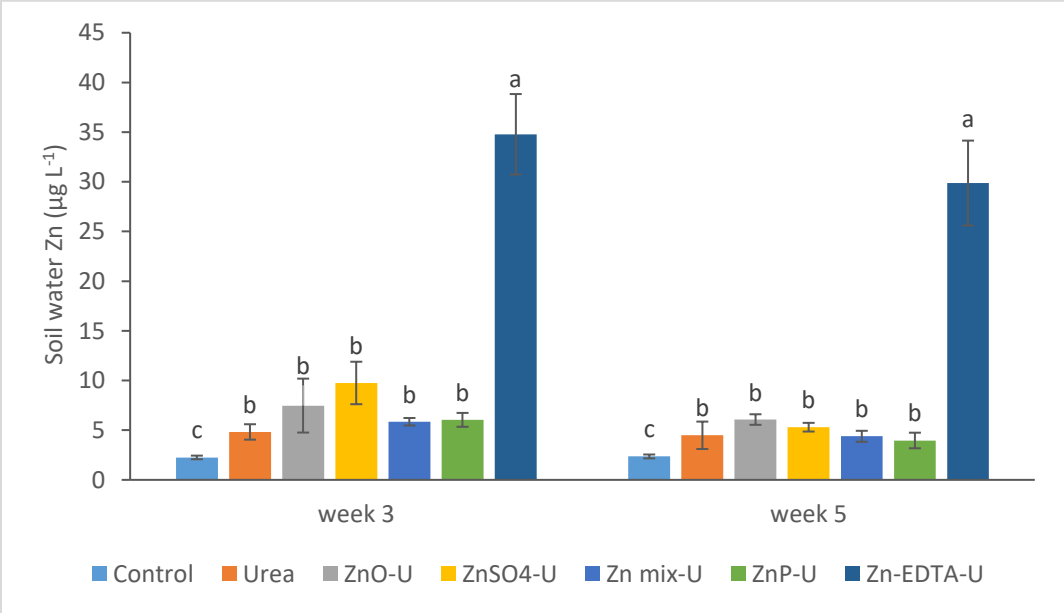


**Figure 3.14 Soil water Zn data collected at the end of third and fifth week of the study for Zn-coated urea treatments in the greenhouse study. Control= no-Zn; Urea = urea control**

**containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a time period containing the same letter are not significantly different at $P \leq 0.05$ according to Tukey pairwise comparison. Error bars represent the standard error of three replicates.**

Rehman et al. (2018c) and Rehman et al. (2018d) also reported that root exudation increased the solubility and availability of nutrients due to changes in the pH of the rhizosphere and soil microbial activity. Therefore, enhanced phytosiderophore production and thus Zn acquisition by the plants. Zinc-EDTA coated urea treatment had significantly greater soil water Zn than other Zn-coated urea treatments due to reasons mentioned above in Zn only sources- its high-water solubility and tendency to avoid Zn sorption.

#### 3.3.2.3 Soil pH

*Zn only*

Soil samples for pH for the greenhouse study were analyzed at the end of the study after plants were harvested. For the Zn-only greenhouse experiment, significantly lower pH was observed for ZnEDTA treatment for point of application (Figure 3.7). This decrease in pH is due to the pKa values of EDTA, as discussed in section 3.3.1.2. The Zn mix treatment had a significantly greater pH than the control for section 4. This can be explained based on the Zn mix constituting ZnO predominantly. As mentioned before, on its dissociation, ZnO releases $OH^-$ ions, which increase soil pH (Su et al., 2019).

$ZnO + H_2O \rightarrow Zn^{2+} + OH^-$

*Zinc coated urea*

Urea showed a significant decrease in soil pH compared to control in all soil sections in the greenhouse study (Figure 3.8). All Zn-coated urea treatments significantly reduced soil pH for section 2 (application section) compared to control. These results agree with soil pH results

from Zn-coated urea incubation study. As mentioned before, the reasons for lower pH for urea and Zn-coated urea fertilizers were due to the pH reduction via urea hydrolysis and addition of Zn fertilizer granules, as discussed in section 3.3.1.2.

#### 3.3.2.4 Diffusion of added zinc

*Zn only*

Zinc movement in soil can be observed by sampling soil sections at increasing distances from the Zn fertilizer application point (Ghosh, 1990; Hettiarachchi et al., 2010). From these experiments, two different zones have been identified based on their different zinc concentrations. Zone adjacent to the fertilizer application is Zn saturated zone, whereas the zone is farther away from the point of application with low Zn concentrations (Ghosh, 1990). Due to different Zn concentrations, various chemical reactions may occur. Zinc precipitation may be observed in the Zn saturated zone, whereas adsorption reactions might occur in the low Zn concentration zone. The greenhouse study results complemented the incubation study results. For Zn only study, the percent Zn recovered of added in each section is shown in Figure 3.9, and soil total Zn concentrations are shown in Figure 3.11. Out of all the treatments, ZnEDTA treatment permeated out to a larger soil volume. The percent Zn recovered from the point of application (section 2) was significantly lower for ZnEDTA treatment, followed by $ZnSO_4$ treatment. A significantly greater percent Zn added was recovered from sections 3 and 4 for ZnEDTA treatment than all other treatments signifying greater Zn diffusion. This explains the greater Zn diffusion in ZnEDTA treatment. On the other hand, 60-70% of the added Zn was recovered from the section of application (section 2) for treatments ZnO, $ZnSO_4$, Zn mix, and ZnP reflecting less Zn movement in these treatments.

*Zinc coated urea*

Zinc, when applied as coated onto granular nitrogen fertilizers, undergo a little different reaction route. Zinc and nitrogen reactions products are water-soluble compounds (Lehr, 1972). Results from a column dissolution study showed that the cumulative Zn release from the ZnO-coated urea granules was comparable to that of Zn release from ZnO alone (Milani et al., 2012). For Zn-coated urea study, the percent Zn added recovered in each section is shown in Fig 3.10, and soil total Zn concentrations are shown in Figure 3.12. For ZnO-U treatment, about 80% of the added Zn was recovered from the second section (application section). Zinc recovered from section of application was in order ZnO-U> ZnP-U> Zn mix-U> $ZnSO_4$-U> ZnEDTA-U. Thus, Zn diffusion was in reverse order, greatest Zn diffusion in ZnEDTA-U treatment. The current research has proven the progressive approach Zn-coated urea. The coating helps the fertilizer to dissolve gradually in the soil.

#### 3.3.2.5 Extractable Zinc

*Zn only*

Results from DTPA ext. (extractable) Zn for Zn only greenhouse study are shown in Fig 3.13. In terms of DTPA extractable Zn, ZnO, and ZnP behaved in the similar manner, i.e., a large portion of ext. Zn was present in the second section (section of application). Extractable Zn was significantly lower for the section- 2 for ZnEDTA as compared to all other Zn-only treatments. Zinc remained extractable for the first, third, and fourth sections of ZnEDTA treatment. Zinc extractability from ZnEDTA depends on inactivation of $Zn^{2+}$ involving its displacement by other cations such as Ca with subsequent metal precipitation. This reaction depends on the soil pH, ZnEDTA stability, and the solubility products of the inorganic metal compounds (Parsad et al., 1975). Zinc-EDTA has a stability constant of 17.5 greater than that of Ca-EDTA (11.6) (Norvell, 1991). Therefore, even in soils with high free $Ca^{2+}$ ions, Zn can preferably remain bound to

EDTA. In this case, ZnEDTA remains effective because Zn is not available for substitution or adsorption to negatively charged soil colloids. Thus ZnEDTA may have very little or no interaction with the soil components, thus preventing the irreversible fixation of Zn into the solid phase. The $ZnSO_4$ treatment did not perform well in terms of extractability, because of its sensitivity to get adsorbed when was added to calcareous soil (Rico et al., 1996). When $ZnSO_4$ is added to calcareous soil, the presence of active $CaCO_3$ results in Zn adsorption, leading to Zn precipitation as Zinc hydroxide or carbonate or the formation of insoluble calcium zincate (Rico et al., 1996). Because of these reasons, most of the applied Zn remained fixed in calcareous soils during a brief period (Shivay et al., 2008; Oliveira et al., 2010). Similar results were reported by Modaihsh et al. (1990), who found that when $ZnSO_4$ and ZnEDTA were applied at the same application rates, Zn diffusion was greater for ZnEDTA.

*Zinc coated urea*

Zinc oxide coated urea, and ZnP-U behaved similarly, i.e., a large portion of ext. Zn was present in the second section (section of application) (Figure 3.14). Another interesting result found in our study was the Zn mix-U extractability. Extractable Zn for the first section was significantly greater than ZnO-U, ZnP-U, and Urea. To the authors' knowledge, the acidulation effect of urea hydrolysis depressed the ZnO characteristics of Zn mix despite of its greatest proportion. The acidulation effect helped dissolving ZnO, stimulated Zn mix to act more like $ZnSO_4$ when applied as Zn mix-U. These results were also confirmed by XANES analyses (section 3.3.1.5).

## 3.4. Conclusions

This study provides a better understanding of the diffusion and transformation processes of different Zn only, and Zn-coated urea fertilizers in a calcareous soil in the absence and

presence of plants. The efficiency of the Zn fertilizers depends on their solubility, resultant pH, tendency to diffuse and avoid sorption reactions. Chemical reactions of cationic Zn with carbonates and hydroxides affected the solid-phase speciation of Zn in the soil and hindered its diffusion and availability. The superior agronomic effectiveness of ZnEDTA in calcareous soils resulted from its enhanced distribution, extractability, and solubility in soil. Zinc oxide and Zn mix treatment are relatively less soluble, and therefore, acted as slow-release fertilizers when added to calcareous soil. Low solubility for ZnO and Zn mix does not act as a barrier, but it keeps Zn soluble enough to provide adequate amounts of Zn to have plant biomass and plant Zn uptake similar to ZnEDTA. Our study showed that the application of Zn as Zn-coated urea offer benefit in terms of Zn dissolution and diffusion in calcareous soils. Zinc coating onto urea granules works via a synergistic mechanism. Reduced soil pH by urea addition promotes Zn solubility and availability, and in turn, Zn coating may delay nitrogen transformations in soil, reducing urea losses. The study also highlights changes in solid-phase speciation of Zn from different Zn-coated urea fertilizers associated with macronutrient fertilizers in soil only environment. X-ray absorption near-edge structure spectroscopy analysis does hint that Zn application with co-additive urea may be impacting Zn dissolution and adsorption and precipitation of Zn as $ZnCO_3$ is responsible for hindering Zn movement for several treatments. Zinc speciation was not discernable by the wet chemical methods to assess plant availability used in this study. Moreover, this study confirmed that the combined use of synchrotron-based research techniques with conventional wet chemical analyses is helpful for the examination of complex soil-fertilizer reactions and can aid our understanding of the reaction pathways of micronutrients applied to the soil.

## 3.5. References

Aciksoz, S. B., L. Ozturk, O.O. Gokmen, V. RÖmheld, and I. Cakmak. 2011b. Effect of nitrogen on root release of phytosiderophores and root uptake of Fe(III)-phytosiderophore in Fe-deficient wheat plants. Physiol. Plant 142: 287-296.

Adriano, D.C. 2001. p. 867. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals. 2nd Edition, Springer, NY.

Allison, L. and C. Moodie. 1965. 1965. p. 149-157. Cation-exchange capacity. In A.G. Norman (ed.) Methods of Soil Analysis. Part-2. Chemical and Microbiological Properties. SSSA, Madison, WI.

Alvarez, J. M., M.I. Rico. 2003. Effects of zinc complexes on the distribution of zinc in calcareous soil and zinc uptake by maize. J. Agric. Food Chem. 51:5760−5767.

Alloway B.J. 1995. p. 38-56. Heavy metals in soils. 2nd ed. Blackie Academic. Professional, London, UK.

Alloway, B.J. 2009. Soil factors associated with zinc deficiency in crops and humans. Environ Geochem Health. 31. 537-48.

Amrani M., D.G. Westfall, and G.A. Peterson. 1999. Influence of water solubility of granular zinc fertilizers on plant uptake and growth. J. Plant Nutr. 22:1815-1827.

Attanayake, C., G. Hettiarachchi, S. Martin, G. Pierzynski. 2015. Potential Bioavailability of Lead, Arsenic, and Polycyclic Aromatic Hydrocarbons in Compost-Amended Urban Soils. J. Environ Qual. 44: 930-944.

Cakmak, I. 2004. p 1-28. Identification and correction of widespread zinc deficiency in Turkey, A success story. IFS Proceedings No. 552, International Fertiliser Society, York, UK.

Cakmak, I. 2009. Enrichment of fertilizers with zinc: An excellent investment for humanity and crop production in India. J. Trace Elem. Med. Biol. 23(4):281-9.

Cakmak, I., M. Kalayci, Y. Kaya, A.A. Torun, N. Aydin, and Y. Wang. 2010a. Biofortification and localization of zinc in wheat grain. J. Agri. Food Chem. 58: 9092-9102.

Cakmak, I. and U.B. Kutman. 2018. Agronomic biofortification of cereals with zinc: a review. Eur. J. Soil Sci. 69: 172-180.

Coleman, W.F. 2008. Molecular Models of EDTA and Other Chelating Agents. J. Chem. Edu. 85: 1296.

Degryse, F., A. Voegelin, O. Jacquat, R. Kretzschmar, and E. Smolders. 2011. Characterization of zinc in contaminated soils: complementary insights from isotopic exchange, batch extractions and XAFS spectroscopy. Eur. J. Soil Sci. 62:318-33.

Degryse, F., R. Baird, and M. McLaughlin. 2015. Diffusion and solubility control of fertilizer-applied zinc: chemical assessment and visualization. Plant Soil. 386.

Degryse, F., R. Silva, Rodrigo, R. Baird, I. Cakmak, A. Yazici, and M. McLaughlin. 2020. Comparison and modelling of extraction methods to assess agronomic effectiveness of fertilizer zinc. J. Plant Nutr. Soil Sci. 183.

Ellis, B.G., J.F. Davis, and W.H. Judy. 1965. Effect of Method of Incorporation of Zinc in Fertilizer on Zinc Uptake and Yield of Pea Beans (Phaseolus vulgaris). Soil Sci. Soc. Am. J. Proc. 29: 225-228

FAO/WHO. 2011. Report of the Fifth Session of the Codex Committee on Contaminants in Foods, The Hague, the Netherlands, 21-25 March 2011. Food and Agriculture Organization (FAO) Rome; World Health Organization (WHO), Geneva, Switzerland.

Gangloff, W. J., D.G. Wesfall, G.A. Peterson, and J.J. Mortvedt. 2002. Relative availability coefficients of organic and inorganic Zn fertilizers. J. Plant Nutr. 25:259−273.

Gilkes, R.J., R.C. Young, J.P. Quirk. 1975. Leaching of copper and zinc from trace element superphosphate. Soil Res. 13:33-41.

Giordano, P.M., and J.J. Mortvedt. 1973. Zinc Sources and Methods of Application for Rice. Agron. J. 65:51-53.

Goos R.J., B.E. Johnson, and M. Thiollet. 2000. A comparison of the availability of three zinc sources to maize (Zea mays L.) under greenhouse conditions. Biol. Fert. Soils. 31: 343- 347.

Gupta G.N. and D.L. Deb. 1984. Effect of chelating agents on zinc diffusion in two soils. J. Plant Nutr. Soil Sci. 147:533- 539

Hamza, S. and A.K. Sadanandan. 2005. Effect of source and method of application of zinc on yield and quality of black pepper (Piper nigrum L.). J. Spices Aromatic Crops. 14: 117- 121.

Hettiarachchi, G. M., E. Lombi, M.J. McLaughlin, D. Chittleborough, and P. Self. 2006. Density changes around phosphorus granules and fluid bands in a calcareous soil. Soil Sci. Soc. Am. J. 70:960-966.

Hettiarachchi, G.M., M.J. McLaughlin, K.G. Scheckel, D.J. Chittleborough, M. Newville, S. Sutton. 2008. Evidence for different reaction pathways for liquid and granular micronutrients in a calcareous soil. Soil Sci. Soc. Am. J. 72(1): 98-110.

Hettiarachchi, G. M., E. Lombi, M.J. McLaughlin, D.J. Chittleborough, and C. Johnston. 2010. Chemical behavior of fluid and granular Mn and Zn fertilisers in alkaline soils. Soil Res. 48:238-247.

Imran, M., S. Kanwal, S. Hussain, T. Aziz, M.A. Maqsood. 2015. Efficacy of zinc application methods for concentration and estimated bioavailability of Zn in grains of rice grown on a calcareous soil. Pak. J. Agri. Sci. 52(1):169-175.

Jacquat, O., A. Voegelin, A. Villard, M. Marcus, and R. Kretzschmar. 2008. Formation of Zn-rich phyllosilicate, Zn-layered double hydroxide and hydrozincite in contaminated calcareous soils Geochim. Cosmochim. Acta. 72:5037-5054.

Jacquat, O., A. Voegelin, and R. Kretzschmar. 2009. Soil properties controlling Zn speciation and fractionation in contaminated soils Geochim. Cosmochim. Acta. 17:5256-5272.

Jadon, P., R. Selladurai, S.S. Yadav, M.V. Coumar, M.L. Dotaniya, and A.K. Singh. 2018. Volatilization and leaching losses of nitrogen from different coated urea fertilizers. J. Soil Sci. Plant Nutr. 18:1036-1047.

Jenkinson, D., and D. Powlson. 1976. The effects of biocidal treatments on metabolism in soil V: A method for measuring soil biomass. Soil Biol. Biochem. 8:209-213.

Jiang, Z., Q. Zeng, B. Tie, B. Liao, H. Pi, X. Feng, and Y. Sun. 2012. Ammonia volatilization and availability of Cu, Zn induced by applications of urea with and without coating in soils. J. Environ. Sci. 24:177-81.

Kabata-Pendias, A. 2004. Soil-plant transfer of trace elements—an environmental issue. Geoderma. 122:143-149.

Kutman U. B., B. Yildiz, I. Cakmak. 2011. Improved nitrogen status enhances zinc and iron concentrations both in the whole grain and the endosperm fraction of wheat. J. Cereal Sci. 53:118-125.

Lehr, J.R. Chemical Reactions of Micronutrients in Fertilizers. 1972. p. 459-503. In Mortvedt, J.J; Giordano, P.M.; and Lindsay, W.L. (eds.) Micronutrients In Agriculture. Soil Sci. Soc. Amer., Inc., Madison, WI.

Lombi, E., and J. Susini. 2009. Synchrotron-based techniques for plant and soil science: opportunities, challenges and future perspectives. Plant Soil. 320: 1-35.

Lombi, E.; M.J. McLaughlin; C. Johnston; R.D. Armstrong and R.E. Holloway. 2004. Mobility and lability of phosphorus from granular and fluid monoammonium phosphate differs in a calcareous soil. Soil Sci. Soc. Am. J. 68: 682-689.

Lindsay, W. L., and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Am. J. 42:421−428.

Lindsay, W.L. 1991. pp 89-112. Inorganic equilibria affecting micronutrients in soils. In Micronutrients in Agriculture. Eds. Mortvedt, J.J., F.R. Cox, L.M. Shuman and R.M. Welch. 2nd edition. Soil Sci. Soc. Am. J., Madison, WI.

Mehdi, S.M., A.M. Ranjha, and T. Hussian. 1990. Relative efficiency of various sources of zinc. Sarhad J. Agric. 6:103-106.

Modaihsh A. 1990. Zinc diffusion and extractability as affected by zinc carrier and soil chemical properties. Fertilizer res. 25:85-91.

McNear, D.H., R. Tappero, and D.L. Sparks. 2005. Shining light on metals in the environment. Elements. 1: 211-6.

Metwally, A.I., A.S. Mashhady, A.M. Falatah, and M. Reda. 1993. Effect of pH on Zinc Adsorption and Solubility in Suspensions of Different Clays and Soils. J. Plant Nutr. Soil Sci. 156: 131-135.

Mortvedt, J.J., and P.M. Giordano. 1969. Extractability of zinc granu- lated with macronutrient fertilizers in relation to its agro- nomic effectiveness. J. Agric. Food Chem. 17:1272

Milani, N., M.J. McLaughlin, S.P. Stacey, J.K. Kirby, G.M. Hettiarachchi, and D.G. Beak. 2012. Dissolution kinetics of macronutrient fertilizers coated with manufactured zinc oxide nanoparticles. J. Agric. Food Chem. 60:3991-3998.

Milani, N., G.M. Hettiarachchi, J. Kirby, D. Beak, S. Stacey, and M. McLaughlin. 2015. Fate of Zinc Oxide Nanoparticles Coated onto Macronutrient Fertilizers in an Alkaline Calcareous Soil. PLOS ONE. 10(5): e0126275.

Neue, H. U., C. Quijano, D. Senadhira, and T. Sette. 1998. Strategies for dealing with micronutrient disorders and salinity in lowland rice systems. Field Crops Res. 56:139-155.

Norvell, W.A. 1991. Reactions of metal chelates in soils and nutrient solutions, pp. 187-227. In: J.J. Mordvet, F.R. Cox, L.M. Sherman, and R.M. Welch (eds.). Micronutrients in agriculture. Soil Sci. Soc. Am. J., Madison, WI.

Ozkutlu, F., B. Torun, and I. Cakmak. 2006. Effect of zinc humate on growth of soybean and wheat in zinc-deficient calcareous soil. Commun. Soil Sci. Plant Anal. 37: 2769−2778.

Obrador, A., J. Novillo, and J.M. Alvarez. 2003. Mobility and availability to plants of two zinc sources applied to a calcareous soil. Soil Sci. Soc. Am. J. 67:564−572.

Oliveira, E.M.M., H.A. Ruiz, V.H. Alvarez, P.A. Ferreira, F.O. Costa, and I.C. Almeida. 2010. Nutrient supply by mass flow and diffusion to maize plants in response to soil aggregate size and water potential. Rev. Bras. Ciênc. Solo. 34: 317- 327.

Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. U.S. Government Printing Office, Washington, D.C.

Ortiz, E.M.E. and O.A. Garcia. 1998. Process of sorption of zinc in three soils of Colombia cultivated with rice (Oryza sativa) of the dry land variety Llano-5. In: Proceedings of the VIII Congress of Soil Sciences, Santa Marta, Colombia. Suelos-Ecuatoriales. 28: 112117.

Ortiz-Monasterio, I.J., N. Palacios-Rojas, E. Meng, K. Pixley, R. Trethowan and R.J. Pena. 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J. Cereal Sci. 46: 293-307.

Oscarson, P. 2000. The strategy of the wheat plant in acclimating growth and grain production to nitrogen availability. J. Exp. Bot. 51: 1921-1929.

Papadopoulos, P., and D.L. Rowell. 1989. The reactions of copper and zinc with calcium carbonate surfaces. Eur. J. Soil Sci. 40:39-48.

Paterson, E., A. Sim, D. Standing, M. Dorward, and A.J.S. McDonald. 2006. Root exudation from Hordeum vulgare in response to localized nitrate supply. J. Exp. Bot. 57: 2413-2420.

Prasad, B., M.K. Sinha, and N.S. Randhawa. 1975. Effect of mobile chelating agents on diffusion of zinc in soils. Soil Sci. 122: 260- 266.

Prasad, R. 2005. Rice-wheat cropping system. Adv. Agron. 86:255-339.

Rashid, A., and J. Ryan. 2004. Micronutrient constraints to crop production in soils with Mediterranean-type characteristics: A review. J. Plant Nutr. 27: 959−975.

Rehman, A., M. Farooq, M. Naveed, L. Ozturk, and A. Nawaz. 2018c. Pseudomonas-aided zinc application improves the productivity and biofortification of bread wheat. Crop Pasture Sci. 69:659-672.

Rehman, A., M. Farooq, M. Naveed, A. Nawaz, and B. Shahzad. 2018d. Seed priming of Zn with endophytic bacteria improves the productivity and grain biofortification of bread wheat. Eur. J. Agron. 94:98-107.

Rengel, Z., G.D. Batten, and D.E. Crowley. 1999. Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crops Res. 60:27-40.

Rico, M.I., J.M. Alvarez, and J.I. Mingot. 1996. Efficiency of zinc ethylenediamine tetraacetate and zinc lignosulfonate soluble and coated fertilizers for maize in calcareous soil. J Agric. Food Chem. 44:3219- 3223.

Saeed, M. and R.L. Fox. 1977. Relation between suspension pH and zinc solubility in acid and calcareous soils. Soil Sci. Soc Am. J. 124:199-204.

Scheckel, K.G., and J.A. Ryan. 2004. Spectroscopic speciation and quantification of lead in phosphate-amended soils. J. Environ. Qual. 33:1288-1295.

Singh, A., and Y.S. Shivay. 2016. Effect of summer green manuring crops and zinc fertilizer sources on productivity, Zn-uptake and economics of basmati rice. J. Plant Nutr. 39:204-218

Slaton, N.A., R.J. Norman, and C.E. Wilson. 2005. Effect of zinc source and application time on zinc uptake and grain yield of flood-irrigated rice. Agron. J. 97:272−278.

Soil Survey Staff. 2011. Burt, R. (Ed.). Soil Survey Laboratory Information Manual. Soil Survey Investigations. Report No. 45, Version 2.0. Washington, DC: U.S. Department of Agriculture, Natural Resources Conservation Service.

Sharpless, R.G., E.F. Wallihan, and F.F. Peterson. 1969. Retention of Zinc by Some Arid Zone Soil Materials Treated with Zinc Sulfate. Soil Sci. Soc. Am. J. 33:901-904.

Shaver, T., D. Westfall, and M. Ronaghi. 2007. Zinc Fertilizer Solubility and Its Effects on Zinc Bioailability Over Time. J. Plant Nutr. 30:123-133.

Shivay, Y.S., D. Kumar, and R. Prasad. 2008. Effect of zinc-enriched urea on productivity, zinc uptake and efficiency of an aromatic rice-wheat cropping system. Nutr. Cycl. Agroecosys. 81: 229-243.

Su, Y., J. Gao, Y. Qin, Y. Zheng, and D. Zhu. 2019. Interfacial Zinc Phosphate is the Key to Controlling Biocompatibility of Metallic Zinc Implants. Advanced Science. 6.

Viets, F. G., L. C. Boawn, C. L. Crawford, and C. E. Nelson. 1953. Zinc deficiency in corn in central Washington. Agron. J. 45:559-565.

Voegelin, A., S. Pfister, A.C. Scheinost, M.A. Marcus, and R. Kretzschmar. 2005. Changes in zinc speciation in field soil after contamination with zinc oxide. Environ. Sci. Technol. 39:6616-6623.

Voegelin, A., O. Jacquat, S. Pfister, K. Barmettler, A.C. Scheinost, and R. Kretzschmar. 2011. Time-dependent changes of zinc speciation in four soils contaminated with zincite or sphalerite. Environ. Sci. Technol. 45:255-61.

Watson, M.E., and J.R. Brown. 1998. p. 13-17. pH and lime requirement. In J. R. Brown (Ed.), Recommended chemical soil test procedures for the North Central Region Missouri. Agricultural Experiment Station, University of Missouri, Columbia, MO.

Westfall, D., and B. Gangloff. 2001. Zinc fertilizers, is there a difference? Agron News. 21(6):8.

Westfall, D., J. Mortvedt, G. Peterson, and W. Gangloff. 2005. Efficient and Environmentally Safe Use of Micronutrients in Agriculture. Commun. Soil Sci. Plant Anal. 36: 169-182.

Whitney, D.A. 1998. p. 59-60. Soil salinity. In Brown, J.R., Ed., Recommended Chemical Soil Test Procedures for the North Central Region. SB 1001. Missouri Agricultural Experiment Station, Columbia, MO.

Werner, F., and J. Prietzel. 2015. Standard Protocol and Quality Assessment of Soil Phosphorus Speciation by P K -Edge XANES Spectroscopy. Environmental science & technology. 49.

Wright, A. and J. Bailey. 2001. Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 Dry Combustion Analyzer. Communications in Soil Science and Plant Analysis. Commun. Soil Sci. Plant Anal. 32:3243-3258.

Xue, Y. F., S.C. Yue, D.Y. Liu, W. Zhang, X.P. Chen, and C.Q. Zou. 2019. Dynamic Zinc Accumulation and Contributions of Pre- and/or Post-Silking Zinc Uptake to Grain Zinc of Maize as Affected by Nitrogen Supply. Front. Plant. Sci. 10:1203.

Yadav, R., M. Pandey, P. Pandey, Prasad, Bishnu, R. Ojha, amd D. Bahadur, J. Shrestha, and K. Ghimire. 2019. Evaluation of Bio-fortified Wheat Genotypes for Grain Yield, Zinc and Iron Content. Syrian J. Agri. Res. 6:528-537.

Zarcinas, B.A., M.J. McLaughlin, and M.K. Smart. 1996. The effect of acid digestion technique on the performance of nebulization systems used in inductively coupled plasma spectrometry. C. Soil Sci. Plant Anal. 27:1331-1354.

## 3.6 Supplementary Information

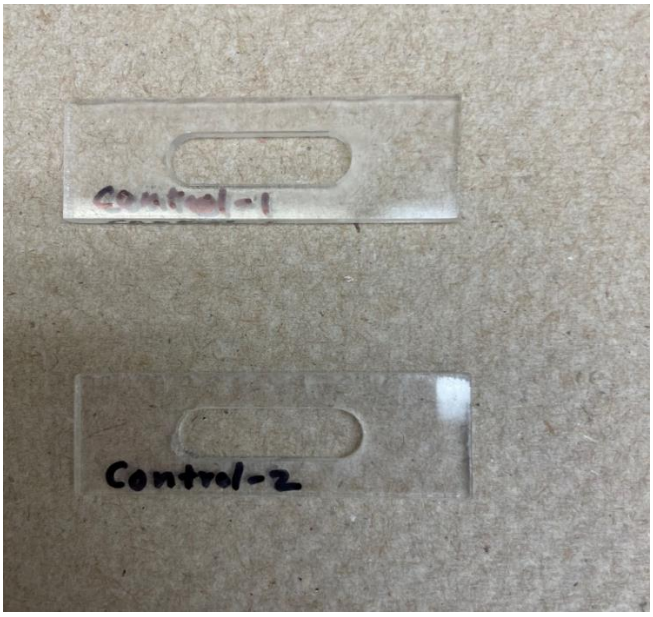


**Figure S3.1 Plexiglas holder labeled with sample name**

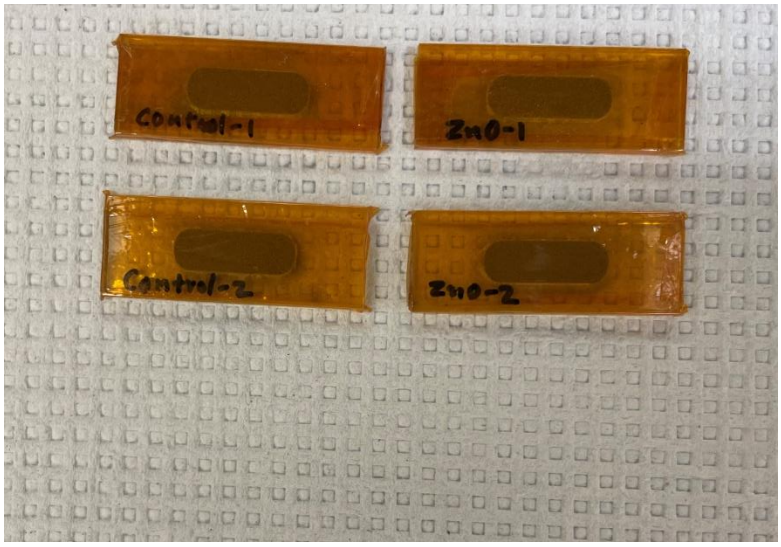


**Figure S3.1  Soil packed in Plexiglas holder and sealed with Kapton tape**

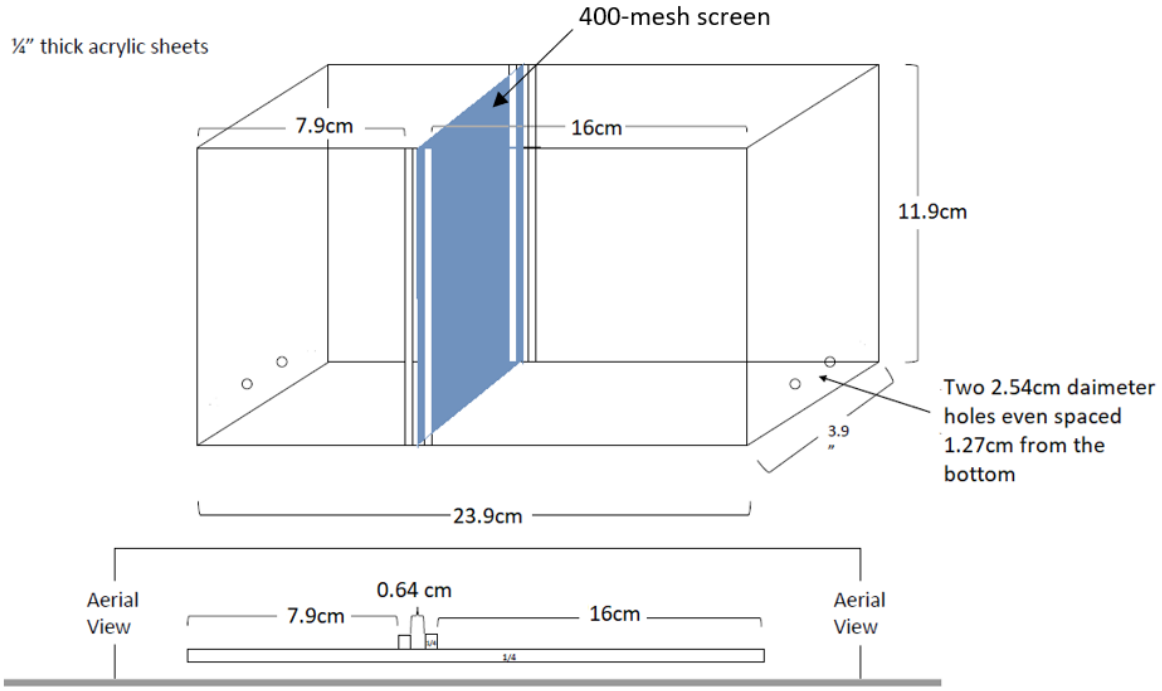


**Figure S3.2 Acrylic container used for greenhouse experiment**

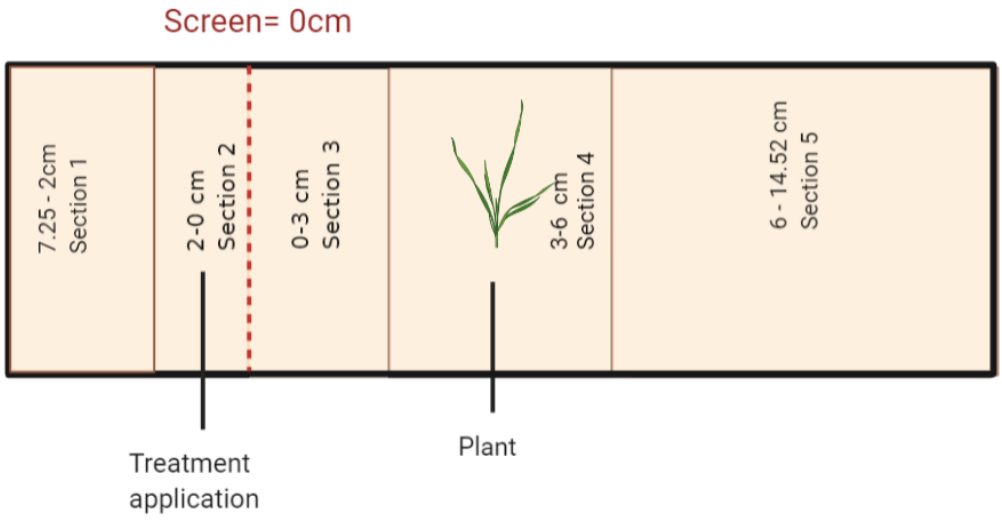


**Figure S3.3 Soil sectioning for end of the greenhouse experiment**

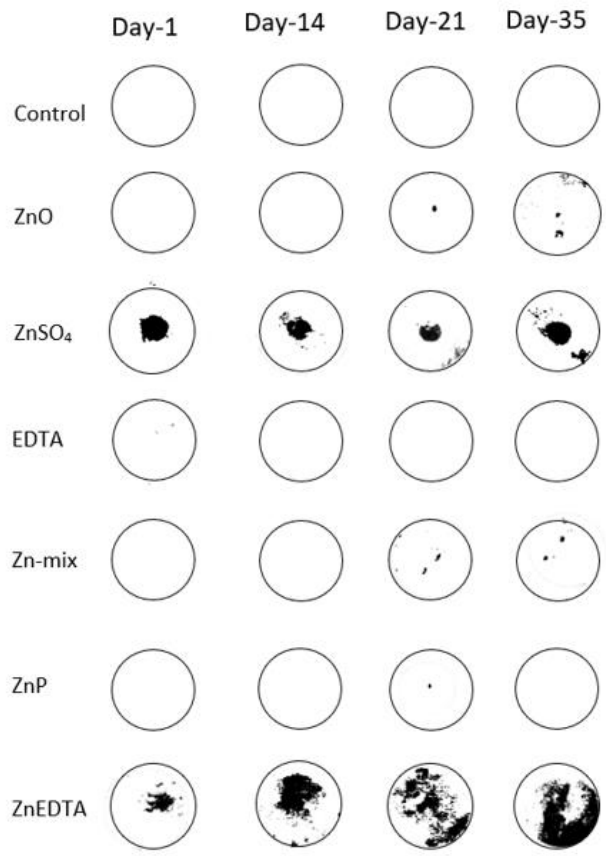


**Figure S3.5 Images analyzed using GIMP software for Zn only treatments at day-1, 7, 21, and 35. Control= no-Zn; ZnO= zinc oxide; ZnSO4= zinc sulfate; EDTA= ethylenediamine tetra acetic acid with no-Zn; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid.**

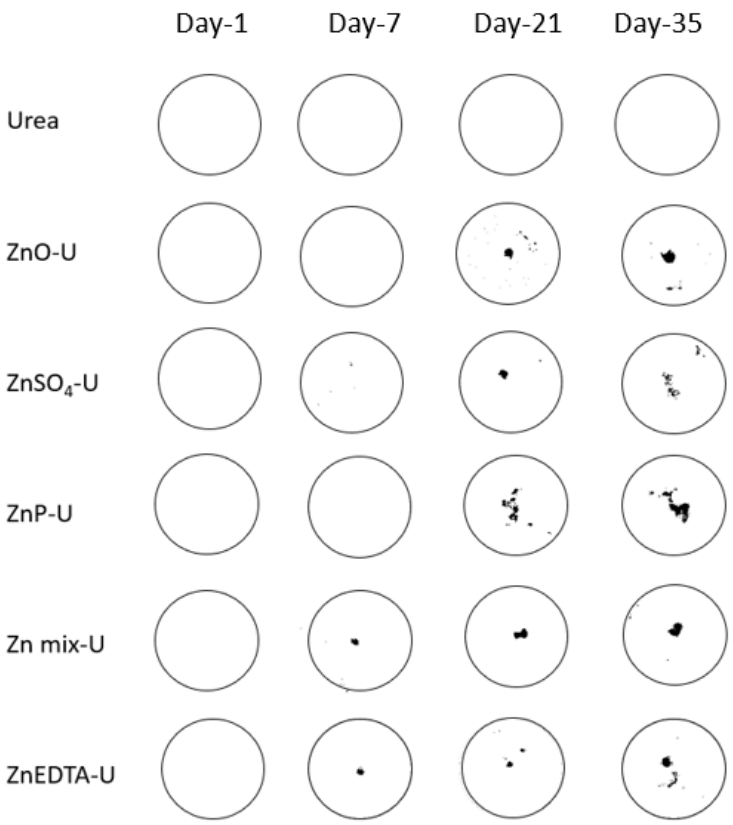


**Figure S3.6 Images analyzed using GIMP software for Zn-coated urea treatments. Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea.**

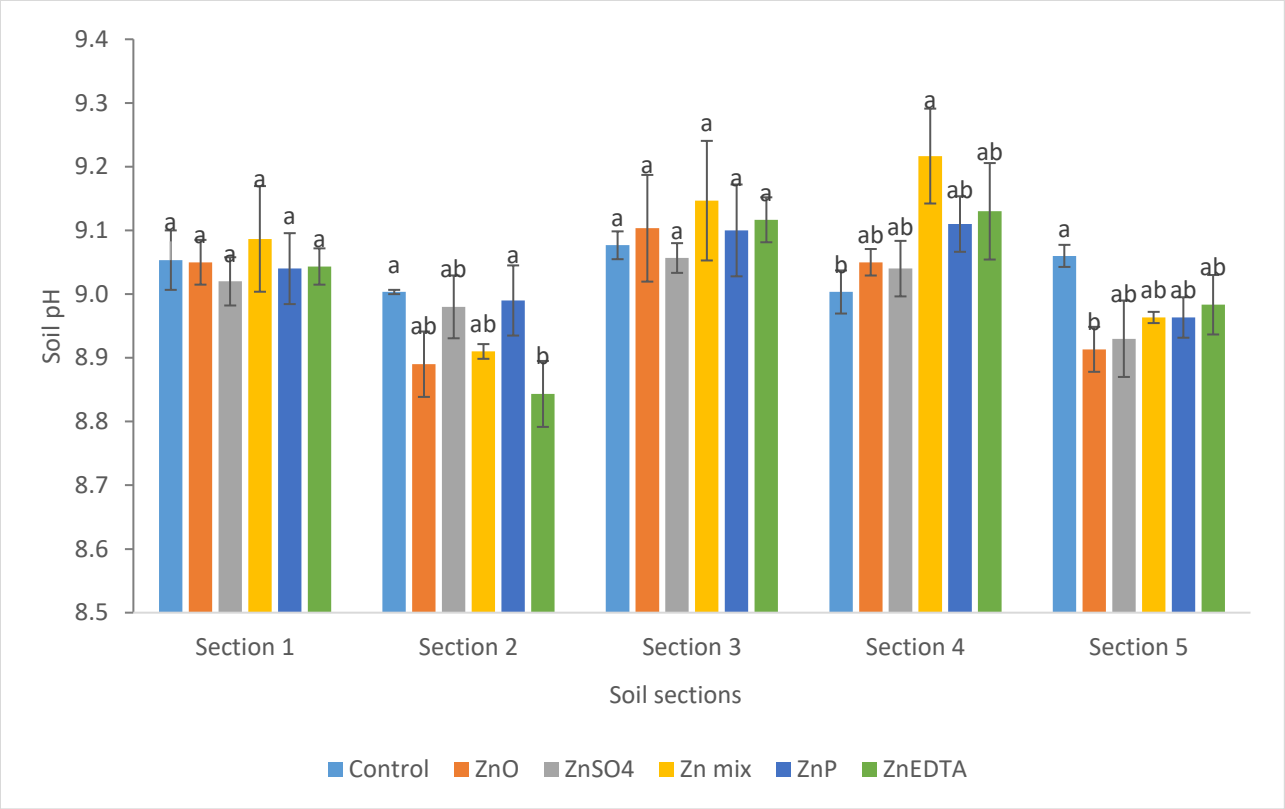


**Figure S3.7 Soil pH for Zn only experiment in greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

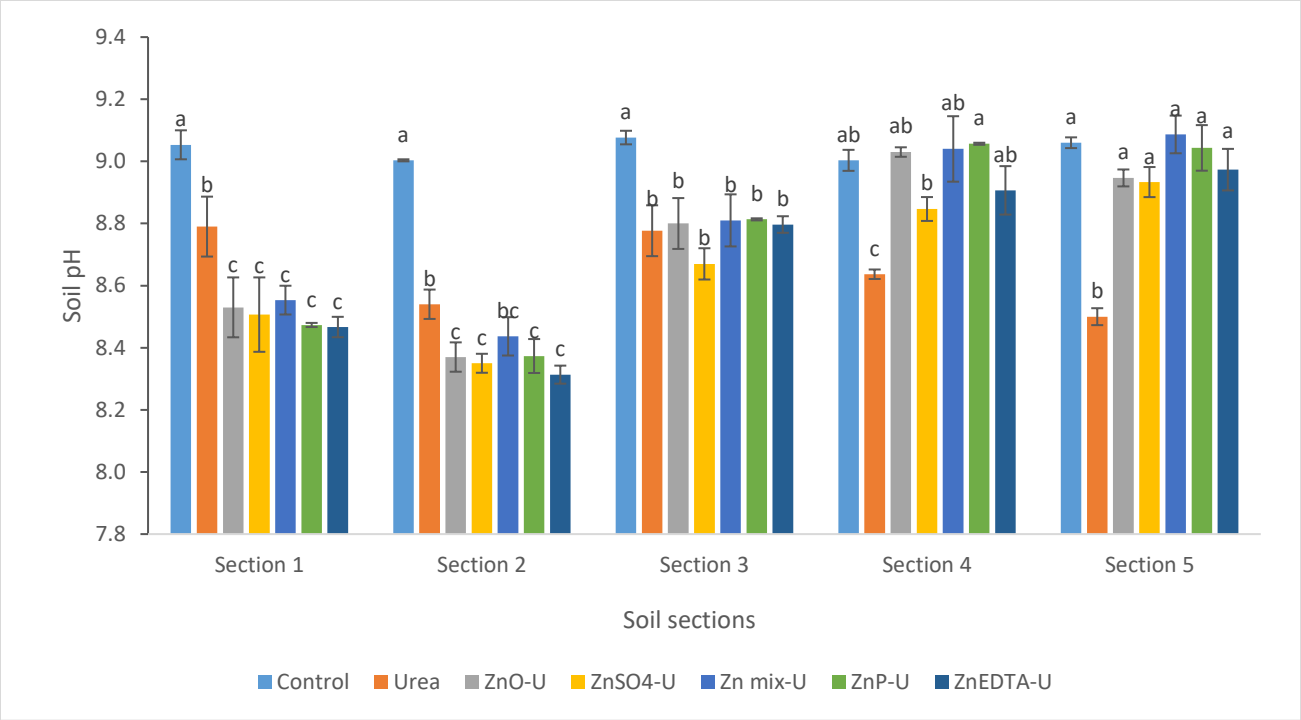


**Figure S3.8 Soil pH for Zn-coated urea treatments at the end of the greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

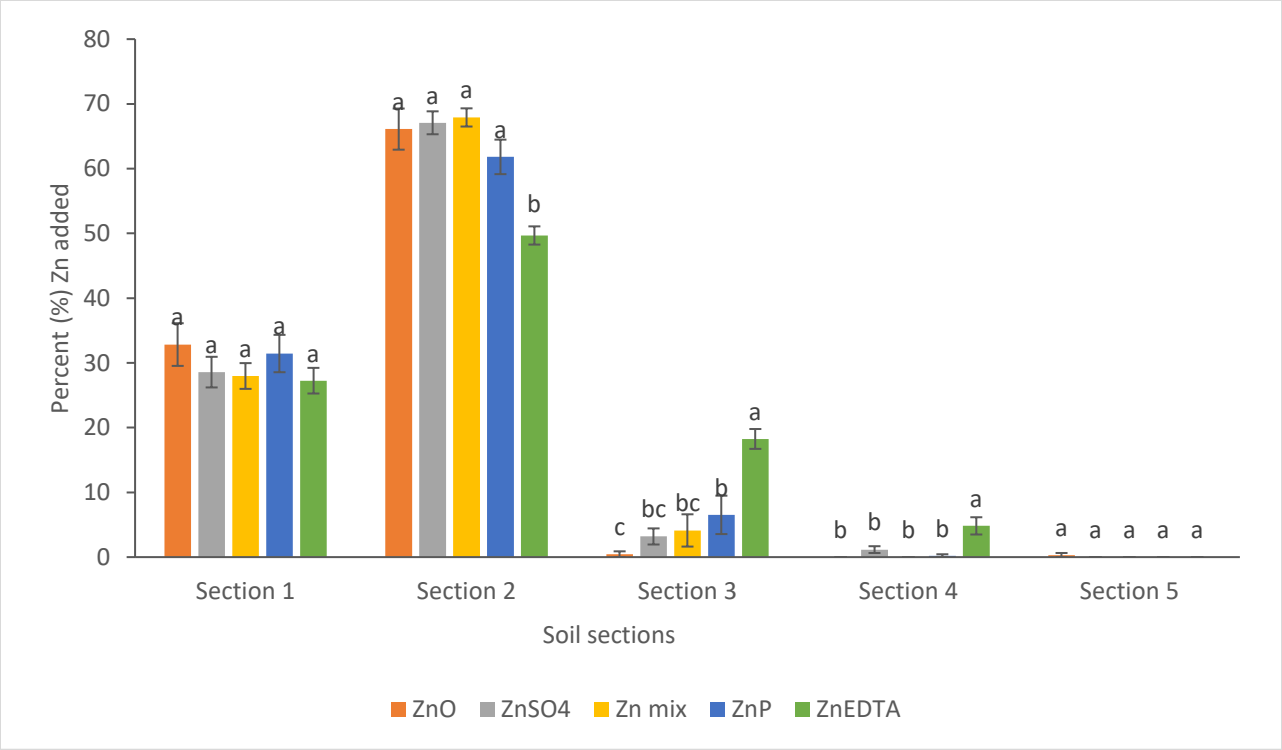


**Figure S3.9 Percentage of Zn added recovered in each section for Zn only experiment in the greenhouse study. ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

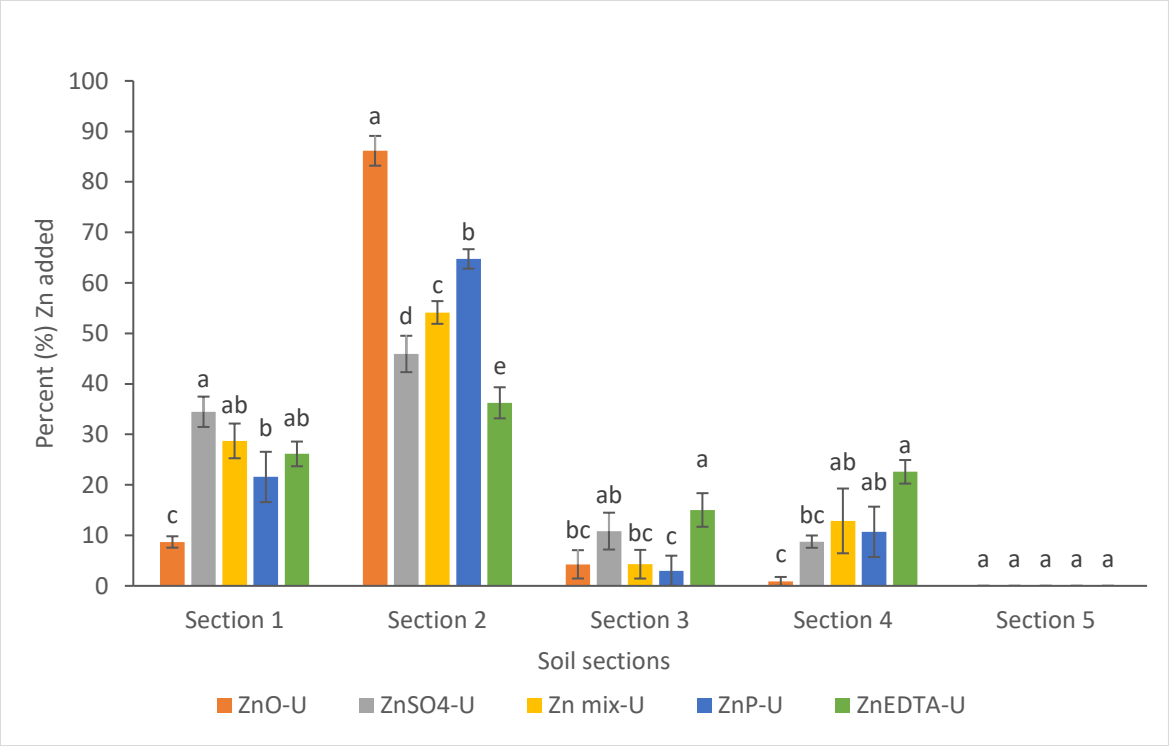


**Figure S3.10 Percentage of Zn added recovered in each section for Zn-coated urea experiment in the greenhouse study. ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

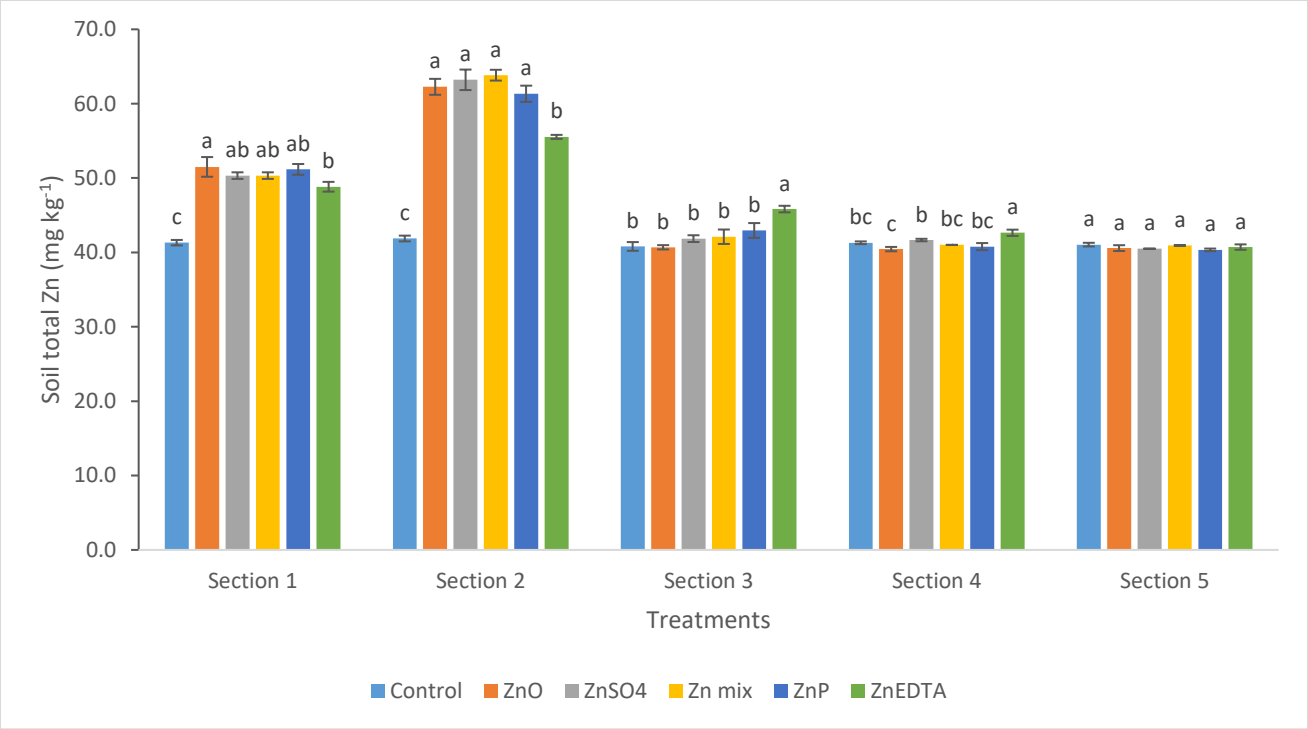


**Figure S3.11 Soil total Zn for Zn only treatments in the greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

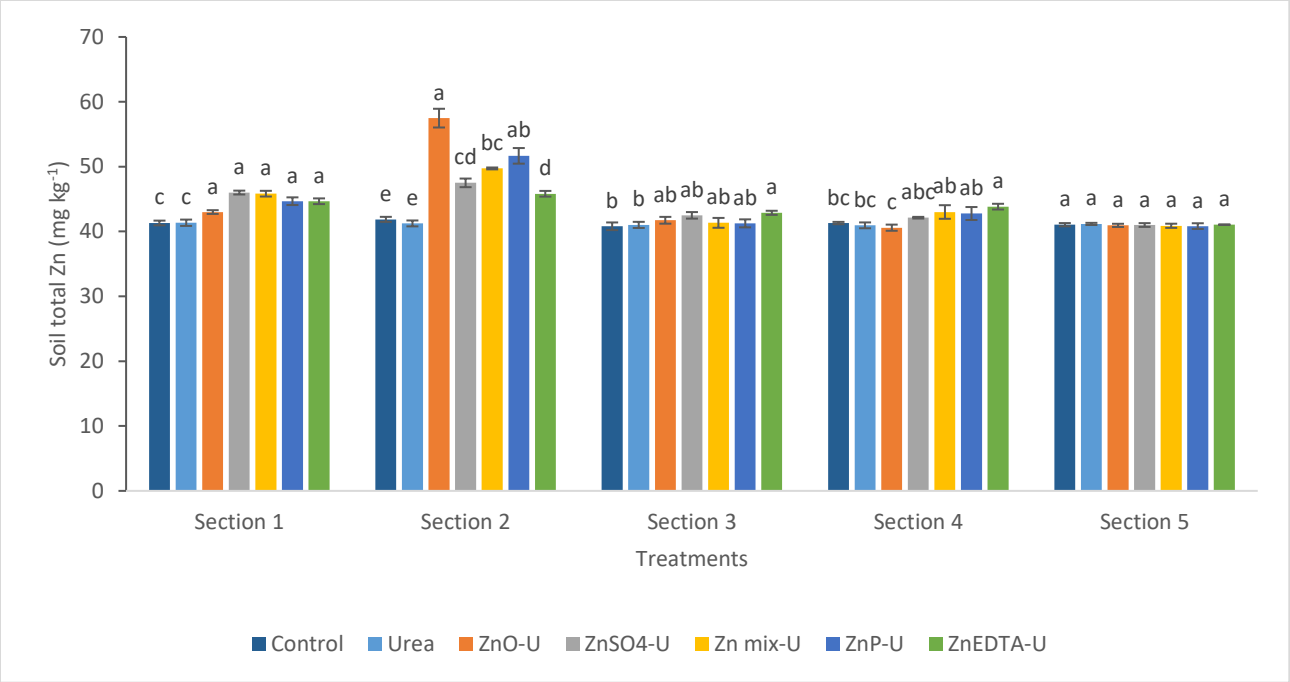


**Figure S3.12 Soil total Zn for Zn-coated urea experiment in the greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

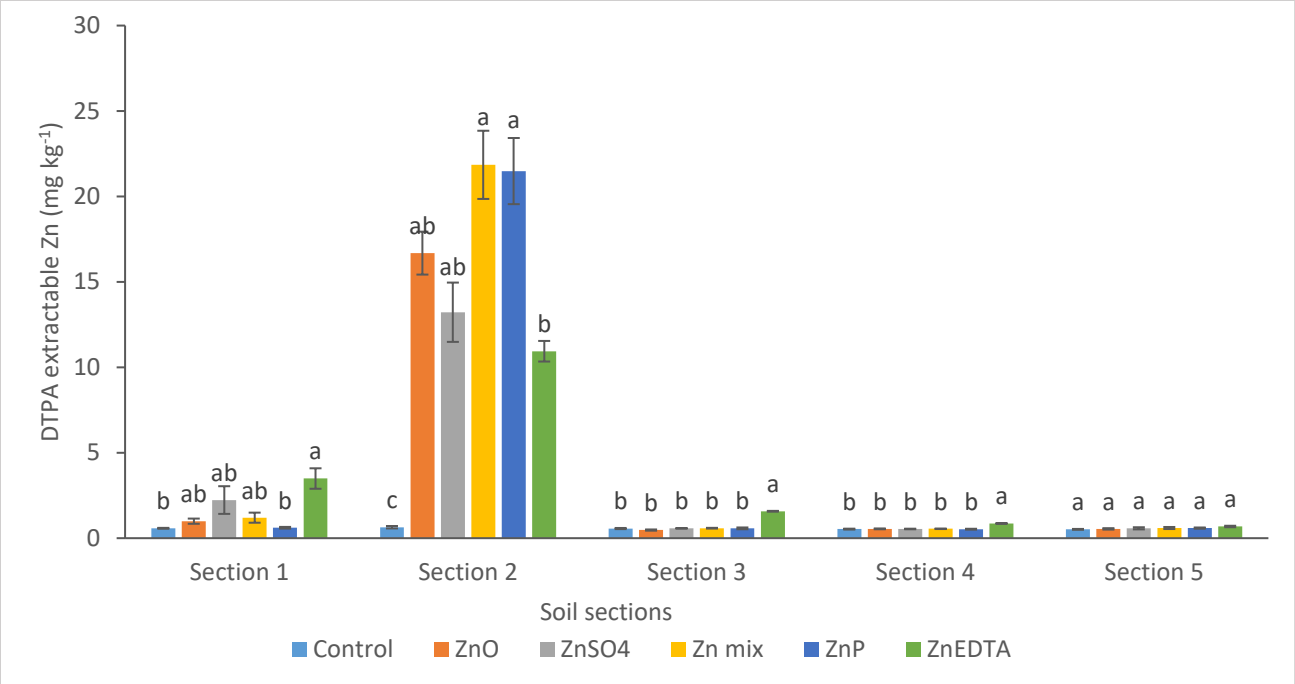


**Figure S3.13 Extractable Zn using DTPA for Zn only experiment in the greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

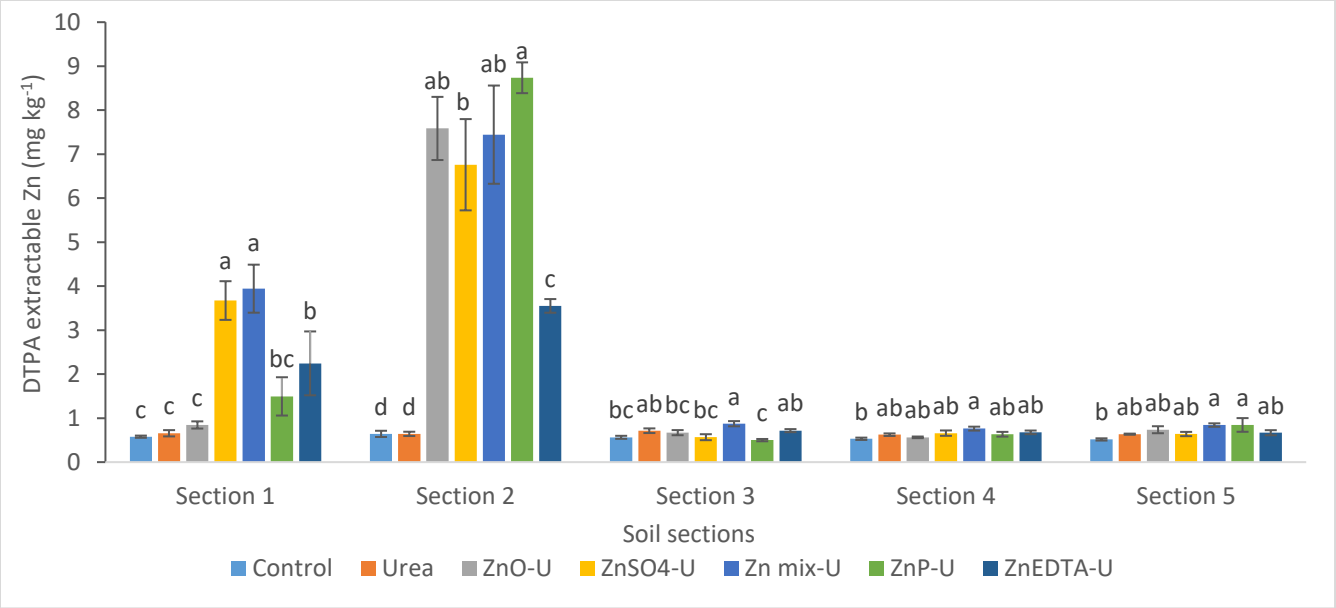


**Figure S3.14 Extractable Zn using DTPA for Zn-coated urea experiment in the greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

# Chapter 4 - Chemical speciation of zinc in fertilized neutral soils governs its lability and Phyto availability


## Abstract

Applied Zn involves multiple physical, chemical, and biological reactions that control available Zn concentrations in the soil. The choice of the Zn fertilizer depends on the chemical reactions zinc undergoes- adsorption-desorption, precipitation-dissolution, complexation-dissociation. The overall objective was to compare four different Zn fertilizers (ZnO, $ZnSO_4.7H_2O$, ZnEDTA to a newly introduced Zn product- Zn mix (mixture of ZnO, $ZnSO_4$ and EDTA) along with control (No-Zn) and EDTA (No-Zn + EDTA) based on relative Zn diffusion, water extractability, plant biomass, and plant Zn uptake involving incubation (soil only) and greenhouse (soil + plant) studies. Speciation affects Zn mobility/bioavailability and therefore, XANES (X-ray absorption near edge structure) was used to evaluate the reaction products in soil. Zinc sulfate and ZnEDTA showed greater Zn diffusion, water extractability, plant Zn concentration, and DTPA extractability from sections away from the point of application than ZnO and Zn mix treatments. The XANES analysis showed Zn was mainly present as Zn aluminum nitrate double-layered hydroxides (ZnAlDHnitrate) in all other treatments except ZnEDTA, complementing our understanding from the incubation and greenhouse studies that Zn gets sorbed to specific metals oxides or soil surfaces. In contrast, ZnEDTA minimizes sorption processes and can turn out to be the most agronomically efficient Zn fertilizer in this neutral soil.


## 4.1 Introduction

Zinc mobility in soil and sediment environments is often dictated by the complexation of Zn ions at the interface of solid surfaces and the surrounding solution. In soils, Zn exists as

numerous sorbent phases capable of adsorbing metal ions, namely clay minerals, metal oxides and oxyhydroxides, and organic matter. The sorbent phase may play an important role in the strength, and reversibility of the Zn complex formed and, therefore, dictate to what degree Zn can either be sequestered or mobilized. Fate of Zn in soil depends on chemical reactions that transform Zn into more or less available forms. Studies on Zn have revealed that the extent and nature of Zn transformations are governed by the amount of Zn added, Zn source, reaction time in the soil, clay mineral type, and the extent of organic matter content in the soil (Viets, 1962; McBride, 1989). Therefore, the crop response to Zn fertilization depends on various soil physicochemical factors and the Zn fertilizer source applied (Boawn, 1973; Westfall et al., 2000). Zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO) are the two most common inorganic Zn sources that are commonly employed for fertilization (Montalvo et al., 2016; Singh and Shivay, 2016). Zinc-ethylenediamine tetra acetic acid (ZnEDTA) is the most common chelated Zn source used for crop production (Singh and Shivay, 2016; Arai and Sparks, 2007). The choice of the Zn fertilizer should depend on the relative agronomic effectiveness of the source applied to a given soil. Metal-chelating agents play an essential role in transporting Zn from solid phases to the surface of plant roots, but chelated Zn is much more expensive than $ZnSO_4$ (Boawn, 1973; Karak et al., 2005; Alloway, 2008b). Several researchers reported the better efficiency of ZnEDTA versus $ZnSO_4$, the availability of chelated Zn is at least double that of $ZnSO_4$.

As discussed above, several researchers reported that some Zn sources are superior to others in alkaline or calcareous soils. These studies give conflicting data regarding the different Zn sources' effectiveness in crop yield and Zn uptake by wheat. For example, some studies performed on wheat plants showed that Zn uptake from the ZnEDTA source was faster compared to $ZnSO_4$ (Zhao et al., 2018), but according to Goos et al. (2000), $ZnSO_4$ showed

better effectiveness than ZnEDTA (soil pH 8.1). However, fewer studies exist on the use of different Zn fertilizers in neutral soils.

When micronutrient fertilizer is added to the soil, as a concentrated product, it alters the chemical composition of the soil solution and modifies the chemical availability of the micronutrient. Over time, zinc gets transformed into less available forms, and therefore availability of Zn to plants reduces (Obrador et al., 2003). Various soil factors such as pH, texture, soil carbonates content- influence the availability of these Zn sources (Adriano, 2001; Alvarez et al., 2001). Sandy soils with low total and plant-available zinc concentrations are highly prone to zinc deficiency. Therefore, it is imperative to study the details of the soil and source parameters controlling Zn behavior in soils.

In addition to this, the fate of Zn in soil depends on the chemical reactions zinc undergoes- adsorption-desorption, precipitation-dissolution, complexation-dissociation (Sparks, 2003). Chemical speciation of Zn in soils depends on the above discussed chemical processes and soil properties, specifically soil pH and Zn concentration (Jacquat et al., 2008; Jacquat et al., 2009). Zinc speciation in the soil is not explicit and needs better elucidation. Speciation determines Zn mobility/bioavailability and can be evaluated directly by spectroscopic techniques like synchrotron-based X-ray absorption spectroscopy (XAS) (Minkina et al., 2019; Sparks, 2013). These techniques can help us to have a better understanding of the solid-phase reaction products formed. Although synchrotron-based techniques can provide direct evidence to complement and understand the results from traditional chemical analyses performed in the lab, the literature available regarding using these advanced techniques to understand fertilizer Zn reactions is scarce.

This study aimed to compare efficiency of four different Zn treatments when applied to neutral soil in plant-less, soil incubation-visualization experiments, and greenhouse experiments using a Zn responsive wheat line. The efficiency for different Zn fertilizers was compared based on (i) Zn mobility, and diffusion (ii) plant Zn uptake and availability, (iii) chemical speciation using X-ray absorption spectroscopy (XAS).

## 4.2 Material and Methods

### 4.2.1 Soil collection and characterization

A Belvue silt loam rarely flooded neutral soil from Ashland Bottoms, KS was used for this study. The surface soil (0 to 15 cm depth) was collected and for basic soil properties, the soil was air-dried and sieved to < 2 mm. Soil pH was determined using a 1:2 soil: Milli Q water extract (Watson and Brown, 1998); extractable P was determined by Olsen method (Olsen et al., 1954); cation exchange capacity was determined by displacement method (Soil Survey Staff, 2011); soil carbonates were determined by Allison and Moodie (1965); and TOC content of the soil was measured by dry combustion method (Wright and Bailey, 2001). The maximum water holding capacity (MWHC) was determined using Jenkinson and Powlson method (1976). Cation exchange capacity, soil carbonates, TOC content determinations were done by the Soil testing laboratory, Kansas State University

**Table 4.1 Initial chemical and physical properties of neutral soil.**

| Parameter | Value |
|---|---|
| pH (1:2 soil: Milli Q water) | 6.7±0.1 |
| CEC*, $cmol_c/kg$ | 6.8 |
| TOC** % | 0.6 |
| Total Zn, mg $kg^{-1}$ | 25.4±0.25 |
| DTPA Zn, mg $kg^{-1}$ | 0.6±0.02 |
| Available P (Olsen) P, mg $kg^{-1}$ | 53.8 |
| Total- N % | 0.08 |
| MWHC*** | 49±0.2 |
| Sand, Silt, and Clay§ | 50.9, 43.6, 5.5 |
| Soil texture | Sandy loam |

*Cation exchange capacity; **Total organic carbon, ***Maximum Water Holding Capacity

### 4.2.2. Experiment set up

The study consisted of two experiments- an incubation and a greenhouse experiment.

The incubation experiment was composed of 42 Petri dishes (53mm diameter and 13.9mm height). The soil was prewetted to 15% of MWHC (Maximum Water Holding Capacity) to pack the soil with a uniform bulk density of 1.1 g $cm^{-3}$.

The soils were brought to 55% MWHC after packing. The edges were wrapped in Parafilm to avoid moisture loss, and dishes were inverted and allowed to equilibrate at room temperature (≈ 24°C) for at least 24 hours. The next day, treatments were introduced at the center of the petri dish. Treatments were added at a rate of 2.43 mg Zn/Petri dish. The six treatments replicated seven times consisted of Control (No-Zn), ZnO, $ZnSO_4$, zinc mix (mixture of 60%

ZnO, 25% $ZnSO_4$, and ratio of $ZnSO_4$ to EDTA is 8:1), EDTA (No-Zn + EDTA), and ZnEDTA. Three replicates were set up for visualization studies, three replicates were set up for the end of study analyses, and one replicate was set up for synchrotron analysis. Following treatment introduction, Parafilm was again used to seal Petri dishes to mitigate moisture loss. The dishes were wrapped in aluminum foil to prevent light exposure and incubated for five weeks at 25°C. Following incubation, soil sectioning was done for four concentric circular sections with radii of 0-4 mm (section 1), 4-8 mm (section 2), 8-17 mm (section 3), and 17 mm- 26.5 mm (section 4). The sections were then dried at 40°C (Fisher Scientific Drying Oven, Waltham, MA), weighed, and finely ground with a mortar and pestle.

As an ongoing study analysis, Visualization was done at days 1, 7, 21, and 35. Visualization using Degryse et al. (2014) method. By this method, Zn movement was tracked using calcium carbonate ($CaCO_3$) impregnated filter papers. Calcium carbonate sorbs Zn via surface complexation and precipitation. Calcium impregnated papers were prepared using Whatman filter paper no. 1. Filter papers were cut to the exact diameter of the Petri dish. Papers were soaked in 1 M $CaCl_2$ for at least 30 minutes (min) and then transferred to 0.4 M $Na_2CO_3$ for about 10 min, resulting in precipitation of $CaCO_3$ in the paper. The papers are rinsed with DI water, dried, and stored for later use. The deployment of papers was done for 2-hour for contact times between soil and fertilizer up to 1 day, 3-hours for contact times up to 1 week, 4-hours deployment time when the fertilizer has been in contact with the soil for more than one week (day- 21, and 35 for present study). Then, for color development, dithizone dye was prepared. The stock solution was prepared by dissolving 50 mg of dithizone in 0.5 mL of ethanol + 0.1 mL of concentrated ammonia and diluting to 10 mL with deionized water. A pink color appeared in

the high-Zn zone; full-color development took about 20 min. In the end, filter papers were scanned, and the size of the pink zone was quantified using imaging software (GIMP, 2.10.20).

For end of study analyses, soil samples were analyzed for pH (1:10 soil: Milli Q water), water-extractable Zn (1:10 soil: Milli Q water), and total Zn concentration (determined using the aqua regia. For total Zn, 0.5 g of soil digested using 5 mL of aqua regia solution (1:3 v/v $HNO_3$/HCl). Samples were pretreated with an acid mixture and then were allowed to react overnight at room temperature. The soils were digested at the temperature of 75°C for 30 minutes (min), 100°C for 30 min, 110°C for 30 min, and 140°C until the acid volume decreased to ≈ 1.0 mL. The digested samples were cooled, diluted with 25 mL of 0.1% $HNO_3$, and filtered through a Whatman filter paper no. 42 (Zarcinas, 1996). One blank and one sample of standard reference soil material (NIST 2711a - Montana soil) was included as a QA/QC (recoveries about 100±20%) control in each digestion batch. The analysis was done using the inductively coupled plasma emission spectrometry (ICP-OES, Varian 720-ES, Santa Clara, CA)

### 4.2.3. Synchrotron analysis

The samples from the first and second sections of the incubation study were chosen for this study. Soils were prepared by grinding them to a fine powder using an agate mortar and pestle to mix the samples homogeneously for respective sections of the treatments. Soil samples were packed in sample holders with 2 mm thick Plexiglas slits (Fig S4.1). Both sides of the sample holders were sealed with Kapton tape (Bertech, Torrance, CA), as shown. The slit allows the X-ray beam to pass through the sample that was sandwiched in between the two pieces of Kapton tape. Zinc X-ray absorption near-edge spectroscopy (XANES) and spectra were collected at the beamline 5 BM-D (DuPont-Northwestern-Dow Collaborative Access Team, DND-CAT) at the Advanced Photon Source (APS), Argonne National Laboratory, in Argonne, IL. Data were

collected at Zn (≈ 9458 to 10210 eV) in fluorescence mode. Two Al foil layers were installed on the detector to decrease undesired fluorescence signals, especially to suppress the soil Fe signal to minimize any interference from Fe. The angle of the incident X-ray beam was at 45° concerning the sample surface. The energy was calibrated using standard Zn-metal foil. The number of spectra collected per sample was 3 to 10 (mostly 6). Data processing and analysis were done using Athena software version 0.9.26 (Ravel, 2018). This software was used to complete background correction and linear combination fitting of the reported spectra using previously collected standards, according to Werner and Prietzel (2015). Three or more scans were merged to limit noise, and samples were normalized using Athena software. Ten reference spectra were used for linear combination fitting: ferrihydrite ($(Fe^{3+})_2O_3.0.5H_2O$) adsorbed Zn, franklinite ($ZnFe^{3+}{}_2O_4$), $ZnSO_4$, ZnEDTA, ZnO, hopeite ($Zn_3(PO_4)_2.4H_2O$), willemite ($Zn_2SiO_4$), smithsonite ($ZnCO_3$), zinc hydroxide ($Zn(OH)_2$), Zn-Al nitrate layered double hydroxides (ZnAlDHnitrate), gahnite ($ZnAl_2O_4$), hydrozincite ($Zn_5(CO_3)_2(OH)_6$), zincite (ZnO), and hemimorphite ($H_6O_9Si_2Zn_4$),

### 4.2.4. Greenhouse experiment

The greenhouse study experiment was carried out in April 2020 in the Throckmorton Plant Sciences greenhouse at Kansas State University, Manhattan, KS (39°12'N, 96°35'W, 325 m above sea level) by using acrylic containers (23.9 cm length and 11.9 cm height) (Fig S4.2). A screen was mounted to the slits provided, separating the containers into two small compartments. The screen was 400-mesh, and it was designed to avoid the movement of plant roots into the fertilizer treatment compartment.

Wheat (*Triticum aesitvum* L.) seeds were a high-Zn spring wheat line (BW 53856, BW 53856 GRIN-Global (cimmyt.org), developed under the Harvest Plus initiative by Dr. Velu

Govindan (CIMMYT). Seeds were provided by Dr. Mary Guttieri (USDA-ARS) from a Yuma, AZ seed increase, with permission of Dr. Govindan. Seeds were kept at 4° (seeds were planted into germination trays containing a mixture of Ashland bottoms neutral soil and sand (1:2) in a vernalization chamber for three weeks at 4°C).

Before soil packing, the basal dose of nitrogen (N), phosphorous (P), and potassium (K) were given. Nitrogen was added as urea at a rate of 120 kg $ha^{-1}$. Phosphorus was added as potassium dihydrogen phosphate ($KH_2PO_4$) at a rate of 40 kg $ha^{-1,}$ and K was added through $KH_2PO_4$ fertilizer based on the P rate.

Soil packing- The soil was prewetted to 15% of MWHC and packed with the uniform bulk density of 1.1 g $cm^{-3}$, with the aid of a soil press. After packing, the soils were brought to 60% MWHC. The containers were wrapped with polythene sheets and were allowed to equilibrate for at least 24 hours. The next day, 21 days old uniform seedlings were selected and transplanted in the middle of the container (one seedling/container) on one side of the screen (opposite of the treatment addition) and parallel to the treatment introduction point.

The next day after transplantation of the seedlings, treatments were introduced on one side of the screen (1 cm away from the screen and at 1.25 cm depth). The following treatments were used: (1) Control; (2) ZnO; (3) $ZnSO_4$; (4) EDTA; (5) Zn mix (mixture containing 60% ZnO, 25% $ZnSO_4$, and EDTA to $ZnSO_4$ 1:8 ratio); (6) ZnEDTA. Zinc was added at a rate of 10 kg $ha^{-1}$. The experimental design was a randomized complete block design with three replications.

To ensure good growth, containers were weighed, and their weight was subtracted from the weight taken on the first day of the study after bringing them to moisture content of 60% of MWHC. The difference in weight is weight lost and plants were watered with DI water every

day (amount of water= amount of water lost) to all containers throughout the experiment period. The greenhouse temperature was maintained within the range of 20°C to 25°C throughout the experiment. A 12 h daylight and 12 h darkness photoperiod regime was maintained with overhead sodium lights for all five weeks until harvesting. The pots were rotated weekly to ensure that all pots received equal amounts of light.

Soil water samples were collected at the end of the third and fifth week of the greenhouse study using rhizon samplers (Rhizon MOM soil moisture sampler, 19.21.22, Rhizosphere research products, Wageningen, Netherlands). Rhizon samplers were inserted close to the plant roots. Soil water sample was collected directly into the vacutainers. Acidification of soil samples was done by adding one drop of concentrated nitric acid and stored in a refrigerator at 4°C until analysis. Soil water samples were analyzed for Zn using an inductively coupled plasma-mass spectrometry (ICP-MS, 7500 CX, Agilent, Santa Clara, CA, USA). Standard water reference (Trace Elements in Natural Water, SRM1643f) from the National Institute of Science and Technology (NIST, Gaithersburg, MD) was used as part of the quality assurance quality control (QA/QC) protocol (recoveries were within 100 ± 20%).

Processing plant samples- The greenhouse study was five weeks long, and aboveground biomass was harvested at the end of the fifth week of the study using a stainless-steel knife. The harvested plant samples were cleaned with tap water, with deionized water (DI) water, with 5 g $kg^{-1}$ sodium lauryl sulfate [$CH_3(CH_2)_{10}CH_2OSO_3Na$] solution, and again with DI water (Attanayake et al., 2013). Fresh sample weight was recorded. Plant samples were dried at 68ºC for about four days, until they reached a constant weight. Dried sample weights were recorded. Dried plant materials were ground using a Willey Mini Mill-Arthur Thomas-type grinder

(Thomas Scientific) using a sieve size of 250 mm (60 mesh). Dried and ground samples were stored, doubling the small zip lock bags.

Chemical analysis- Ground sub-samples (0.5 g) of aboveground biomass were digested with 10 mL of trace metal grade nitric acid ($HNO_3$) in Pyrex digestion tubes. The next day, samples were heated at 125°C for 4 hours in digestion block, cooled, and the volume was brought up to 10mL mark using Milli Q water. Samples were filtered using Whatman filter no. 2. Standard plant reference material (apple leaves) from the National Institute of Science and Technology (NIST, Gaithersburg, MD) was also digested and analyzed alongside the plant materials as part of the quality assurance quality control (QA/QC) protocol (accuracies within 100 ± 20%). Reagent blanks, internal standards, and spiked digestions were also included for QA/QC. Samples were stored at 4° C until analysis. Samples were analyzed for Zn using an inductively coupled plasma-mass spectrometry (ICP-MS, 7500 CX, Agilent, Santa Clara, CA, USA).

Zinc uptake was calculated as follows:

Zn uptake (g/pot) = (Zn concentration of plant tissue * dry weight of biomass)

Processing of soil samples-

At the end of the greenhouse study, soil in the container was sectioned into eight sections and considering screen as 0 cm, labeling was done as: 7.25-2cm (section 1), 2-1 cm (section 2), 1-0 cm (section 3), 0-1 cm (section 4), 1-2 cm (section 5), 2-3 cm (section 6), 3-6 cm (section 7), 6-14.52 cm (section 8) (Figure S4.3), air-dried and sieved through 2-mm sieve. Samples were analyzed for pH (1:10 soil: Milli Q water), the total elemental concentration in the soil was determined using aqua regia (Zarcinas, 1996), extractable Zn was determined using diethylene-triamine Penta acetic acid. Ten grams of ground, air-dried, passed through 1.0 mm stainless steel

sieve soil sample was taken in 125 mL conical flask, 20 mL of DTPA- triethanolamine (DTPA-TEA) extraction solution was added, each flask was covered with parafilm, and was shaken using a horizontal shaker with 8.0 cm of stroke length and 120 cycles $min^{-1}$ for 2 hours (Lindsay and Norvell, 1978). Suspensions were filtered through Whatman filter paper no. 42, and samples were analyzed for Zn using the ICP-OES.

### 4.2.5 Statistical Analysis

Statistical analysis of all data was done using SAS version 9.4 (SAS Institute Inc., Cary, NC, 2020) One-way ANOVA using PROC GLM was used to analyze soil data. The data analysis was performed as a split-plot completely randomized design. The main plot was Zn fertilizer treatment, and the sub plot factor was soil section. Pairwise comparison was done using LSD at $\alpha \leq 0.05$. One-way ANOVA using PROC GLIMMIX was used to analyze plant data and pairwise comparison was done at $\alpha \leq 0.05$ using LS means.

ANOVA using PROC MIXED was performed to evaluate Type III tests of fixed effects (treatment and time) on visualization and soil water Zn data. The data analysis was performed as a split-plot completely randomized or completely randomized block design with repeated measures, respectively, for visualization and soil water data. The main plot factor was the treatment, and the subplot factor was time. Pairwise comparison was done at $\alpha \leq 0.05$ using Tukey test.

## 4.3. Results and discussions

### 4.3.1. Incubation study

#### 4.3.1.1. Visualization

Visualization was done for days 1, 14, 21, and 35. The results showed that Zn diffusion for ZnEDTA and $ZnSO_4$ treatments were similar (Figure S4.4 and 4.1). In these treatments, Zn diffused to farther sections away from the point of application than ZnO and Zn mix treatments. Interestingly, a significant difference between ZnO and Zn mix treatments was observed at day 14; Zn distribution was greater for Zn mix compared to ZnO. This can be due to the influence of other constituents present in Zn mix (60% ZnO, 25% $ZnSO_4$, and EDTA to $ZnSO_4$, 1:8), but it might have undergone sorption over time and there were no significant differences for day 21 and 35. High Zn diffusion for $ZnSO_4$ was not surprising due to its high solubility and relative low sorption in this soil. Zinc-EDTA tended to stay in complex form and thereby avoided various adsorption and precipitation reactions that lead to Zn loss (Rico et al., 1996; Alloway, 2009). This was confirmed by water-extractable results as well (Figure 4.3). Greater water extractability was observed in the case of $ZnSO_4$ and ZnEDTA compared to ZnO and Zn mix in the farther sections of the Petri dish. Lower Zn diffusion in ZnO and Zn mix was also confirmed by percent Zn recovered (Figure 4.4); most of the added Zn remained in the application section only (section 1). Less Zn diffusion for these treatments can also be due to the greater resultant pH observed for ZnO and Zn mix treatments (Figure 4.2) and, therefore, more Zn adsorption. Zinc adsorption increases with an increase in soil pH (Alloway, 2009; Rashid and Ryan, 2004). No-Zn was added in control and EDTA treatments; a slight pink color observed can be due to adsorption of native soil Zn on calcium impregnated filter papers. Therefore, Zn diffusion accessed by Visualization was in order ZnEDTA≈ $ZnSO_4$> ZnO≈ Zn mix> control≈ EDTA (Figure 4.1).

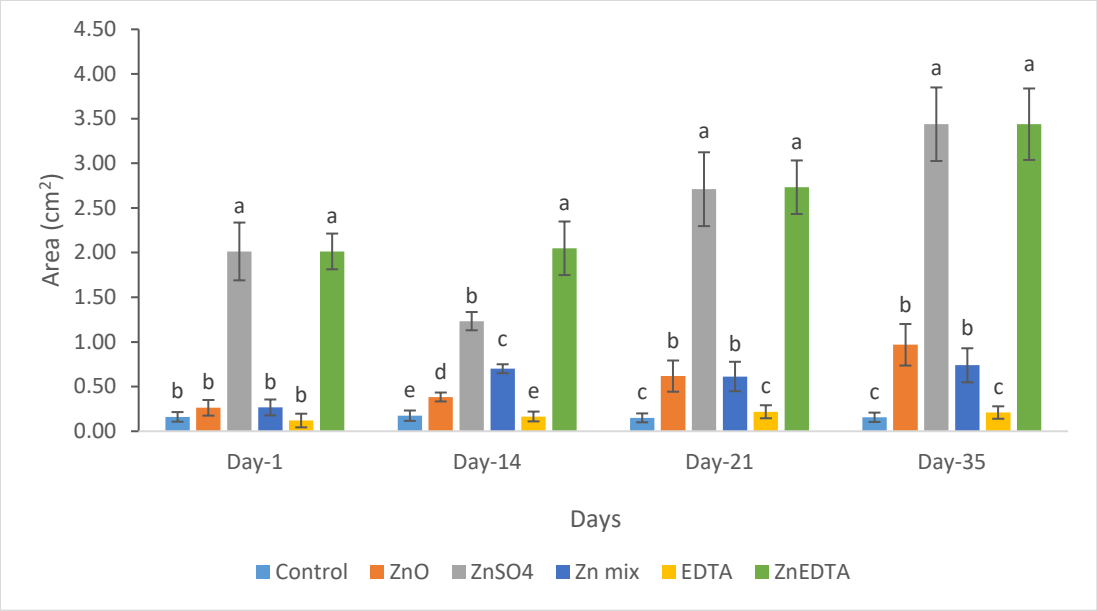


**Figure 4.1 Calculated area of the pink zone using GIMP software for Zn only incubation study at day 1, 7, 21, and 35. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; EDTA= ethylenediamine tetra acetic acid with no-Zn; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a time period containing the same letter are not significantly different at $P \leq 0.05$ according to Tukey pairwise method. Error bars represent the standard error of three replicates.**

4.3.1.2. Soil pH

Cation exchange capacity of this soil was low, i.e., 6.75 $cmol_+ kg^{-1}$, together with low clay content, led to low Zn adsorption, and low pH buffering (Alloway, 2009). The resultant soil pH for ZnO and Zn mix treatments was greater than the control treatment for section 1 (application point) (Figure 4.2).

The reason behind the increase in soil pH for Zn mix (60% ZnO) treatment is that the dissociation of ZnO releases $OH^-$ ions, increasing soil pH (Su et al., 2019).

$ZnO + H_2O \rightarrow Zn^{2+} + OH^-$

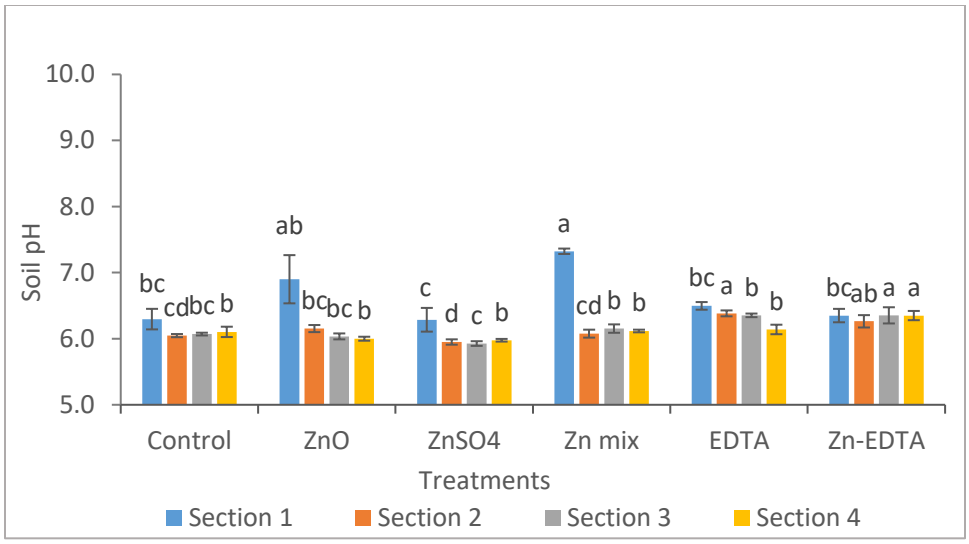


**Figure 4.2 Soil pH for incubation study. Control= no zinc; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix (mixture of ZnO, $ZnSO_4$, and ZnEDTA); EDTA= ethylenediamine tetra acetic acid with no-Zn; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

Although not significant, pH for the first section for $ZnSO_4$ treatment was lower than control. This slight decrease in pH can be explained by when $ZnSO_4$ comes in contact with water, its hydrolysis occurs and forms sulfuric acid and zinc hydroxide. Sulfuric acid is a strong acid, and zinc hydroxide is a weak base, making $ZnSO_4$ acidic.

$ZnSO_4 + H_2O \rightarrow H_2SO_4 + Zn(OH)_2$

#### 4.3.1.3. Water extractable Zn

Water-extractable Zn was significantly greater for ZnO and Zn mix treatments than ZnEDTA for section-1 (POA), indicating that even though ZnO and Zn mix treatments did not move farther, they remained water-extractable (Figure 4.3).

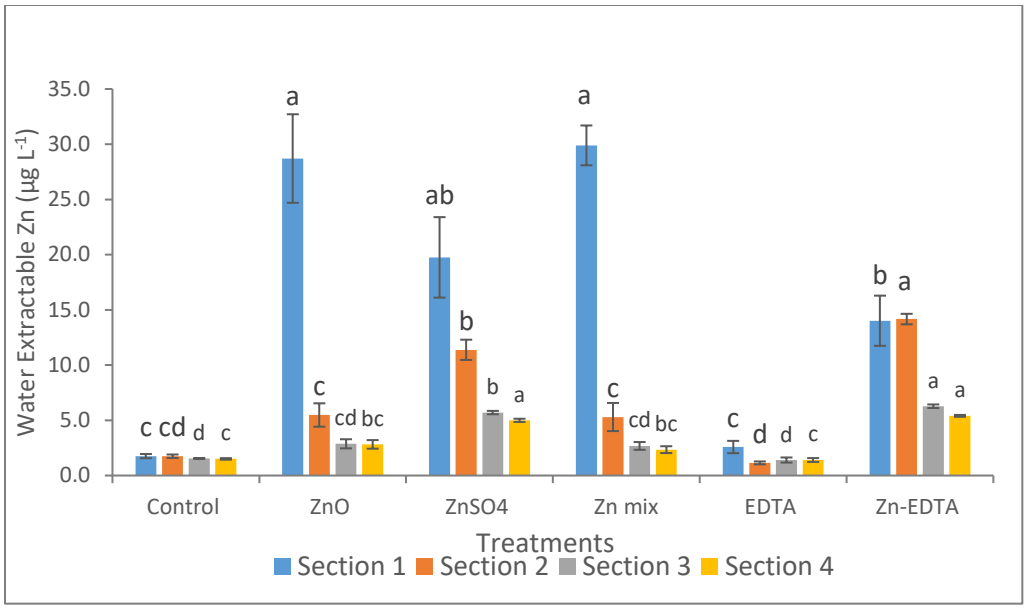


**Figure 4.3 Water extractable zinc for incubation study. Control= no zinc; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix (mixture of ZnO, $ZnSO_4$, and ZnEDTA); EDTA= Ethylenediamine tetra acetic acid with no-Zn; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

Although their proportion, i.e., 85-90% of the Zn added, remained in the first section was high, water extractable Zn was low because of the inherent lower water solubility for these treatments, water extractable Zn for ZnO and Zn mix 'was not significantly different form $ZnSO_4$ treatment. The treatments $ZnSO_4$ and ZnEDTA are highly water-soluble Zn sources, and therefore they remained water-extractable even in the sections away from the point of application. Their solubility can also be explained by their diffusion/movement to other sections, as observed in visualization studies (section 4.4.1.1). Comparing all the treatments, ZnEDTA showed a significantly greater water extractability than other treatments for the third, fourth, and fifth sections. Zinc-EDTA can increase soil Zn concentrations in solution by converting solid-phase labile Zn into soluble Zn or preventing irreversible fixation of Zn into the solid phase (Hamza and Sadnandan, 2005; Alvarez et al., 2006).

#### 4.3.1.4. Zinc diffusion

For percent (%) total Zn, a significant part of the applied Zn remained in the application section (Figure 4.4). More than 80% of the added Zn for ZnO (87.13±0.08%) and Zn mix (89.57±0.24%) treatments recovered only from section- 1 (application point), implying accumulation of Zn in section- 1. Neither of the ZnO and Zn mix treatments resulted in sufficient Zn mobility to distribute Zn throughout the container and thereby did not transport it to the sections away from the point of application. This response was due to the retention of Zn by the solid phase of soil. On the other hand, percent Zn recovered for $ZnSO_4$ (47.50±0.64%), and ZnEDTA (≈ 33.7±0.76%) was significantly lower, which signifies greater Zn mobility for these treatments than ZnO and Zn mix. The product with the most increased Zn mobility through this neutral soil is ZnEDTA, the chelated Zn source used. For ZnEDTA treatment, a significantly greater percentage of Zn was recovered from sections second, third, and fourth section as well, signifying Zn movement into these sections in this soil. Lesser Zn diffusion in other Zn treatments is because of slow dissolution, the retention of Zn by soil components, or its loss by precipitation. Several authors have noted the greater migration capacity of ZnEDTA fertilizer in different soils than other inorganic Zn sources such as ZnO and $ZnSO_4$. Increased Zn mobility in ZnEDTA is by reducing the reaction of Zn with soil colloids (Mikkelsen and Brandon, 1975; Kuo and Mikkelesen, 1979; Alvarez and Gonzalez, 2006).

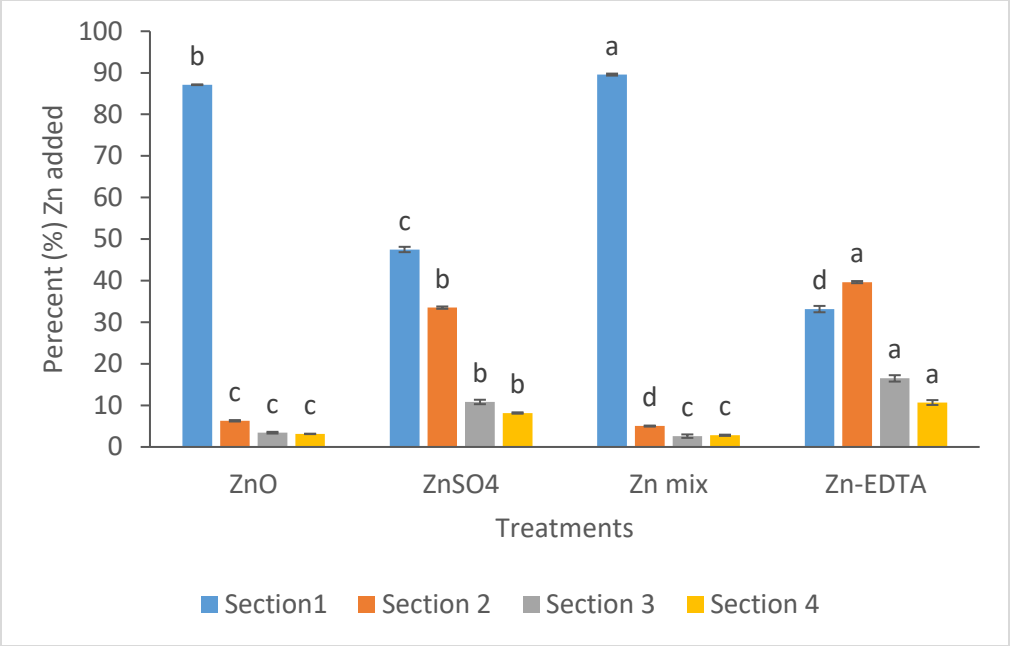


**Figure 4.4 Percent (%) Zn added recovered in respective soil sections and treatments. Control= no zinc; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix (mixture of ZnO, $ZnSO_4$, and ZnEDTA); EDTA= Ethylenediamine tetra acetic acid with no-Zn; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

#### 4.3.1.5. Synchrotron analysis

The XANES technique can be used for the qualitative estimation of the chemical speciation Zn in soil samples. The XANES spectra for several Zn standards help discriminate between the Zn species present in a sample (Figure 4.5). Jacquat et al. (2008) and Jacquat et al. (2009) reported that with increasing soil pH, specific sorption of Zn to organic matter, metal oxides, and carbonates become more relevant. Moreover, the formation of Zn precipitates as zincite (ZnO) and Zn hydroxide ($Zn(OH)_2$) can decrease Zn solubility. In the present study, Zn was observed to be present predominantly as Zn aluminum nitrate double-layered hydroxides (ZnAlDHnitrate) in all other treatments except ZnEDTA (Figure 4.6). In support of this, it has been found that new technogenic phases (Zn-containing phyllosilicates) can also form in slightly

acidic to neutral soil (Juillot et al., 2003; Manceau et al., 2005; Schlegel et al., 2001). In the aluminosilicate-bound (residual) fraction, layered Zn phases were found (Manceau et al., 2003, Manceau et al., 2004; Scheinost et al., 2002), and the mechanism of Zn entering the octahedral structures of layered minerals had been determined. If true for present study, this could be the major factor responsible for less mobility of Zn in this soil. Zinc was also observed to be present as $ZnSO_4$ in all treatments. This can be either due to $ZnSO_4$ as indicated by enhanced Zn solubility or some other missing standard for Zn species, which closely resembles the $ZnSO_4$ spectrum. For treatments ZnO and Zn mix treatments, Zn was present as ZnO as dominant species. In ZnO, treatment was best fitted with a mixture of ZnAlDHnitrate, ZnO, and Gahnite, whereas ZnAlDHnitrate, ZnO, and $ZnSO_4$ for Zn mix treatment (Figure 4.6). Zinc present as ZnO species was 61.4% for section 1, and 41.6% for section- 2 for ZnO treatment. Similarly, Zn present as ZnO species was 66.2% for section 1, and 45.8% of section 2 for Zn mix. These results help to understand low Zn mobility observed for these treatments in the visualization study (section 4.4.1.1) and fertilizer Zn distribution results (section 4.4.1.4). Zinc remaining as ZnO can also be correlated to low solubility for these treatments as observed in water-extractable Zn results (section 4.4.1.3). For both sections of $ZnSO_4$, Zn was present as $ZnSO_4$, ZnALDHnitrate, and ferrihydrite adsorbed Zn. Ferrihydrite adsorbed Zn represented the $Zn^{2+}$ adsorbed onto Fe oxides. Zinc sulfate dissociates to give $Zn^{2+}$ ions. The translocation of the Zn cation can result in the formation Zn inner-sphere adsorption complexes with iron (Fe) oxides.

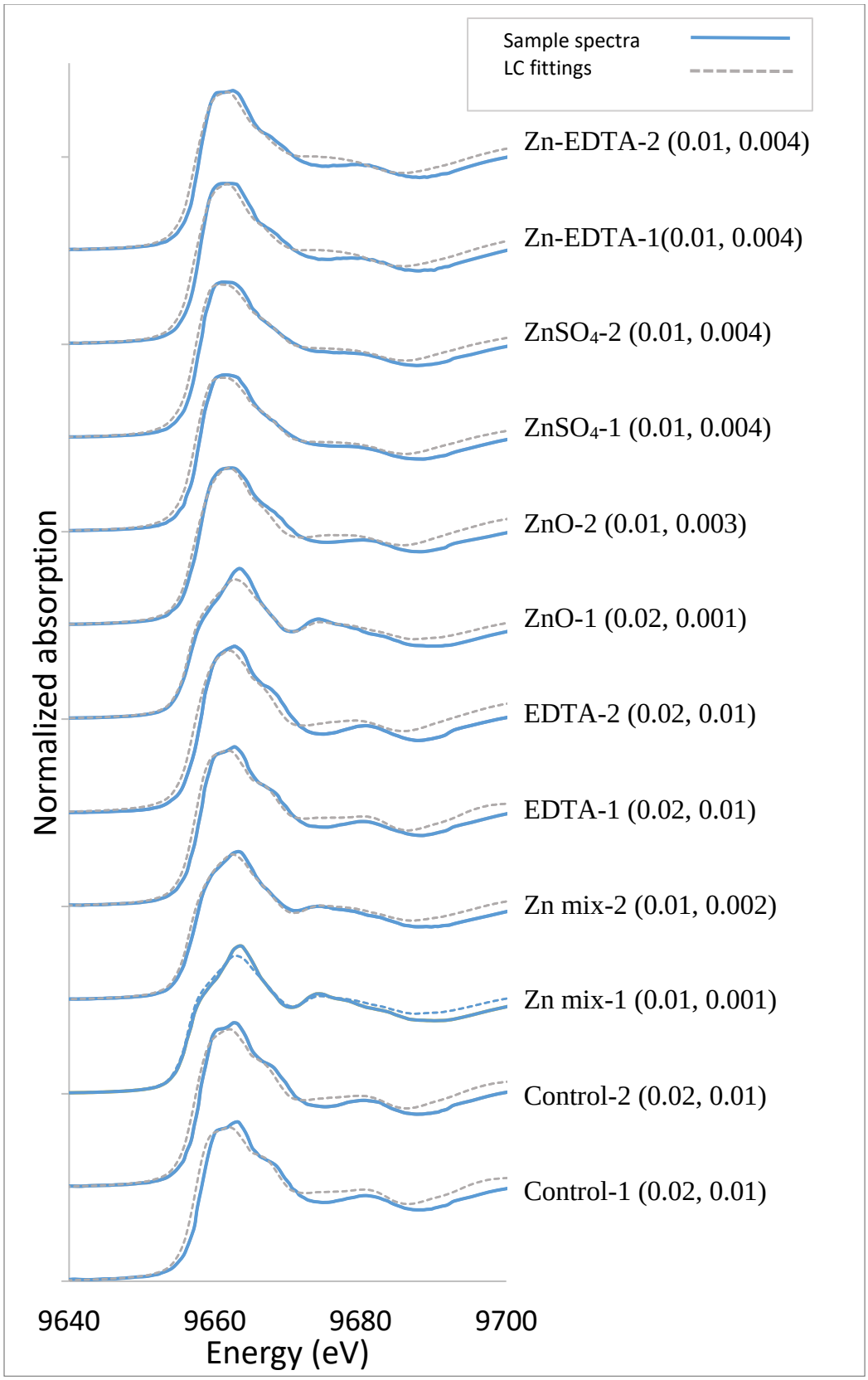
Sample spectra
LC fittings
Zn-EDTA-2 (0.01, 0.004)
Zn-EDTA-1(0.01, 0.004)
ZnSO4-2 (0.01, 0.004)
ZnSO4-1 (0.01, 0.004)
ZnO-2 (0.01, 0.003)
ZnO-1 (0.02, 0.001)
EDTA-2 (0.02, 0.01)
EDTA-1 (0.02, 0.01)
Zn mix-2 (0.01, 0.002)
Zn mix-1 (0.01, 0.001)
Control-2 (0.02, 0.01)
Control-1 (0.02, 0.01)
Normalized absorption
9640
9660
9680
9700
Energy (eV)

**Figure 4.5 Normalized X-ray absorption fine structure (XANES) Zn absorbance spectra of the selected rate of treatments after 35 days. Treatment (x, y) = Treatment (x=R factor, y= reduced chi square). Control-1 = control section 1; Control-2 = control section 2; ZnO-1= zinc oxide section 1; ZnO-2= zinc oxide section 2; $ZnSO_4$-1= zinc sulfate section 1; $ZnSO_4$-2= zinc sulfate section 2; Zn mix-1= zinc mix section 1; Zn mix-2= zinc mix section 2; ZnP-1= zinc phosphate section 1; ZnP-2= zinc phosphate section 2; ZnEDTA-1= zinc-ethylenediamine tetra acetic acid section 1; ZnEDTA-2= zinc-ethylenediamine tetra acetic acid section 2.**

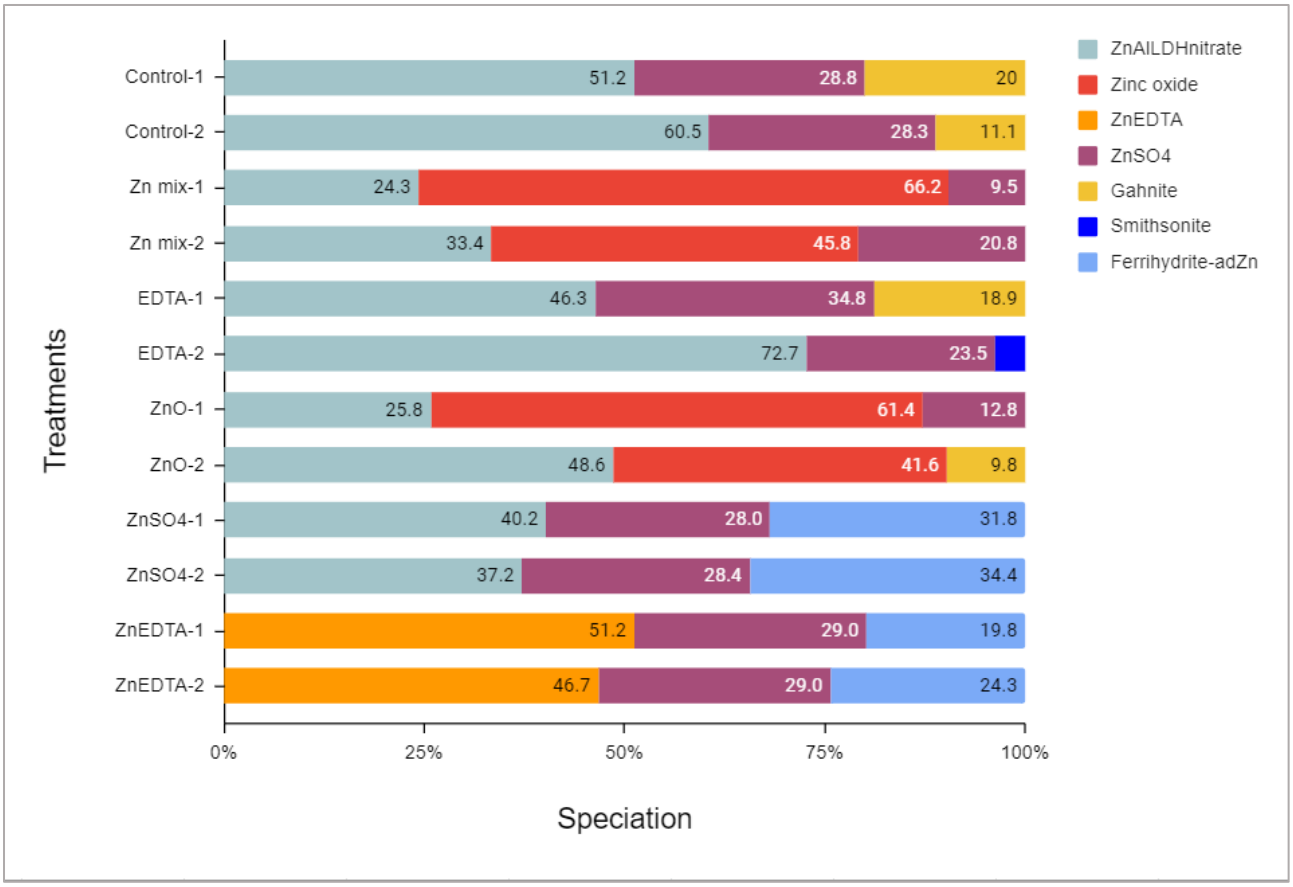


**Figure 4.6 Relative proportions of Zn species as estimated by linear combination fittings on XANES spectra. Control-1 = control section 1; Control-2 = control section 2; ZnO-1= zinc oxide section 1; ZnO-2= zinc oxide section 2; $ZnSO_4$-1= zinc sulfate section 1; $ZnSO_4$-2= zinc sulfate section 2; Zn mix-1= zinc mix section 1; Zn mix-2= zinc mix section 2; ZnP-1= zinc phosphate section 1; ZnP-2= zinc phosphate section 2; ZnEDTA-1= zinc-ethylenediamine tetra acetic acid section 1; ZnEDTA-2= zinc-ethylenediamine tetra acetic acid section 2. ZnALDHnitrate = Zinc aluminum double layered hyrdoxides nitrate; ZnEDTA = Zinc-ethylenediamine tetra acetic acid; ZnSO4 = Zinc sulfate; Gahnite = Zinc and aluminum oxide, $ZnAl_2O_4$; Smithsonite = Zinc carbonate; Ferrihydrite-adZn = Ferrihydrite adsorbed zinc, $((Fe^{3+})_2O_3.0.5H_2O$ adsorbed zinc.**

According to the available literature, several inorganic surface functional groups occur at the surfaces of secondary minerals such as oxides and oxyhydroxides of Fe and Mn (Sparks, 2003). Viets (1962) and Hodgson (1963) also reported that Zn in non-calcareous soils exists as complexed with organic matter, inorganically precipitated with iron or manganese oxide/hydroxide, and entrapped in primary and secondary minerals (Viets, 1962; Hodgson,

1963).For ZnEDTA, the dominant Zn species was ZnEDTA which supports the fact that ZnEDTA is a highly stable complex with very minimal or no interactions with soil components. However, another non-dominant Zn species in ZnEDTA that was observed was ferrihydrite adsorbed Zn. Zinc as ZnEDTA was 51.2% for section- 1, and 46.7% for section- 2. This explains the high Zn mobility and solubility as observed in visualization, water extractable Zn, soil total Zn results (section 4.4.1.1., 4.4.1.3, 4.4.14, respectively).

Applying the X-ray absorption spectroscopy (XANES in our case) in combination with chemical analyses in soil research can provide insight into interactions between the added Zn and soil components, and thus helping to understand fertilizer Zn reaction pathways in soils. The coupled application of conventional chemical techniques and modern advanced methods significantly widen the understanding of the chemical transformations of Zn added to the soils. It is an effective tool to check and confirm the various properties of Zn fertilizers and the reactions they undergo upon their addition to the soil.

### 4.3.2. Greenhouse experiment

#### 4.3.2.1 Plant biomass and Zn uptake

Only $ZnSO_4$ treatment showed a significantly greater biomass than control and EDTA treatments (Fig 4.7). No significant differences could be seen among all Zn treatments. The plant Zn uptake results indicated that Zn uptake by the wheat plants was driven by added Zn irrespective of the source applied. Zinc oxide and zinc mix treatments were also equally effective. The results of the present research support previous observations that Zn applied as a chelate such as ZnEDTA is much effective in terms of Zn accumulation by plants.

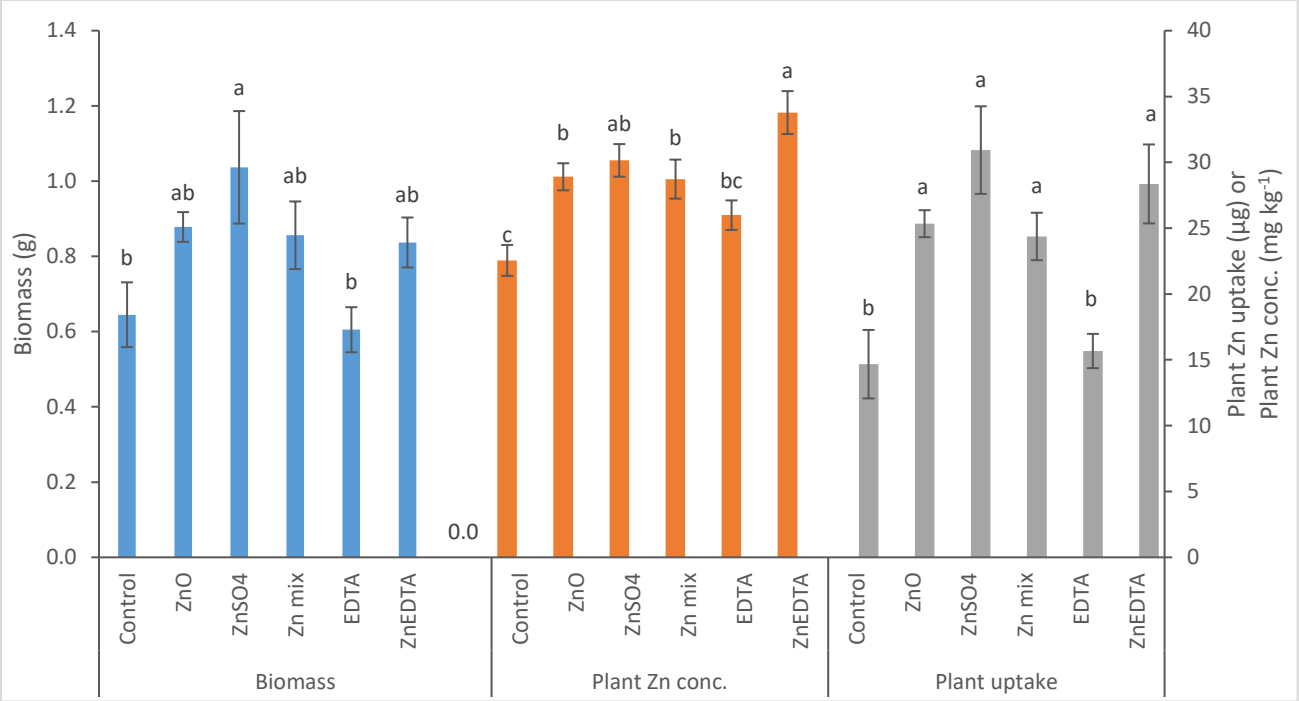


**Figure 4.7 Plant Zn uptake, plant Zn concentration, and biomass results from greenhouse study. Plant biomass is plotted against the primary axis, and plant Zn uptake and concentration are plotted against the secondary axis. Control= no zinc; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix (mixture of ZnO, $ZnSO_4$, and ZnEDTA); EDTA= Ethylenediamine tetra acetic acid with no-Zn; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means within response variable are not significantly different at $P \leq 0.05$ according to LS-means. Error bars represent the standard error of three replicates.**

For plant Zn concentration, Zn concentration for ZnEDTA was significantly greater from ZnO, Zn mix, EDTA, and control treatment (Figure 4.4). Although not significant, Zn concentration for ZnEDTA was greater than $ZnSO_4$ as well. Zinc availability for $ZnSO_4$ seems to be mainly driven by high water solubility of $ZnSO_4$.The plant Zn concentration was driven by water-soluble plus exchangeable fraction in neutral soil. Consequently, the effectiveness of Zn sources applied depends on their capacity to maintain the Zn in a labile form that plants can easily uptake. Synchrotron X-ray XANES data showed that when Zn−EDTA was applied, a major part of it remained as ZnEDTA most likely due to the greater stability of this complex in this soil, explaining that Zn remained more labile or plant available form (Fig 4.6). Therefore,

the improved response of the wheat, concerning Zn concentration, to the treatment with ZnEDTA might be attributed to the stability of the ZnEDTA molecule in neutral soil and the ability of this fertilizer source to provide greater Zn distribution within the soil (Figure S4.7)

#### 4.3.2.2. Soil water Zn

A significantly greater amount of soil water Zn was observed for ZnEDTA compared to all other treatments for both samples collected at the end of the third and fifth week (Figure 4.8). This response is attributed to ZnEDTA can increase soil Zn concentrations in solution by preventing irreversible fixation of Zn into the solid phase or converting solid-phase labile Zn into soluble Zn (Hamza and Sadnandan, 2005; Gupta and Deb, 1984; Parsad et al., 1975; Alvarez et al., 2006). The decrease in soil water Zn with time (three weeks versus five weeks) could be because of either Zn uptake by wheat plants or Zn losses due to adsorption and precipitation reactions in the soil. The neutral pH-induced low solubility to metals probably played a role in retaining metals in the soil layers (Alvarez and Gonzales, 2006). This was also confirmed by XANES analysis, as discussed in section 4.4.1.5.

#### 4.3.2.3. Soil pH

The soil pH was similar for sections away from the point of application. So, only two sections are shown (Figure S4.5). Complementing results from incubation study, significantly greater soil pH was seen for ZnO and Zn mix for the section of Zn application as compared to other Zn treatments but not significantly different from control. Reasons for high pH for ZnO and Zn mix treatments are explained in section 4.4.1.2.

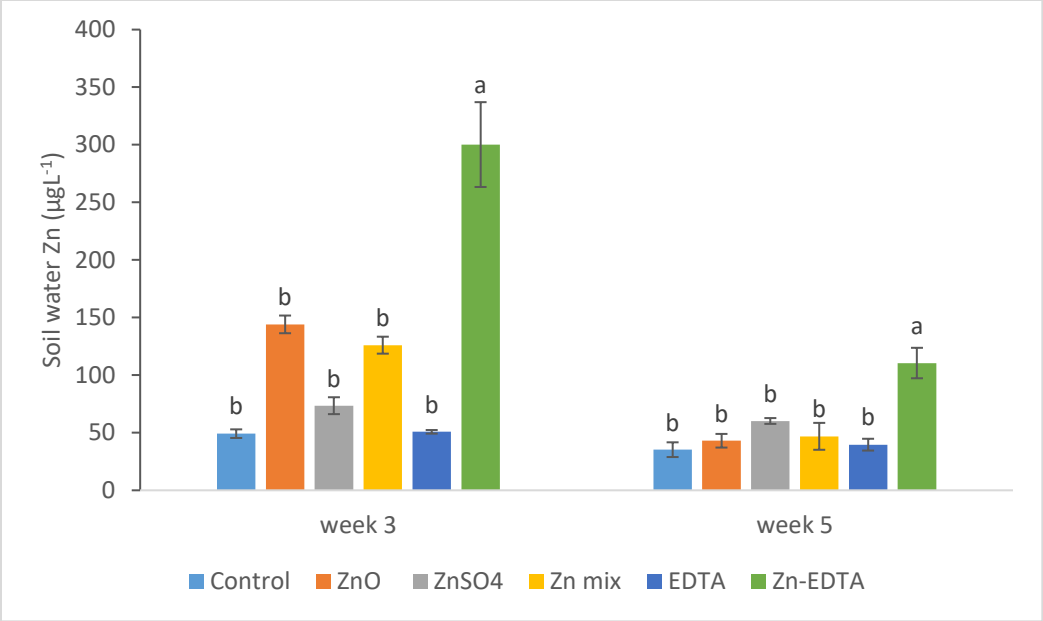


**Figure 4.8 Soil water zinc collected at the end of third and fifth week of the greenhouse study. Control= no zinc; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix (mixture of ZnO, $ZnSO_4$, and ZnEDTA); EDTA= Ethylenediamine tetra acetic acid with no-Zn; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a time period containing the same letter are not significantly different at $P \leq 0.05$ according to Tukey pairwise method. Error bars represent the standard error of three replicates.**

.

#### 4.3.2.4. Zinc diffusion

More than 80% of the added Zn for ZnO (84.4±6.2%) and Zn mix (85.19±2.6%) treatment recovered in the point of application, referring to very low Zn diffusion for these treatments (Figure S4.6 and S4.7). This response was due to slow dissolution and the retention of Zn by the solid phase of soil. The percent Zn recovered for $ZnSO_4$ (70.1±6.2%), and ZnEDTA (47.6±3.7%) was significantly lower for the application section, which signifies greater Zn mobility for these treatments. The product with the most increased Zn mobility for this neutral soil was ZnEDTA, a chelated Zn source. For ZnEDTA treatment, a significantly greater percentage of Zn was recovered from sections- third, fourth, and fifth. The reasons for this response are explained in detail in section 4.4.14. Another noteworthy observation was significantly greater soil total Zn observed for $ZnSO_4$ and Zn mix treatments than control, ZnO,

and EDTA for section 3. Adriano (2001) reported that in neutral pH soil, metals are retained in the soil layers, and this has probably played a major role in inducing low solubility of metals in soil. This was also confirmed by XANES results (section 4.4.1.5). Zinc presents predominantly as ZnAlLDnitrate hinted to its retention in the soil layer and therefore reduced diffusion and mobility.

#### 4.3.2.5. Extractable Zn

Average concentrations of labile Zn fraction measured by this method varied with the source of Zn applied. More than 80% of the DTPA extractable Zn was contained in the section of the application for ZnO and Zn mix treatments (section 2) (Figure S4.8). Even though more than 80% of the added Zn recovered from section 2, DTPA-Zn for ZnO and Zn mix was still not significantly different from ZnEDTA treatment for the same section (Figure S4.6). The lower DTPA ext. Zn in ZnO and Zn mix can be because of low solubility and the greater resultant soil pH upon their addition. Stahl and James (1991) reported that Zn activity decreases gradually with an increase in soil pH. McBride and Blasiak (1979**)** observed that Zn concentrations were reduced by 97% for each unit increase in soil pH between 5 and 7. Sachdev et al. (1992) observed a 99% decrease in $Zn^{2+}$ activity for each unit increase in pH. In the present study, available Zn, this Zn fraction diminished as we move away from the application point. Extractable Zn was significantly greater for ZnEDTA treatment for sections third, fourth, fifth, and sixth. The decreasing level of DTPA extractable Zn for ZnEDTA for father sections can be because of the plant uptake. Plant uptake of Zn resulted in lower DTPA Zn concentrations in soil. The decreasing trend can also be attributed to the fact that ZnEDTA is highly water-soluble and has negative charges on it. Therefore, most of it remains as water-soluble Zn making it most mobile and available.

## 4.4. Conclusions

Water solubility is the primary factor that affects the movement of Zn in Zn fertilizers in neutral soils, not total Zn concentration. The addition of low water-soluble Zn sources (ZnO and Zn mix) resulted in lower water-extractable Zn, soil water Zn, and DTPA extractable-Zn compared with the greater water-soluble Zn sources ($ZnSO_4$ and ZnEDTA) in the sections away from point of application. Mobility for greater water-soluble Zn fertilizers was also greater than fertilizers with low water solubility.

When considering all parameters measured, the Zn sources evaluated can be divided into two categories. The more mobile Zn sources- ZnEDTA and $ZnSO_4$ and less mobile sources- ZnO and Zn mix. The greatest concentrations of the most labile forms of Zn occurred with ZnEDTA treatment in this neutral soil. The high effectiveness of ZnEDTA could be due to high mobility of the complex as observed in total Zn, DTPA Zn, Water extractable Zn, and soil water Zn. This greater effectiveness can result from the larger quantities of labile forms of Zn in the soil, as observed by XANES results. The results of this study have shown that the most crucial factor to consider is the resulting water solubility for the reaction products of the micronutrient sources when added to the soil.

## 4.5 References

Adriano, D. C. 2001. Trace Elements in the Terrestrial Environment, Springer-Verlag, NY.

Allison, L. and C. Moodie. 1965. p. 149-157. Cation-exchange capacity. In A.G. Norman (ed.) Methods of Soil Analysis. Part-2. Chemical and Microbiological Properties. SSSA, Madison, WI.

Alloway, B.J. 2009. Soil factors associated with zinc deficiency in crops and humans. Environmental geochemistry and health. 31:537-48.

Alloway, B.J. 2008b. Zinc in soils and crop nutrition. International Zinc Association & International Fertilizer Industry Association. Brussels, Belgium and Paris, France.

Alvarez, J. M., J. Novillo, A. Obrador, L.M. Lopez-Valdivia. 2001. Mobility and leachability of zinc in two soils treated with six organic zinc complexes. J. Agric. Food Chem. 49: 3833−3840.

Alvarez, J. M., Lopez-Valdivia, L. M., Novillo, J., Obrador, A.; Rico, M. I. 2006. Comparison of EDTA and sequential extraction tests for Phyto availability prediction of manganese and zinc in agricultural alkaline soils. Geoderma. 132:450−463.

Alvarez, J.M., and D. Gonzales. 2006. Zinc Transformations in Neutral Soil and Zinc Efficiency in Maize Fertilization. J. Agric. Food Chem. 54: 9488-9495.

Attanayake, C., G. Hettiarachchi, S. Martin, and G. Pierzynski. 2015. Potential Bioavailability of Lead, Arsenic, and Polycyclic Aromatic Hydrocarbons in Compost-Amended Urban Soils. J. Environ. Qual. 44: 930-944

Arai, Y., and D.L. Sparks. 2007. p. 135-179. Phosphate reaction dynamics in soils and soil components: a multiscale approach. D. Sparks (Ed.), Advances in Agronomy, Vol. 14, Academic Press, San Diego.

Asad, A., and R. Rafique. 2000. Effect of zinc, copper, iron, manganese and boron on the yield and yield components of wheat crop in Tehsil Peshawar. Pak. J. Bio. Sci. 310: 1615-1620.

Boawn, L. C. 1973. Comparison of zinc sulphate and Zn EDTA as zinc fertilizer sources. Soil Sci. Soc. Am. J. 37:111−115.

Gangloff, W. J., D.G. Wesfall, G.A. Peterson, and J.J. Mortvedt. 2002. Relative availability coefficients of organic and inorganic Zn fertilizers. J. Plant Nutr. 25:259−273.

Goos, R.J., B.E. Johnson, and M. Thiollet M. 2000. A comparison of the availability of three zinc sources to maize (Zea mays L.) under greenhouse conditions. Biol. Fert. Soils. 31: 343- 347.

Gupta, G.N. and D.L. Deb. 1984. Effect of chelating agents on zinc diffusion in two soils. J. Plant Nutr. Soil Sci. 147:533- 539.

Hamza, S. and A.K. Sadanandan. 2005. Effect of source and method of application of zinc on yield and quality of black pepper (Piper nigrum L.). J. Spices Aromatic Crops. 14:117- 121.

Hao, H.L., Y.Z. Wei, X.E. Yang, Y. Feng, and C.Y. Wu. 2007. Effects of different nitrogen fertilizer levels on Fe, Mn, Cu and Zn concentrations in shoot and grain quality in rice (Oryza sativa). Rice Sci. 14: 289-294.

Hodgson, J. F. 1963. Chemistry of micronutrient elements in soils. Adv. Agron. 15: 119−159.

O. Jacquat, A. Voegelin, A. Villard, M. Marcus, and R. Kretzschmar. 2008. Formation of Zn-rich phyllosilicate, Zn-layered double hydroxide and hydrozincite in contaminated calcareous soils Geochim. Cosmochim. Acta. 72:5037-5054

O. Jacquat, A. Voegelin, and R. Kretzschmar. 2009. Soil properties controlling Zn speciation and fractionation in contaminated soils Geochim. Cosmochim. Acta. 17:5256-5272.

Jenkinson, D., and D. Powlson. 1976. The effects of biocidal treatments on metabolism in soil V: A method for measuring soil biomass. Soil Biol. Biochem. 8:209-213.

Jones, J. B., 2001. Jr. In Laboratory Guide for Conducting Soil Tests and Plant Analysis. CRC Press, Boca Raton, FL.

Juillot, F., G. Morin, P. Ildefonse, T.P. Trainor, M. Benedetti, L. Galoisy, G. Calas and G.E. Brown. 2003. Occurrence of Zn/Al hydrotalcite in smelter-impacted soils from northern France: Evidence from EXAFS spectroscopy and chemical extractions. Am. Mineral. 88: 509-526.

Karak, T., U.K. Singh, S. Das, D.K. Das, and Y. Kuzyakov. 2005. Comparative efficacy of $ZnSO_4$ and ZnEDTA application for fertilization of rice (*Oryza sativa* L.). Arch. Agron. Soil Sci. 51:253-264.

Kuo, S. and D.S. Mikkelsen. 1979. Zinc adsorption by two alkaline soils. Soil Sci.128: 274-279.

Lindsay, W. L., and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Am. J. 42:421−428.

Manceau, A., N. Tamura, R.S. Celestre, A.A. MacDowell, N. Geoffroy, G. Sposito and H.A. Padmore. 2003. Molecular-scale speciation of Zn and Ni in soil ferromanganese nodules from loess soils of the Mississippi Basin. Environ. Sci. Technol. 37: 75-80.

Manceau, A., M.A. Marcus, N. Tamura, O. Proux, N. Geoffroy, and B. Lanson. 2004. Natural speciation of Zn at the micrometer scale in a clayey soil using X-ray fluorescence, absorption, and diffraction. Geochim. Cosmochim. Acta. 68: 2467-2483.

Manceau, A., C. Tommaseo, S. Rihs, N. Geoffroy, D. Chateigner, M. Schlegel, D. Tisserand, M.A. Marcus, N. Tamura and Z.S. Chen. 2005. Natural speciation of Mn, Ni, and Zn at the micrometer scale in a clayey paddy soil using X-ray fluorescence, absorption, and diffraction. Geochim. Cosmochim. Acta. 69: 4007-4034.

McBride, M. B. 1989. Reactions controlling heavy metals solubility in soils. Adv. Soil Sci. 10:1−56.

McBride, M.B. and J.J. Blasiak. 1979. Zinc and Copper Solubility as a Function of pH in an Acid Soil. Soil Sci. Soc. Am. J. 43: 866-870.

Mikkelsen, D.S., and D.M. Brandon. 1975. Zinc deficiency in California rice. Calif. Agric. 29:8-9.

Minkina, T., D. Nevidomskaya, V. Shuvaeva, T. Bauer, A. Soldatov, S. Mandzhieva, A. Trigub, Y. Zubavichus, and K. Ghazaryan. 2019. Molecular characterization of Zn in Technosols using X-ray absorption spectroscopy Appl. Geochem. 104:168-175.

D. Montalvo, F. Degryse, R.C. da Silva, R. Baird, and M.J. McLaughlin. 2016. Agronomic effectiveness of zinc sources as micronutrient fertilizer. Adv. Agron. 139:215-267.

Obrador, A., J. Novillo, J.M. Alvarez. 2003. Mobility and availability to plants of two zinc sources applied to a calcareous soil. Soil Sci. Soc. Am. J. 67:564−572.

Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. Government Printing Office, Washington D.C.

Rashid, A., and J. Ryan. 2004. Micronutrient constraints to crop production in soils with Mediterranean-type characteristics: A review. J. Plant Nutr. 27: 959−975.

Rico, M.I., J.M. Alvarez, and J.I. Mingot. 1996. Efficiency of zinc ethylenediamine tetraacetate and zinc lignosulfonate soluble and coated fertilizers for maize in calcareous soil. J Agric. Food Chem. 44: 3219- 3223.

Sachdev, P., W.L. Lindsay, and D.L. Deb. 1992. Activity measurements of zinc in soils of different pH using EDTA. Geoderma. 55: 247-257.

Scheinost, A.C., R. Kretzschmar, S. Pfister and D.R. Roberts. 2002. Combining selective sequential extractions, x-ray absorption spectroscopy, and principal component analysis for quantitative zinc speciation in soil. Environ. Sci. Technol. 36: 5021-5028.

Schlegel, M.L., A. Manceau, L. Charlet, D. Chateigner, and J.L. Hazemann. 2001. Sorption of metal ions on clay minerals. III. Nucleation and epitaxial growth of Zn phyllosilicate on the edges of hectorite. Geochim. Cosmochim. Acta. 65: 4155-4170.

Singh, and Y.S. Shivay. 2016. Effect of summer green manuring crops and zinc fertilizer sources on productivity, Zn-uptake and economics of basmati rice. J. Plant Nutr. 39:204-218

Sparks, D.L. 2003. p. 352. Environmental Soil Chemistry (Second edition), Academic Press.

Sparks, D.L. 2013. p. 93-114. Advances in the use of synchrotron radiation to elucidate environmental interfacial reaction processes and mechanisms in the 'Earth's critical zone J. Xu, D. Sparks (Eds.), Molecular Environmental Soil Science, Progress in Soil Science.

Soil Survey Staff. 2011. Burt, R. (Ed.). Soil Survey Laboratory Information Manual. Soil Survey Investigations. Report No. 45, Version 2.0. Department of Agriculture, Natural Resources Conservation Service, Washington, DC.

Stahl, R., and B. James. 1991. Zinc Sorption by B Horizon Soils as a Function of pH. Soil Sci. Soc. Am. J. 55.

Su, Y., J. Gao, Y. Qin, Y. Zheng, and D. Zhu. 2019. Interfacial Zinc Phosphate is the Key to Controlling Biocompatibility of Metallic Zinc Implants. Adv. Sci. 6.

Viets, F. G. 1962. Chemistry and availability of micronutrients in soils. J. Agric. Food Chem. 10: 174−178.

Watson, M.E., and J.R. Brown. 1998. p. 13-17. pH and lime requirement. In J. R. Brown (Ed.), Recommended chemical soil test procedures for the North Central Region Missouri. Agricultural Experiment Station, University of Missouri, Columbia, MO.

Werner, F., and J. Prietzel. 2015. Standard Protocol and Quality Assessment of Soil Phosphorus Speciation by P K -Edge XANES Spectroscopy. Environmental science & technology. 49.

Wright, A. and J. Bailey. 2001. Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 Dry Combustion Analyzer. Commun. Soil Sci. Plant Anal. 32:3243-3258.

Westfall, D. G., W.J. Gangloff, G.A. Peterson, G. A., and J.J. Mortvedt. 2000. Organic and inorganic fertilizers: Relative availability. Technical Bulletin, TB01-1; Colorado Agricultural Experimental Station, CO.

Zhao, S. Yang, B. Wang, X. Tian, Y. Zhang. 2018. Effects of $ZnSO_4$ and ZnEDTA broadcast or banded to soil on Zn bioavailability in wheat (*Triticum aestivum* L.) and Zn fractions in soil. Chemosphere. 205:350-360.

Zarcinas, B.A., M.J. McLaughlin, and M.K. Smart. 1996. The effect of acid digestion technique on the performance of nebulization systems used in inductively coupled plasma spectrometry. C. Soil Sci. Plant Anal. 27:1331-1354.

## 4.6 Supplementary Information

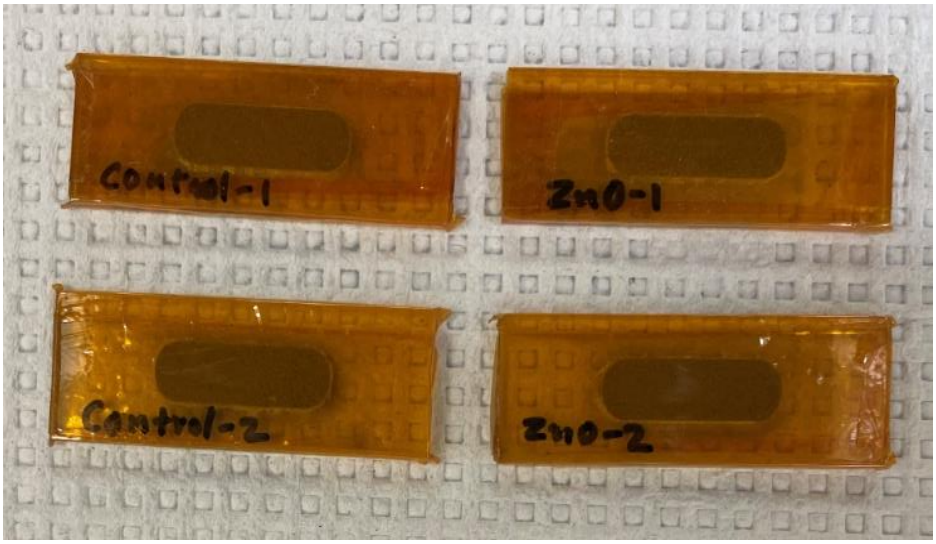


**Figure S4.1 Plexiglas holder labeled with sample name and soil packed in Plexiglas holder and sealed with Kapton tap**

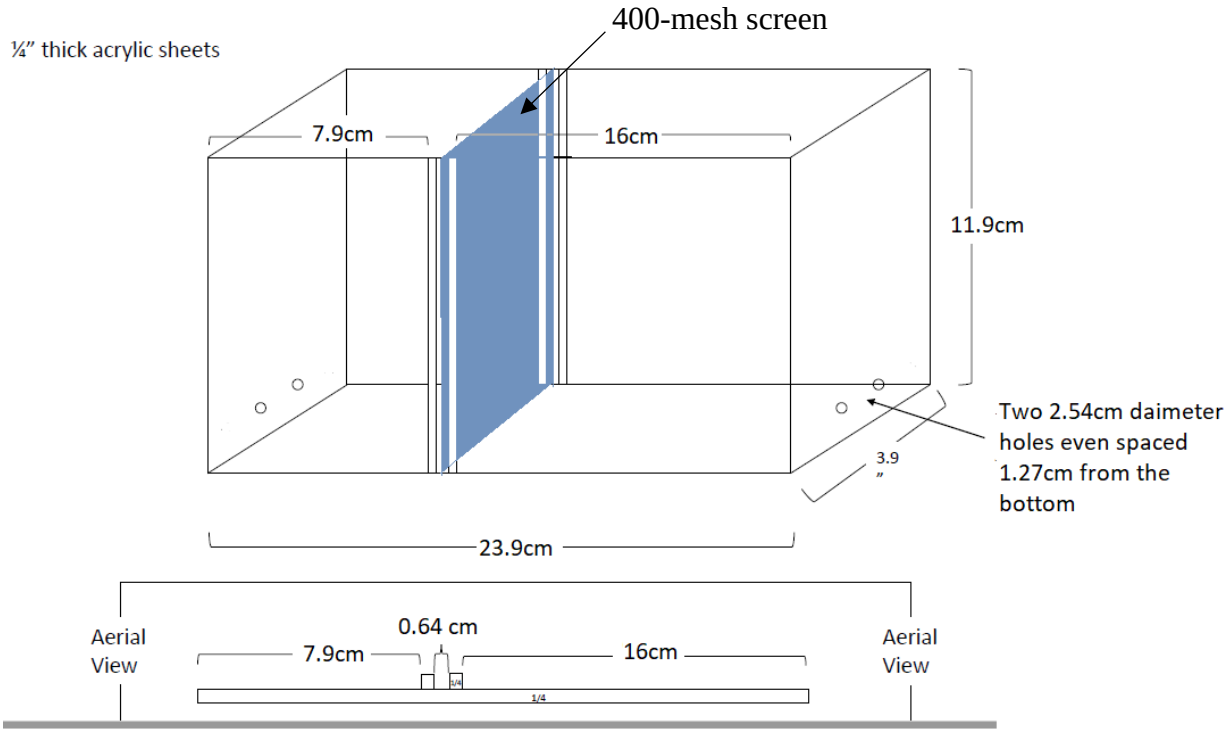


**Figure S4.2 - Acrylic containers used for the greenhouse study**

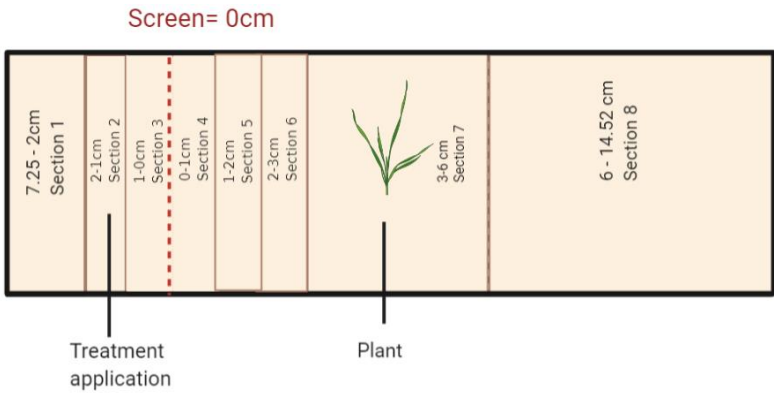


**Figure S4.3 Soil sectioning at the end of the experiment**

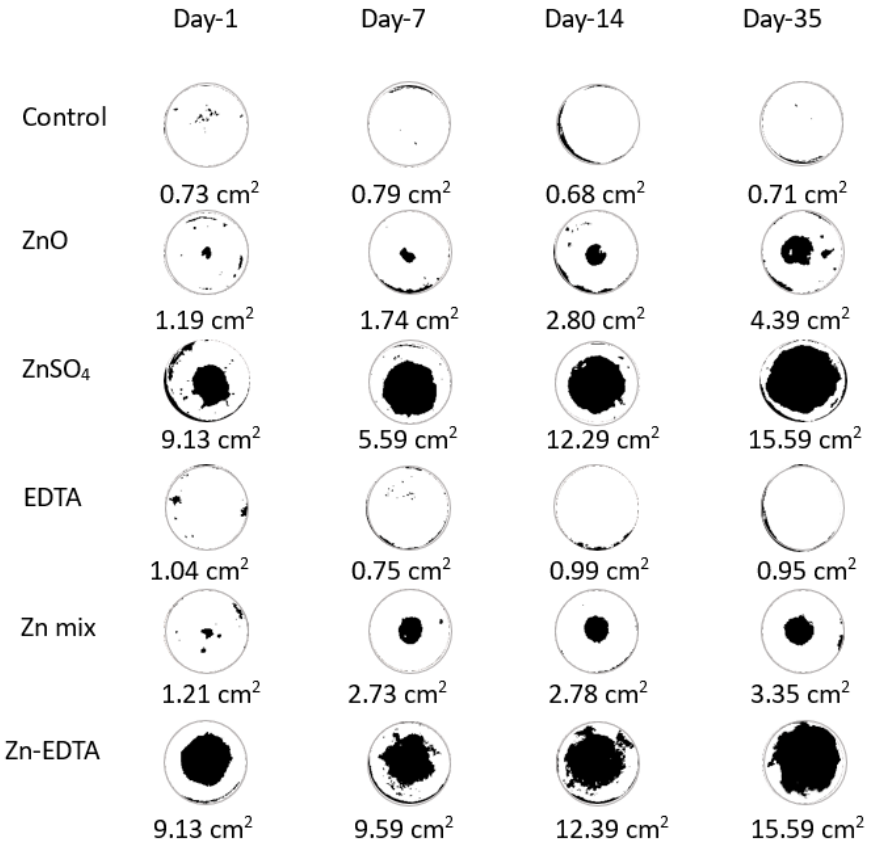


**Figure S4.4 Visualization study- images scanned using scanner and then pink color quantified using GIMP 2.10 software at day 1, 7, 21, and 35. Control= no zinc; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; EDTA= ethylenediamine tetra acetic acid with no-Zn; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid.**

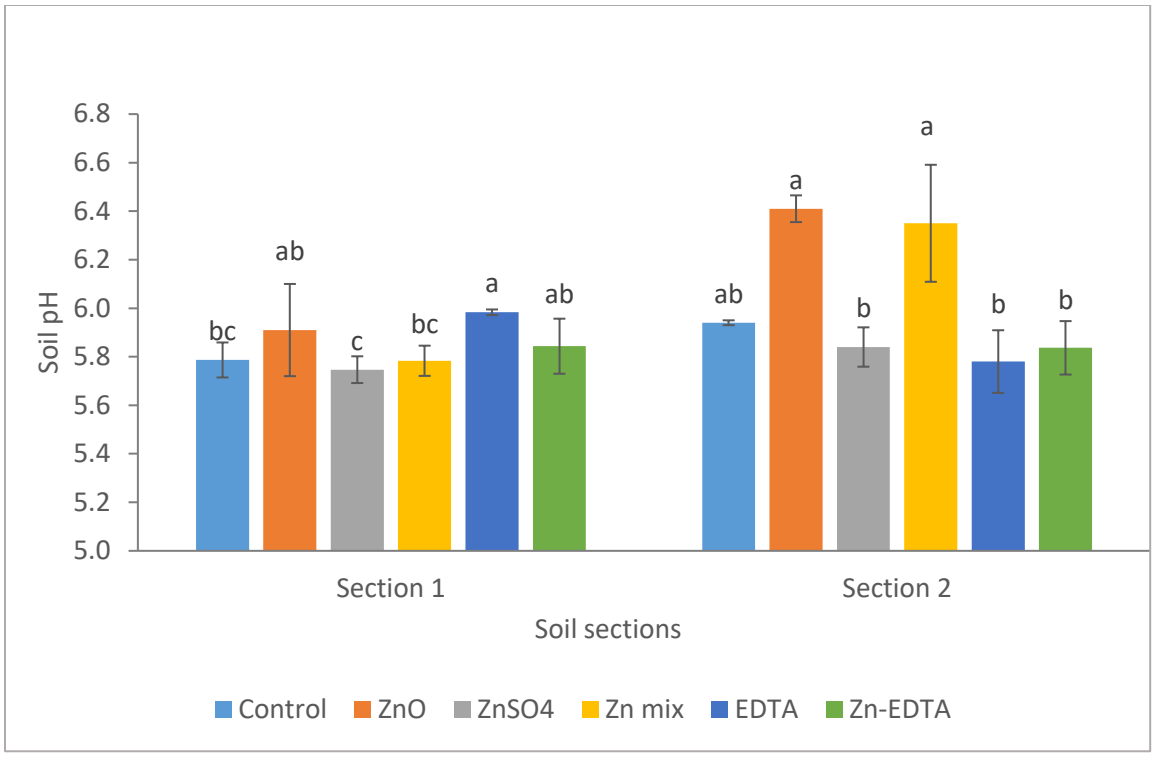


**Figure S4.5 Soil pH at the end of the greenhouse study. Control= no zinc; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix (mixture of ZnO, $ZnSO_4$, and ZnEDTA); EDTA= Ethylenediamine tetra acetic acid with no-Zn; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

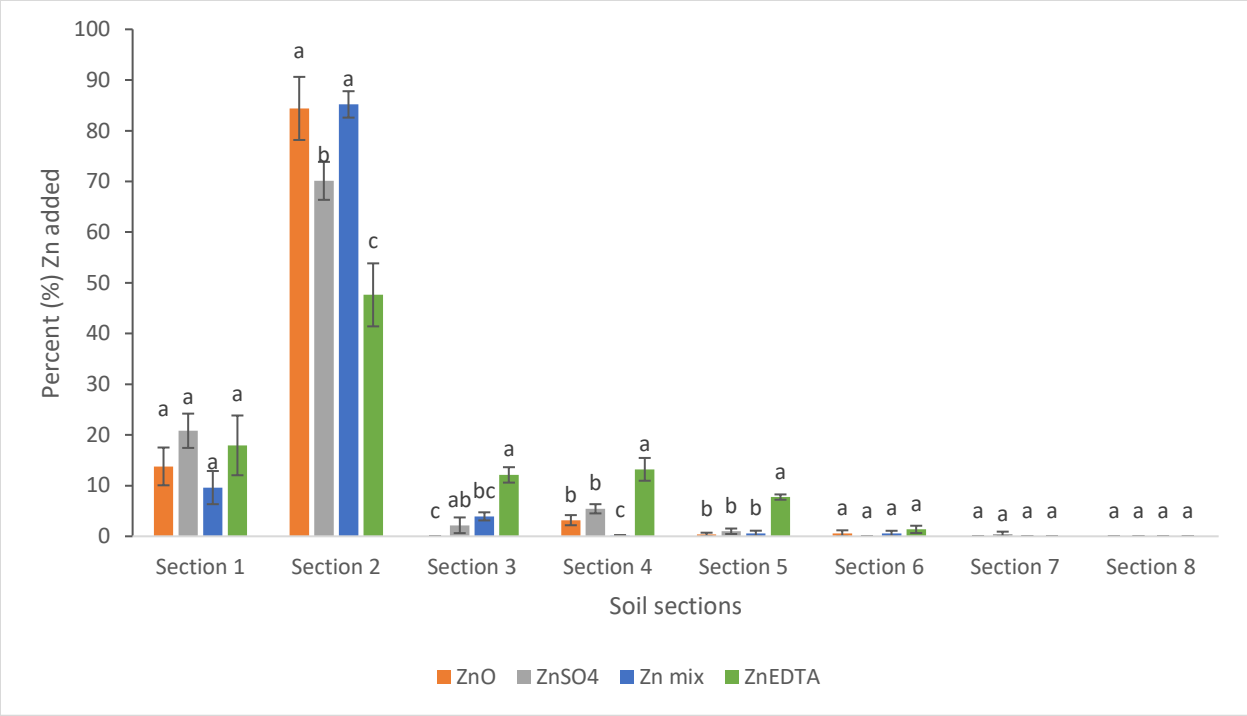


**Figure S4.6 Percent (%) Zn added recovered from respective soil section at the end of the greenhouse study. ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix (mixture of ZnO, $ZnSO_4$, and ZnEDTA); ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

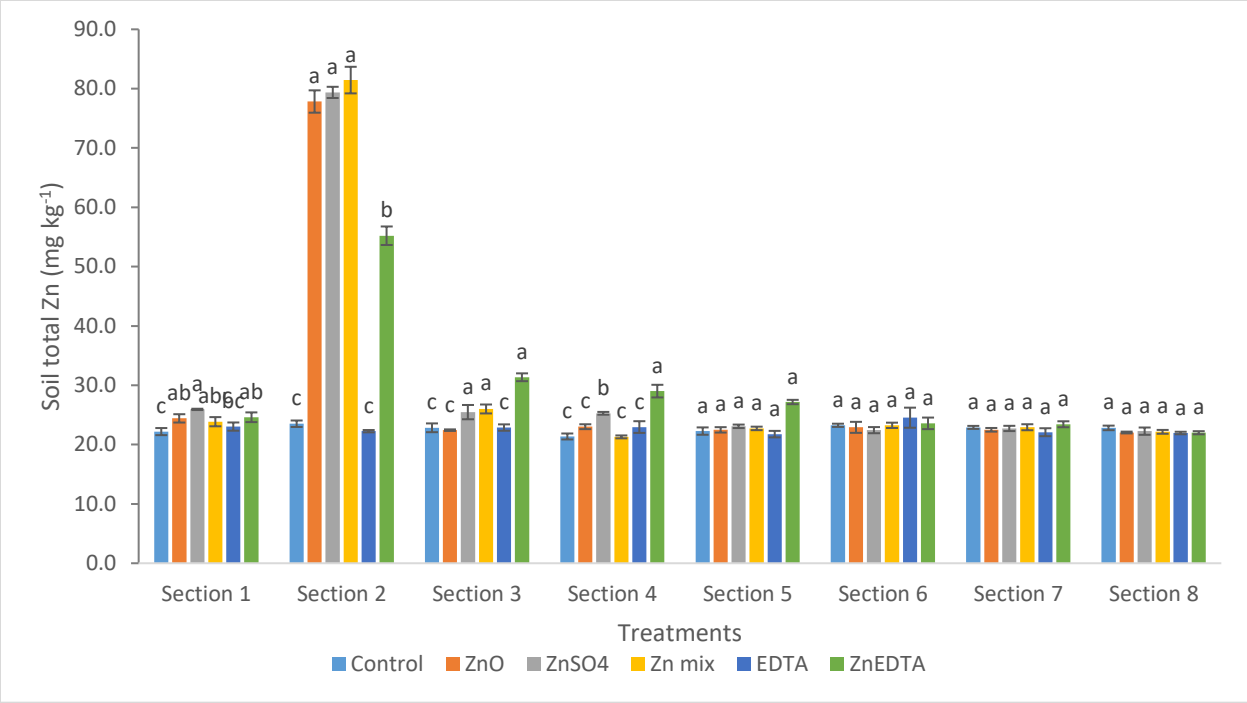


**Figure S4.7 Soil total Zn concentration at the end of the greenhouse experiment. Control= no zinc; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix (mixture of ZnO, $ZnSO_4$, and ZnEDTA); EDTA= Ethylenediamine tetra acetic acid with no-Zn; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

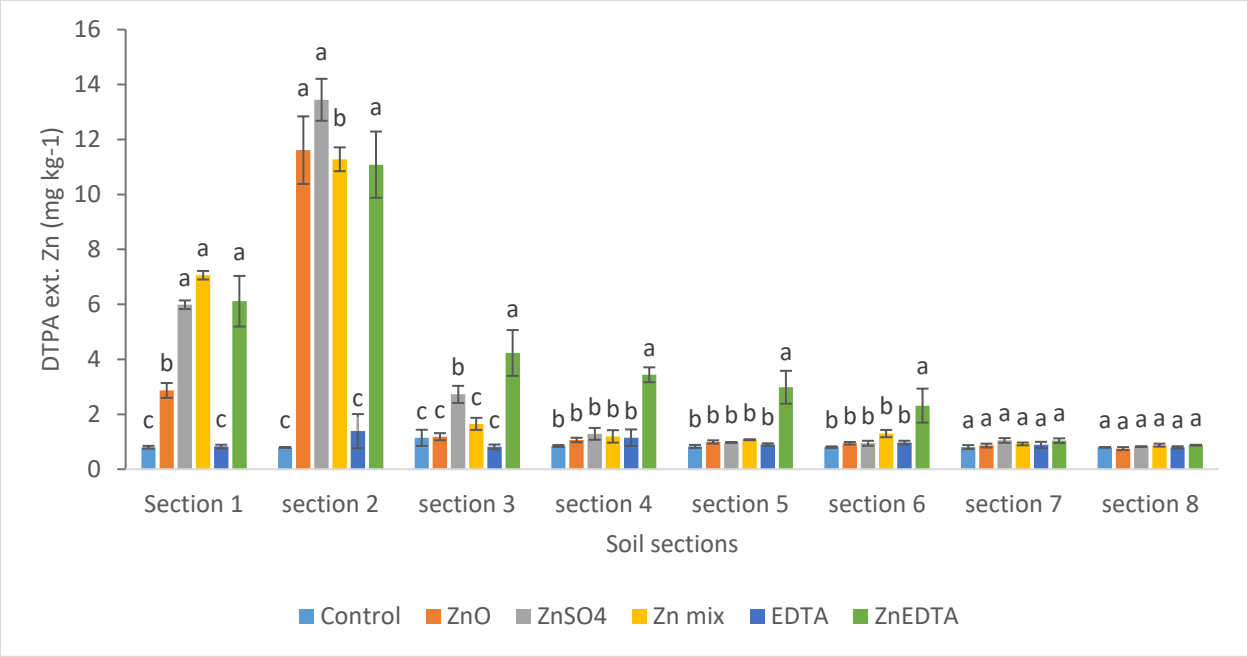


**Figure S4.8 DTPA extratable zinc. Control= no zinc; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix (mixture of ZnO, $ZnSO_4$, and ZnEDTA); EDTA= Ethylenediamine tetra acetic acid with no-Zn; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

# Chapter 5 - Efficiency of different zinc sources in two contrasting soils- Is Zinc oxide under-rated?


## Abstract

Zinc deficiency is common in many different types of soil. Zinc deficiency mainly occurs due to its low plant availability. It is driven by strong zinc sorption onto $CaCO_3$ in calcareous soils and hydroxy-Al interlayered montmorillonite and sorption by phosphorus under acidic conditions. Experiments under laboratory (incubation-visualization) and greenhouse conditions were conducted to study the response of a high Zn responsive wheat line (*Triticum aestivum*) in two soils. The effect of Zn sources (ZnO, $ZnSO_4.7H_2O$, Zn mix (mixture of ZnO, $ZnSO_4$, and EDTA) and ZnEDTA) in a silt loam alkaline calcareous soil, and Zn only (ZnO, $ZnSO_4.7H_2O$, Zn mix, ZnP, and ZnEDTA) and Zn-coated urea sources (ZnO-U, $ZnSO_4.7H_2O$-U, Zn mix-U, ZnP-U, and ZnEDTA-U) in a silt loam acidic soil were investigated. The sources were compared based on their concentration and plant uptake, Zn diffusion, pH, solubility, and availability. Our results showed that for Zn only sources, the application of ZnO and ZnEDTA fertilizers significantly increased plant Zn uptake in calcareous soil, but no significant differences were observed among Zn only sources in acid soil. For Zn-coated urea sources in acid soil, ZnO-U, $ZnSO_4$-U, and ZnEDTA-U had significantly greater plant Zn uptake than control and Urea treatment. Rate of Zn added for Zn-coated urea sources was half the rate used for Zn only. Still, Zn-coated urea sources managed to have plant Zn uptake comparable to Zn only sources. Zinc-ethylenediaminetetraacetate (EDTA) showed the greatest labile Zn distribution throughout the soil as observed in the greenhouse and incubation experiment. We found that zinc oxide act as slow-release when applied as Zn only source in both soils but becomes more soluble when applied as ZnO-U in acid soil. Zinc mix treatment effectively increased biomass and Zn uptake

comparable to highly soluble $ZnSO_4$ and chelated Zn treatment for calcareous and acid soil but showed significantly lower plant biomass and Zn uptake when coated onto urea granules. When added alone, Zn mix and Zn oxide can provide adequate amounts of Zn to fulfill the plant Zn requirements comparable to the chelated source of Zn (i.e., ZnEDTA).

## 5.1 Introduction

Zinc is a micronutrient vital for humans and plants (Kaya et al., 1999; Hao et al., 2007). Zinc deficiency is one of the essential micronutrient disorders acting as a barrier for sustainable crop production and food quality (Kumssa et al., 2015; Lindsay, 1979). Zinc (Zn) availability and mobility in soils is controlled by its interaction with the soil matrix and components. Most important are soil factors influencing Zn availability are high pH, low organic matter content, high clay content, phosphorus supply, and high calcium carbonates ($CaCO_3$) content (Rashid and Ryan, 2004). High soil pH reduces Zn solubility in soil, and high $CaCO_3$ is responsible for adsorption and precipitation of Zn (Alloway, 2009; Rashid and Ryan, 2004). Adsorption and precipitation reactions regulate Zn concentration in soil solution. Zinc adsorption in various soils is well documented by several researchers (Kaya and Ören, 2005; Tang et al., 2009). The buffering capacity of a soil can considerably affect quantity, and intensity which determine the capacity of the soil to supply plant nutrients. The unavailability of Zn in soil has been attributed to the entrapment of the metal into particle aggregates or clay mineral structures, the strong surface adsorption by oxides, and/or the formation of insoluble metal precipitates (Hamon et al., 2002). The non-liability of Zn is connected to its reaction with carbonates and form $ZnCO_3$ precipitates in calcareous soil (Wang and Harrell, 2005); the formation of Zn-Fe precipitates such as $ZnFe_2O_4$ (franklinite) in both acid and calcareous soils (Scheinost et al., 2002); and formation of Zn-phosphate precipitation such as $Zn_3(PO_4)_2.4H_2O$ (Hopeite) in acid soil (Pérez-Novo et al., 2011).

Therefore, there is a need to understand the pathways Zn cation undergoes in different soils. Better understanding will help to provide efficient solutions to correct Zn-deficiency in specific soil. One of the most critical factors influencing the effectiveness of Zn fertilizer is its

solubility, which depends on its inherent properties as well as the properties of soil to which it is applied, finally affecting plant growth and development (Amrani et al., 1999; Shaver et al., 2007; Westfall et al., 2005).

Zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO) are the two most common inorganic Zn sources that employ for fertilization (Montalvo et al., 2016). Zinc EDTA is the most common chelated Zn source used for crop production (Arai and Sparks, 2007). Zinc application has been proven to successfully remediate Zn deficient soils. Ideally, the choice of the Zn fertilizer should depend on the relative agronomic effectiveness of the source applied to a given soil. For example, previous researchers reported the better efficiency of ZnEDTA in calcareous soils compared to $ZnSO_4$, the availability of chelated Zn is at least double than that of $ZnSO_4$, but chelated Zn is much more expensive than $ZnSO_4$ (Boawn, 1973; Karak et al., 2005; Alloway, 2008b). Zinc fertilizers are typically blended with, incorporated into, or coated onto macronutrient fertilizer because it allows more uniform spread with conventional application equipment. Costs also are reduced by eliminating need for a separate application.

Few studies have directly compared an inorganic Zn source with synthetic Zn chelate. A new granular Zn mix fertilizer with 62% Zn was recently introduced in the central part of the United States. Although it needs to be proven through research, Zn mix should inherit the good characteristics from the components used in its formation, e.g., greater solubility- $ZnSO_4$, slow-release-ZnO, and greater mobility-ZnEDTA. It is very important to understand the role of physical and chemical factors in the availability of these sources when applied directly to the soil.

Total Zn concentration in soil provides inadequate information to assess the bioavailability of this micronutrient. The various chemical pool of Zn exists in noncalcareous

soils concerning its bioavailability: water-soluble, exchangeable, complexed with organic matter, inorganically precipitated/occluded with iron or manganese oxide, and entrapped in primary and secondary minerals (Viets, 1962; Hodgson, 1963). Therefore, along with total Zn concentrations, the assessment of Zn availability or bioavailability can also be determined using other approaches such as DTPA extractable Zn, water-soluble Zn, and its uptake by plants.

This study aimed to compare (i) four different Zn treatments- zinc oxide (ZnO), zinc sulfate ($ZnSO_4$), zinc mixture of ZnO, $ZnSO_4$, and ZnEDTA (Zn mix), and zinc-ethylenediamine tetra acetic acid (ZnEDTA) compared to a control (No zinc) in an alkaline calcareous soil. (ii) five different Zn sources- zinc oxide (ZnO), zinc sulfate ($ZnSO_4$), zinc mixture, zinc phosphate, $Zn_3(PO_4)_2.4H_2O$ (ZnP), and zinc-ethylenediamine tetra acetic acid (ZnEDTA) compared to control (No-Zn) in an acid soil (iii) five different Zn-coated urea treatments- zinc oxide coated urea (ZnO-U), zinc sulfate coated urea ($ZnSO_4$-U), zinc mix coated urea (Zn mix-U), zinc phosphate coated urea (ZnP-U), and zinc-ethylenediamine tetra acetic acid-coated urea (ZnEDTA-U) compared to two controls (No-Zn+ urea as basal application) and urea (No-Zn + urea added to application section as other fertilizer treatments) in an acid soil. The comparison was made based on zinc diffusion, plant uptake efficiency, and availability of Zn.

We hypothesize that Zn mix treatment, which is a mixture of ZnO, $ZnSO_4$, and ZnEDTA, will- (i) show greater solubility due to the presence of the $ZnSO_4$ component, (iii) stay in the soil for more prolonged periods, acting as slow-release fertilizer due to the ZnO component (iii) have greater mobility due to the presence of the EDTA component.

## 5.2 Material and Methods

### 5.2.1 Soil collection

Two soils were used for the experiment- Silt loam alkaline calcareous soil from Garden City, KS and Smolan silt loam, acid soil from Manhattan, KS. The surface soil (0 to 15 cm depth) was collected from both locations, air-dried, and sieved to < 4 mm by passing through a stainless-steel mesh. The soil was analyzed for pH, total organic carbon (TOC), cation exchange capacity (CEC), and texture. The pH was measured in a 1:10 soil: Milli Q water extract (Watson and Brown, 1998); extractable phosphorus was determined by Olsen method (Olsen et al., 1954); cation exchange capacity was determined by displacement method (Soil Survey Staff, 2011); soil carbonates were determined by Allison and Moodie, (1965); and total organic carbon content was measured by dry combustion method (Wright and Bailey, 2001). The maximum water holding capacity (MWHC) was determined using Jenkinson and Powlson method (1976). Cation exchange capacity, soil carbonates, TOC content determinations were done by the Soil testing laboratory, Kansas State University.

**Table 5.1 Initial chemical and physical properties of calcareous soil and acid soil**

| Parameter | Calcareous soil | Acid soil |
|---|---|---|
| pH (1:10 soil: water) | 8.4±0.1 | 5.4±0.1 |
| CEC*, $cmol_c/kg$ | 17.0 | 22.3 |
| $CaCO_3$, % | 5.7 | - |
| Total Zn, mg $kg^{-1}$ | 58.5±1.2 | 38.8±1.3 |
| DTPA Zn, mg $kg^{-1}$ | 0.7±0.1 | 0.6±0.1 |
| Available P (Olsen) P, mg $kg^{-1}$ | 37.6 | 57.3 |
| Total Organic Carbon, % | 1.5 | 1.75 |
| Sand, Silt, and Clay, % | 18.4, 58.2, 23.4 | 10.8, 55.7, 33.5 |
| MWHC** | 57.8±0.1 | 56±0.2 |
| Soil texture | Silt loam | Silt loam |

*Cation Exchange Capacity; **Maximum Water Holding Capacity

## 5.2.2 Experiment set up

### 5.2.1.1 Greenhouse study

Two separate greenhouse experiments were carried out using two soils. Calcareous soil, was used for greenhouse experiment 1 (GH-1), carried out in November 2019, and acid soil, was used for greenhouse experiment 2 (GH-2). The experiments were conducted in the Throckmorton Plant Sciences Greenhouse Complex at Kansas State University, Manhattan, KS (39°12'N, 96°35'W, 325 m above sea level) by using acrylic containers (23.9 cm length and 11.9 cm height) (Figure S5.1).

The acrylic containers contained two slits to mount a 400-mesh screen (37µm) to restrict the movement of plant roots towards the treatment. Wheat (*Triticum aesitvum* L.) seeds were a

high-Zn spring wheat line (BW 53856, BW 53856 GRIN-Global (cimmyt.org), developed under the Harvest Plus initiative by Dr. Velu Govindan (CIMMYT). Seeds were provided by Dr. Mary Guttieri (USDA-ARS) from a Yuma, AZ seed increase, with permission of Dr. Govindan. Seeds were planted into germination trays containing a mixture of soil and sand (1:2) and kept in a growth chamber for three weeks at 4°C.

Before soil packing, the basal dose of nitrogen (N), phosphorous (P), and potassium (K) were given for both experiments. For GH-1 with calcareous soil, Nitrogen was added as urea at a rate of 120 kg $ha^{-1}$. Phosphorus was added as potassium dihydrogen phosphate ($KH_2PO_4$) at a rate of 40 kg $ha^{-1}$ , and K was added through $KH_2PO_4$ fertilizer based on the P rate.

For GH- 2 with acid soil, nitrogen was added as urea at a rate of 480 kg $ha^{-1}$, For the Zn only experiment, urea was added as basal fertilizer dose and mixed with soil. For the Zn-coated urea experiment, the amount of urea added with Zn-coated urea fertilizers was calculated. Urea was added as basal dose to balance the nitrogen concentration in all treatments. Phosphorus was added as $KH_2PO_4$ at a rate of 40 kg $ha^{-1}$, and K was added as $KH_2PO_4$ fertilizer based on the P rate.

Acrylic containers were packed with pre-wetted soil to achieve a uniform bulk density. For GH- 1, the soil (calcareous) was prewetted to 12% of MWHC and packed with a uniform bulk density of 1.15 Mg $m^{-3}$. For GH- 2, the soil (acid) was prewetted to 11% of MWHC and packed with a uniform bulk density of 1.01 Mg $m^{-3}$. After packing, the soils were brought to 60% MWHC. The containers were weighed and covered with polythene sheets to equilibrate the moisture for at least 24 hours. The next day, three weeks old uniform seedlings were selected and transplanted in the middle of the container (one seedling/container) on one side of the screen (opposite of the treatment addition) parallel to the treatment introduction point. The next day

after transplantation of the seedlings, treatments were introduced on one side of the screen (1 cm away from the screen and at 1.25 cm depth).

The following treatments were used:

For calcareous soil (GH-1)- (1) Control (No-Zn); (2) ZnO; (3) $ZnSO_4$; (4) Zn mix (mixture containing 60% ZnO, 25% $ZnSO_4$, and EDTA to $ZnSO_4$ 1:8 ratio); (5) ZnEDTA. For acid soil (GH- 2): Zn only experiment- (1) Control (No-Zn + No Urea); (2) ZnO; (3) $ZnSO_4$; (4) Zn mix; (5) ZnP; (6) ZnEDTA. Zin coated urea experiment- (7) Urea; (8) ZnO coated urea; (9) $ZnSO_4$ coated urea; (10) Zn mix coated urea; and (11) ZnP coated urea; (12) ZnEDTA coated urea. The experimental design was a randomized complete block design with three replications. Zinc rate was10 kg $ha^{-1}$ for Zn only treatments for both calcareous and acid soil while it was 5 kg $ha^{-1}$ for Zn-coated urea treatments (through treatments 8 to 12) for acid soil.

To ensure good growth, containers were weighed, and their weight was subtracted from the weight taken on the first day of the study after bringing them to moisture content of 60% of MWHC. The difference in weight is weight lost and plants were watered with DI water every day (amount of water= amount of water lost) to all containers throughout the experiment period. The greenhouse temperature was maintained within the range of 20°C to 23°C throughout the experiments. A 12 h daylight and 12 h darkness photoperiod regime was maintained with overhead sodium lights for all five weeks until harvesting. The containers were rotated weekly to ensure that all pots received equal amounts of light.

Soil water samples were collected at the end of the third and fifth week of the greenhouse studies using rhizon samplers (Rhizon MOM soil moisture sampler, 19:21:22, Rhizosphere research products, Wageningen, Netherlands). Rhizon samplers were inserted close to the plant roots. Soil water sample was collected directly into the vacutainers. Samples were acidified by

adding one drop of concentrated nitric acid immediately upon collection and stored in a refrigerator at 4°C until analysis. Soil water samples were analyzed for Zn, Ca, P, and Fe using an inductively coupled plasma-mass spectrometry (ICP-MS, 7500 CX, Agilent, Santa Clara, CA, USA). Standard water reference (Trace Elements in Natural Water, SRM1643f) from the National Institute of Science and Technology (NIST, Gaithersburg, MD) was used as part of the quality assurance quality control (QA/QC) protocol (recoveries were within 100 ± 20%).

Processing and chemical analysis of plant samples: Above-ground biomass was harvested at the end of the fifth week of the greenhouse studies using stainless steel knife. The harvested above-ground biomass of plant samples was washed with tap water, followed by deionized water (DI) water, followed by 5 g $kg^{-1}$ sodium lauryl sulfate [$CH_3(CH_2)_{10}CH_2OSO_3Na$] solution (Attanayake et al., 2014). After cleaning, samples were placed in pre-weighed paper bags. Fresh sample weight was recorded. Plant samples were dried at 68°C for about four days until they reached a constant weight. Dried sample weights were recorded. Dried plant materials were ground using a Willey Mini Mill-Arthur Thomas-type grinder (Thomas Scientific), sieve size of 250 mm (60 mesh), and secured and stored using double zip lock bags.

A sub-sample (0.5 g) of ground above-ground biomass was digested with 10 mL of trace metal grade nitric acid ($HNO_3$) in Teflon tubes by microwave (CEM, Matthews, NC). The digestion unit was ramped up to 200° C and held for 15 minutes (1600 W at 100%, 15-minute ramp time, 15-minute cooling time). Standard plant reference material (wheat flour, 1567b) from the National Institute of Science and Technology (NIST, Gaithersburg, MD) was used as part of the quality assurance quality control (QA/QC) protocol (recoveries were within 100 ± 20%). Internal standards, Reagent blanks, and spiked digestions were also included for QA/QC. Sample digests were filtered with Whatman filter paper no. 42 under the fume hood in 15mL centrifuge

tubes and stored at 4° C until analysis. Based on their concentrations, samples were analyzed for Zn, P, and Fe using an inductively coupled plasma-mass spectrometry ICP-MS or inductively coupled plasma-optical emission spectroscopy (ICP-OES).

Zinc uptake was calculated as follows:

Zn uptake (g/pot) = (Zn concentration of plant tissue * dry weight of biomass)

Processing of soil samples-

Calcareous soil was sectioned into eight sections, considering screen as 0 cm. Labeling was done as (Figure S5.2): 7.25-2cm (section 1), 2-1 cm (section 2), 1-0 cm (section 3), 0-1 cm (section 4), 1-2 cm (section 5), 2-3 cm (section 6), 3-6 cm (section 7), 6-14.52 cm (section 8). Acid soil was sectioned into five sections, considering screen as 0 cm. Soil sectioning was done as (Figure S5.3): 7.25-2 cm (section 1), 2- 0 cm (section 2), 0-3 cm (section 3), 3-6 cm (section 4), 6-14.52 cm (section 5). The soil was air-dried and sieved through a 2-mm sieve. For DTPA-Zn, ground, air-dried soil sample was passed through 1.0 mm stainless steel. Ten gram of soil sample was taken in 125 mL conical flask, 20 mL of diethylene-triamine Penta acetic acid-triethanolamine (DTPA-TEA) extraction solution was added, each flask was covered with parafilm, and was shaken using a horizontal shaker with 8.0 cm of stroke length and 120 cycles min-1 for 2 hours (Lindsay and Norvell, 1978). Suspensions were filtered through Whatman filter paper no. 42, and samples were analyzed using the ICP-OES. The end of study analyses includes- pH (1:10 soil: water), the total elemental concentration in the soil using aqua regia (Zarcinas, 1996), and extractable Zn using DTPA as described before.

#### 5.2.1.2 Incubation experiment

Calcareous (soil 1) was air-dried, and soil passed through a 2 mm sieve was used for the incubation experiment. This experiment was composed of 30 Petri dishes (53 mm diameter and

13.9 mm height). The soil was prewetted to 12% of the MWHC and then packed with the uniform bulk density of 1.10 Mg $m^{-3}$. Water was added according to 55% of the MWHC of the soil. The Petri dishes were covered, and the edges were wrapped using Parafilm. Petri dishes were allowed to equilibrate at room temperature (≈24°C) for at least 24 hours. The next day, treatments were introduced at the center of the dish at a rate of 2.43 mg Zn/Petri dish. The five treatments replicated three times consisted of control (No-Zn), ZnO, $ZnSO_4$, Zn mix, and ZnEDTA. Following treatment introduction, Petri dishes were again covered, and Parafilm was employed to the edges to mitigate moisture loss. The dishes were wrapped in aluminum foil to prevent light exposure and incubated for five weeks in the dark at 25°C.

#### 5.2.1.3 Visualization experiment

For ongoing study analyses, Visualization was done for days 1, 7, 21, and 35. It was done using Degryse et al. (2015) method. By this method, Zn movement was investigated using calcium carbonate ($CaCO_3$). Calcium carbonate sorbs Zn through surface complexation and precipitation. Calcium impregnated papers were prepared using Whatman filter paper no. 1. Filter papers were cut to the exact diameter of the Petri dish. Papers were soaked in 1 M $CaCl_2$ for at least 30 minutes (min) and then transferred to 0.4 M $Na_2CO_3$ for about 10 min, resulting in precipitation of $CaCO_3$ in the paper. The papers are rinsed with DI water, dried, and stored for later use. The deployment of papers was done for 2-hour for contact times between soil and fertilizer up to 1 day, 3-hours for contact times up to 1 week, 4-hours deployment time when the fertilizer has been in contact with the soil for more than one week (day- 21, and 35 for present study). For color development, dithizone dye was prepared. The stock solution was prepared by dissolving 50 mg of dithizone in 0.5 mL of ethanol + 0.1 mL of concentrated ammonia and diluting to 10 mL with deionized water. A pink color appeared in the high-Zn zone; full-color

development took about 20 min. In the end, filter papers were scanned, and the size of the pink zone was quantified using imaging software (GIMP, 2.10.20).

For the end of study analyses, soils in dishes were sectioned into four concentric circular sections with radii of 0-4 mm, 4-8 mm, 8-17 mm, and 17 mm- 26.5 mm. The soils were then dried at 40°C (Fisher Scientific Drying Oven, Waltham, MA), weighed, and finely ground with a mortar and pestle. Soil samples were analyzed for pH (1:10 soil: Milli Q water), water-extractable Zn (1:10 soil: Milli Q water), and the total Zn concentration in the soil using the aqua regia; 0.5 g of soil digested using 5 mL of aqua regia solution (1:3 (v/v) $HNO_3$/HCl). Samples were pretreated with aqua regia solution and allowed to react overnight at room temperature. The soils were digested- at the temperature of 75°C for 30 minutes (min), 100°C for 30 min, 110°C for 30 min, and 140°C until the acid volume decreased to ≈1.0 mL. The digested samples were cooled, diluted with 25 mL of 0.1% $HNO_3$, and filtered through a no. #42 Whatman filter paper (Zarcinas, 1996). In each digestion batch, one blank, and one sample of standard reference soil material (NIST 2711a - Montana soil) was included as a QA/QC control. The analysis was done using ICP-OES.

### 5.2.2 Statistical Analysis

Statistical analysis of all data was done using SAS version 9.4 (SAS Institute Inc., Cary, NC, 2020) One-way ANOVA using PROC GLM was used to analyze soil data. The data analysis was performed as a split-plot completely randomized design. The main plot was Zn fertilizer treatment, and the sub plot factor was soil section. Pairwise comparison was done using LSD at $\alpha \leq 0.05$. One-way ANOVA using PROC GLIMMIX was used to analyze plant data and pairwise comparison was done at $\alpha \leq 0.05$ using LS means.

ANOVA using PROC MIXED was performed to evaluate Type III tests of fixed effects (treatment and time) on visualization and soil water Zn data. The data analysis was performed as a split-plot completely randomized or completely randomized block design with repeated measures, respectively, for visualization and soil water data. The main plot factor was the treatment, and the subplot factor was time. Pairwise comparison was done at $\alpha \leq 0.05$ using Tukey test.

5.3 Results and discussions

## 5.3.1 Greenhouse study

### 5.3.1.1. Calcareous soil

#### *5.3.1.1.1 Plant Zn biomass and uptake*

Zinc fertilizers significantly increased plant biomass in comparison to control treatment. Plant biomass was in order ZnO> ZnEDTA≈$ZnSO_4$≈ Zn mix> control (Figure 5.1). Compared to control, plant biomass was six times greater for ZnO treatment.

Concerning plant Zn concentrations, ZnO and ZnEDTA treatments were significantly different than $ZnSO_4$ and control treatments. No significant differences were observed among ZnO, Zn mix (60% ZnO), and ZnEDTA. Greater plant Zn concentration in ZnEDTA was reflected by greater soil water Zn (Figure 5.2) and greater Zn distribution observed in greenhouse study (Figure S5.5, S5.6) and incubation study (Figure S5.23). Greater plant Zn concentration in ZnO treatment implies that Zn solubility is not the sole factor responsible for Zn availability to plants. Our results showed despite being sparingly soluble, ZnO tends to have greater efficacy in calcareous soils. This can be due to the high extractable Zn present in ZnO treatment (Figure S5.7). Haslett et al. (2001) also observed greater plant Zn concentrations for ZnEDTA and ZnO treatments than $ZnSO_4$.

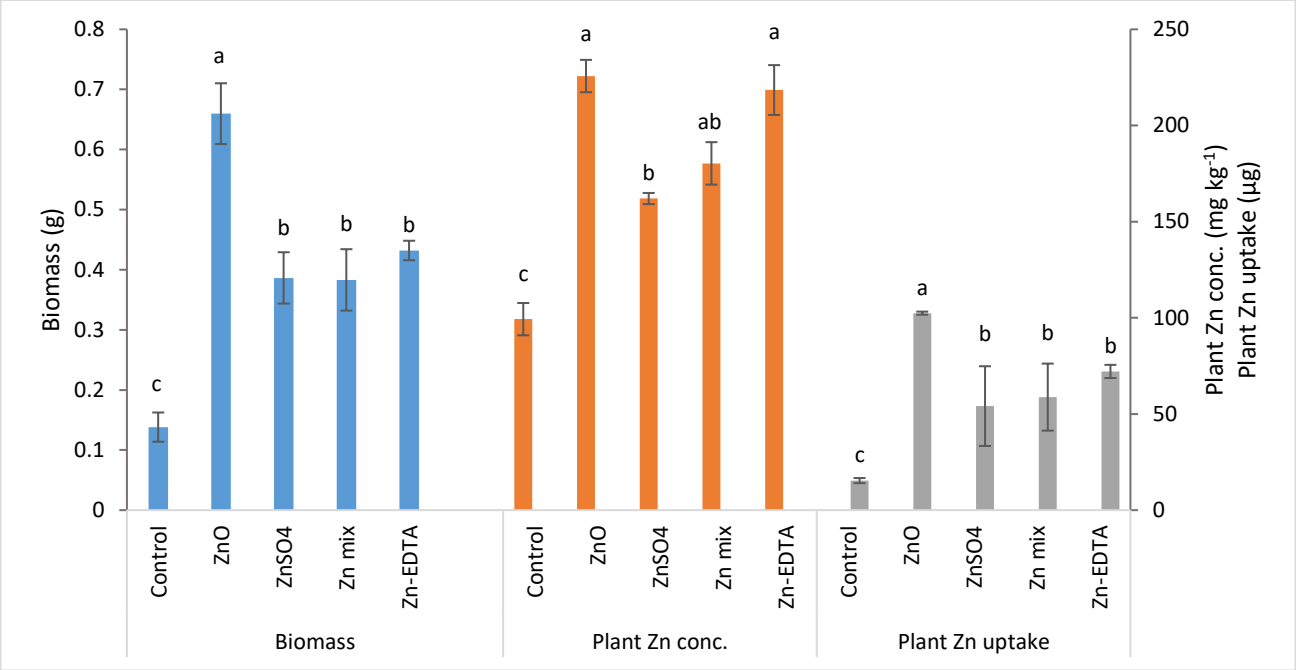


**Figure 5.1 Plant Zn uptake, plant Zn concentration, and biomass results for the calcareous soil greenhouse study. Plant biomass is plotted against the primary axis, and plant Zn uptake and concentration are plotted against the secondary axis. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means within response variable containing the same letter are not significantly different at $P \leq 0.05$ according to LS-means. Error bars represent the standard error of three replicates.**

The poorer performance of $ZnSO_4$ compared to ZnO can be due to its rapid fixation when applied to calcareous soil (Martín-Ortiz et al., 2008). The presence of active $CaCO_3$ induces sorption of soluble $ZnSO_4$ followed by Zn precipitation as zinc carbonate or insoluble zinc-hydroxide (Rico et al., 1996). Whereas, on the other hand, high CEC can buffer soil pH increase because of ZnO dissolution (which limits Zn solubility) and provides a sink (through sorption) for $Zn^{2+}$ ions released through the dissolution process, thereby complete dissolution of ZnO. Similar results were observed in our previous study, where different calcareous soil was used. In the previous study, we observed that plant biomass was significantly greater for ZnO treatment than $ZnSO_4$, and no significant differences were observed concerning plant Zn uptake and Zn concentrations between these two treatments. Plant Zn uptake is the product of biomass and plant Zn concentration Plant Zn uptake was greatest for ZnO. Plant Zn uptake was increased by 400% when Zn was added as ZnO. Due to high biomass and plant Zn concentration, great amount of

plant Zn uptake was observed in ZnO treatment. Referring to water extractable results (Figure S5.22), we can infer that solubility of ZnO was low which led to its slow-release effect and hence low chances of Zn getting adsorbed or precipitated.

#### *5.3.1.1.2 Soil water results*

Soil water collected by rhizon samplers was analyzed for soil water Zn via ICP-MS, which measured all dissolved species of Zn in soil solution, including ZnEDTA complexes. For the end of the third week, soil water Zn was significantly greater for ZnEDTA treatment compared to all other treatments (Figure 5.2). This is not surprising, as chelated-Zn is capable of avoiding fixation and stay in soil solution. Norvell and Lindsay (1969) also reported that ZnEDTA was stable in alkaline soils. Therefore, the availability of Zn in ZnEDTA would not likely be limited by the instability of the complex. Wallace and Romney (1970) concluded that ligands like EDTA remain almost entirely in the soil solution and move staying in the liquid phase (Cheng et al., 1970). Zinc oxide, Zn mix (60% ZnO), and $ZnSO_4$ did not show any significant differences despite the high solubility of $ZnSO_4$. Soil water Zn is an outcome of a few different processes. Dissolution, sorption, and plant uptake. For the end of the fifth week, all Zn fertilizers were significantly greater than control. This response might be because of the mentioned processes. Additional Zn remained in soil water after 5 wk of plant uptake. At week 3, for $ZnSO_4$, Zn seems to be dissociated to cationic Zn form and got adsorbed onto soil components/clay minerals. The increased Zn at the fifth week can be because of the adsorbed Zn might have been desorbing due to plant growth and rhizosphere induced changes (such as reduced pH), and Zn desorption increased soil water Zn in Zn treatments. This did not work in case of control because no additional Zn was added in that treatment. Therefore, native Zn uptake by the plant was most likely negated the increased Zn availability due to rhizosphere effects.

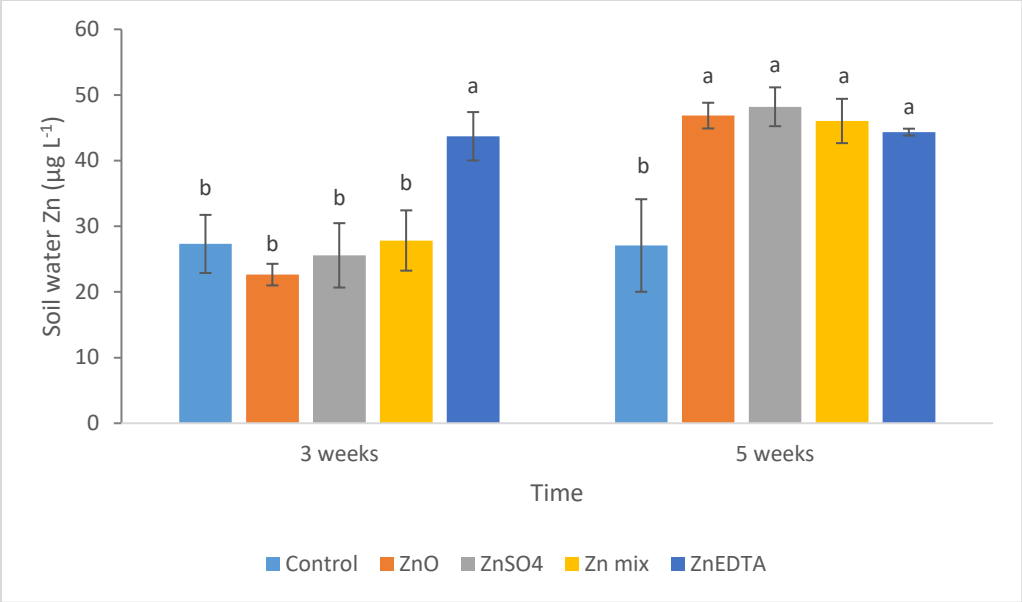


**Figure 5.2 Soil water Zn in calcareous soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a time period containing the same letter are not significantly different at $P \leq 0.05$ according to Tukey pairwise method. Error bars represent the standard error of three replicates.**

#### *5.3.1.1.3 Soil pH*

Soil pH was analyzed at the end of the experiment after plant harvest. Soil pH, being a very sensitive parameter, might have changed a lot in five weeks. We did not see any significant differences for any of the Zn fertilizer treatments as compared to the control in the end (Figure S5.7). This can be due to the high pH buffering capacity of this soil. Zinc fertilizer addition might have influenced the soil pH, but it changed/returned to the original value because of the high CEC and clay content for this soil. Soil pH effects for respective treatments were better studied with incubation experiment done using this calcareous soil due to the greater Zn rate used for the study (section 5.3.3.2).

#### *5.3.1.1.4. Zinc diffusion*

The vast majority of Zn added in this calcium-rich soil remained within the section of the application. Comparing Zn treatments, there were no significant differences among the treatments for the application section (section 2) (Figure S5.5). For section 1, the percent Zn recovered was significantly greater for ZnEDTA than other Zn treatments directing its diffusion to this section of the soil. This may be due to the very little or no interaction of chelated Zn source (ZnEDTA) between soil components preventing various reactions responsible for Zn loss as compared to soil treated with $ZnSO_4$, which results in greater fixation and adsorption of Zn (Giordano and Morvedt, 1973). For section 3, the percent Zn recovered was significantly greater for ZnO treatment. This is due to the soil driving its dissolution and therefore increasing Zn mobility, as mentioned above in section. For section 4, the percent Zn recovered in the Zn mix was significantly greater from ZnO and ZnEDTA. The percentage of Zn recovered from this section was also greater for $ZnSO_4$, even though it was not statistically different from ZnO and ZnEDTA. To the author's knowledge, one reason can be Zn moved to this section for these treatments, but it remained in adsorbed and fixed forms which are neither available nor DTPA extractable.

#### *5.3.1.1.5 Extractable Zn*

In general, DTPA extractable Zn for control soil (0.95±0.04 mg $kg^{-1}$) increased compared to initial DTPA Zn values for this soil (0.7±0.1 mg $kg^{-1}$). This increase in soil DTPA Zn might be attributed to plant growth that might have induced rhizosphere induced changes (such as acidification). Root-induced soil pH changes in the rhizosphere result from the balance between $H^+$ and $HCO_3^-$ ($OH^-$) excretion (Hinsinger et al., 2003). This balance of $H^+$ and $HCO_3^-$ ($OH^-$) plant roots depend on the cation/anion uptake ratio (Tang and Rengel, 2003; Jing et al., 2012). Greater

the $H^+$ excretion, greater absorption of cations over anions and this results in rhizosphere acidification.

Zinc fertilization significantly improved DTPA extractable Zn content in the soil, irrespective of the Zn source in some sections (Figure S5.7). The application of EDTA-chelated Zn was found to be superior in terms of increasing DTPA-extractable Zn. Zinc remained DTPA extractable even in the sections away from the point of the application when applied as the chelated source. For sections 4 and 7, DTPA-Zn for ZnEDTA treatment was significantly greater than control and all other Zn treatments. Both ZnO and $ZnSO_4$ treatment showed greater extractability for sections 1, 2, and 3, i.e., the section of the application and sections closer to the application. Extractable Zn for these sections for ZnO and $ZnSO_4$ was comparable to ZnEDTA, implying that even though Zn did not move much for these treatments, it remained extractable. Previously, it has been reported that the availability of Zn from ZnO and $ZnSO_4$ is similar for calcareous soil containing 2.4 % of free $CaCO_3$ (Gómez et al., 2017). Various sorption reactions are known to drive ZnO dissolution towards completion by providing a sink to $Zn^{2+}$ ions released. These mechanisms are essential to study specifically in calcareous soils that can adsorb Zn onto soil components (McBeath and McLaughlin, 2014). The available Zn fraction is meager in calcareous soil, and therefore, the rate of Zn release from the oxides has a low influence in calcareous soil.

#### 5.3.1.2. Acid soil

##### *5.3.2.1. Plant biomass and uptake*

Zinc-only experiment

No significant differences among Zn treatments were observed for biomass, but $ZnSO_4$ and ZnEDTA had significantly greater biomass than the control (Figure 5.3). Greater plant

biomass for ZnEDTA can be associated with greater soil water Zn and Fe concentrations. (Figure 5.5). Enhanced micronutrients uptake might have helped to achieve greater plant biomass. In the literature, ZnEDTA was found to be a most effective source in acidic soil (Alvarez et al., 1996; López-Valdivia et al., 2002). Ortiz and Garcia (1998) also reported that fixation for chelated-Zn was much less than inorganic sources. Srivastava et al. (1999) studied the comparative efficiency of different Zn sources and observed that chelated-Zn is the most effective source out of all. Less Zn was fixed in soil when Zn was applied as ZnEDTA (Ortiz and Garcia, 1998; Chatterjee and Mandal, 1985; Mehdi et al., 1990; Srivastava et al., 1999). High plant biomass for $ZnSO_4$ could be a result of high-water solubility, extractability, and diffusion (Figure 5.5, S.5.18, S.5.15, respectively). Greater diffusion can be attributed to the ionic interaction between $Zn^{2+}$ and $SO_4^{2-}$ ($ZnSO_4^0$) in the soil solution (Santos et al. 2019). Several other researchers also reported that Zn diffusion in the soil is affected by the accompanying anions in the soil solution.

The application of Zn fertilizers significantly increased the Zn concentrations and plant Zn uptake (Figure 5.3). Observed significant differences might be due to the increased Zn in soil solution due to the applied Zn that increased Zn acquisition by plants and therefore increased Zn concentration and consequently plant Zn uptake. In literature, several authors reported the same effectiveness for different Zn sources when applied to acid soil. Both zinc sulfate and zinc oxide were equally effective in acid soil (Brennan and Bolland, 2006). Gonzalez et al. (2008) reported that six organic Zn sources (ZnEDTA-HEDTA, Zn-HEDTA, Zn-*S, S*-EDDS, ZnEDTA, and Zn-EDDHSA) showed similar level of effectiveness in acidic soil.

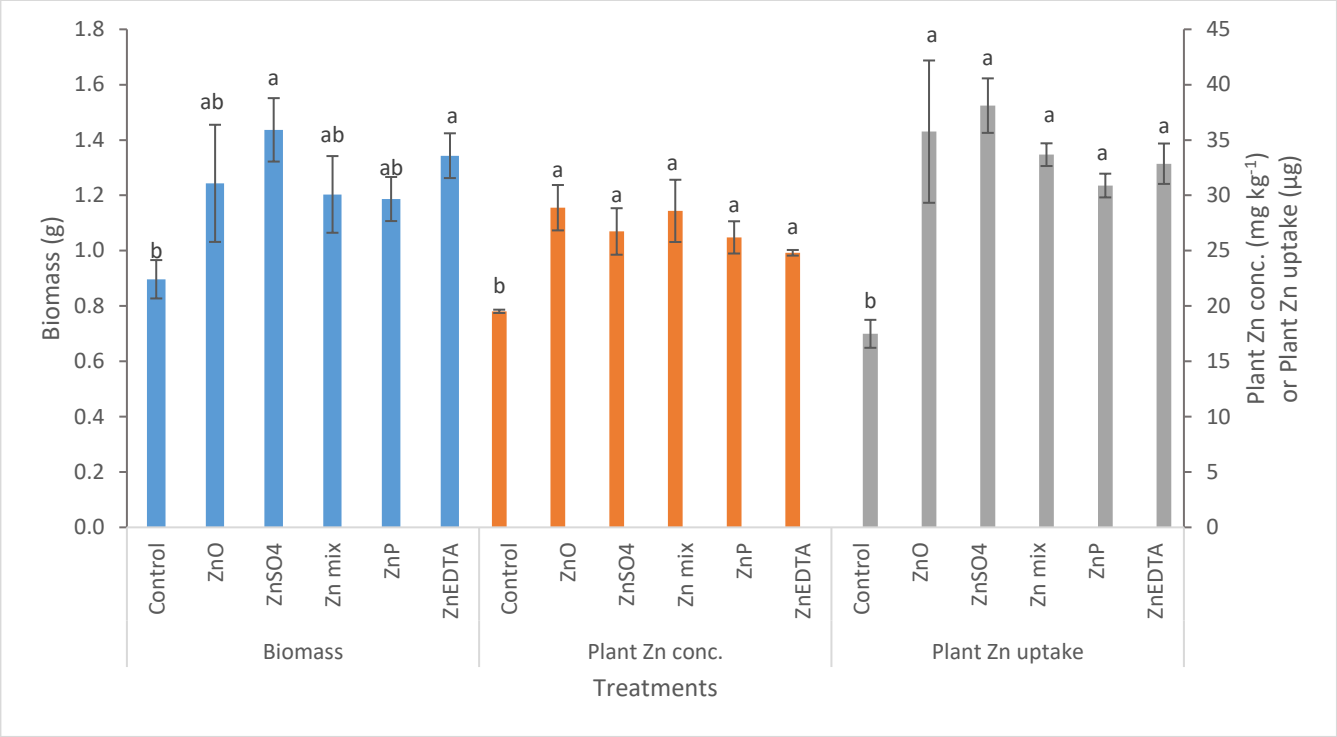


**Figure 5.3 Plant Zn uptake, plant Zn concentration, and biomass results for the acid soil greenhouse study. Plant biomass is plotted against the primary axis, and plant Zn uptake and concentration are plotted against the secondary axis. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means within response variable containing the same letter are not significantly different at $P \leq 0.05$ according to LS-means. Error bars represent the standard error of three replicates.**

Zinc coated urea experiment

For plant biomass, ZnEDTA-U had significantly greater plant biomass than all other treatments except ZnO-U (Figure 5.4). High soil water Zn indicated the high solubility of ZnEDTA-U (Figure 5.6). The greater extractability of ZnEDTA-U was also supported by greater DTPA extractable Zn results (Figure S5.19), suggesting high Zn diffusion and availability of this treatment which could have resulted in high biomass.

Although the difference was not significant, ZnO-U seems to be the second-best treatment based on plant biomass results. Greater biomass can be related to the synergistic

effects of Zn coated onto urea granules. Nano-ZnO coatings reported to reduce $NO_3$-N leaching by 35.1% (Jadon et al., 2018). The decrease in leaching losses from nano-ZnO-coated urea might be due to the antimicrobial properties of nano-ZnO particles (Espitia et al., 2012; Singh and Nanda, 2013). The antimicrobial properties might have influenced the microbial activities that decreased urea hydrolysis and nitrification rates. Several other studies supported the antibacterial activities of nano-ZnO (Buzea et al., 2007; Nair et al., 2011; Raghupathi et al., 2011). The authors assume that the bulk ZnO-coated urea granules used for this study exerted the similar mechanism. In addition to this, some researchers observed that coated fertilizers could increase nutrient availability (Dong et al., 2016) and improve water use efficiency of the production system (Zhang et al., 2017) due to their slow-releasing capacity and tendency to make nutrients available in the soil for prolonged periods. However, this was beyond the scope of the current investigation but warrants further investigation. Plant biomass was significantly lower for urea treatment than control, and all Zn-coated urea treatments. First, this response can be due to the decreased extractability of native Zn as shown in DTPA extractable Zn results (Figure S5.19), and due to greater pH was recorded for urea treatment (Figure S5.9). The second reason can be due to the saturated urea at the application point for the urea treatment.

Although control and urea received equal amounts of nitrogen as urea, the significant difference in biomass can be because of the application method; nitrogen added as basal dose for the control treatment by mixing it with soil, whereas it was added at one location for urea treatment. The addition of urea to one point of application (i.e., without mixing in soil) can result in greater losses by volatilization and other losses versus its mixed application.

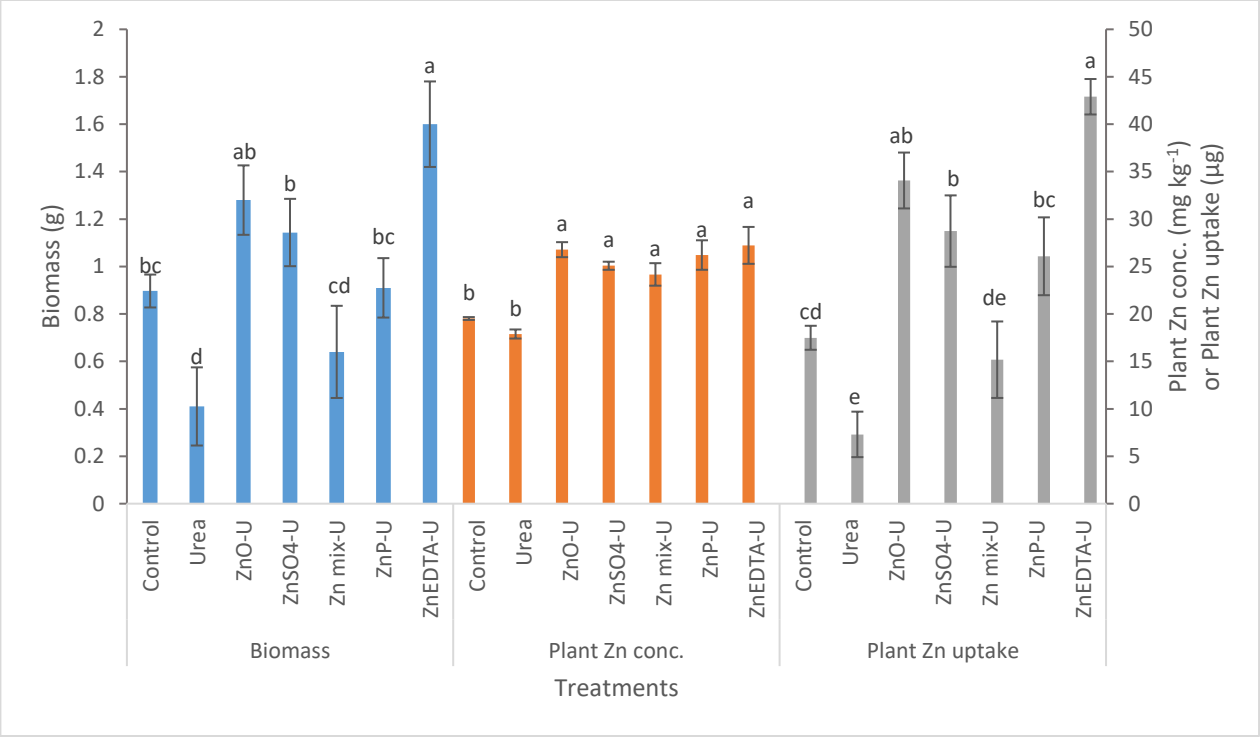


**Figure 5.4 Plant Zn uptake, plant Zn concentration, and biomass results for the acid soil greenhouse study. Plant biomass is plotted against the primary axis, and plant Zn uptake and concentration are plotted against the secondary axis. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means within response variable containing the same letter are not significantly different at $P \leq 0.05$ according to LS-means. Error bars represent the standard error of three replicates.**

Another noteworthy observation was, even though Zn rate used for Zn-coated urea treatments was half the rate used for Zn only sources, plant Zn uptake was comparable for these two. It can be due to the ammonium ions released upon hydrolysis of urea. Wang and Harrell (2005) showed that $NH^{4+}$ decreased Zn sorption; $NH^{4+}$ yielded 4 to 12% more adsorbed Zn in the labile pool than $K^{+}$ in acid soils. For Zn concentration, all Zn-coated urea treatments were significantly different from urea-control and control.

Zinc fertilizers increased available Zn as in soil water Zn concentrations (Figure 5.6), therefore enhancing Zn absorption by plants. Zinc uptake of ZnO-U and ZnEDTA-U treatments

were significantly greater than all other treatments except for $ZnSO_4$ treatment. The greater biomass for these treatments led to increased plant uptake as well.

#### *5.3.1.2.2. Soil water Zn*

Zinc only experiment

At the end of the third week, soil water Zn was significantly greater for ZnEDTA treatment than all other treatments besides $ZnSO_4$ (Figure 5.5). This response is due to the greater water solubility for ZnEDTA and $ZnSO_4$ (Parsad et al., 1975; Alvarez et al., 2006; Westfall et al., 1999) than ZnO, ZnP and Zn mix. Low soil water Zn in ZnP can be explained by presence of Zn and phosphate together. Co-localization of both might have affected the Zn mobility due to enhanced Zn sorption in the section of application.

At the end of the fifth week, the soil water Zn remained significantly greater for ZnEDTA than all other treatments (Figure 5.5) due to the same reasons discussed before. Soil water Zn was greatest for ZnEDTA followed by ZnO. An additional reason for reduction of soil water Zn for $ZnSO_4$ at the fifth week is the greater available Ca amounts competed with Zn for cation exchange sites on the soil colloids, and much of the non-exchangeable Zn probably precipitated. This was confirmed by significantly greater water-soluble Ca for $ZnSO_4$ treatment for both the third and the fifth week of the study (Figure S5.10). Another interesting observed result was significantly greater soil water Fe for ZnEDTA treatment for both the third and the fifth week of the study (Figure S5.12). As mentioned before, this suggested formation of FeEDTA at the expense of some added ZnEDTA (and other micronutrients), enhancing their availability. Low soil water Zn for ZnP treatment can be explained by the presence of Zn and P. Wang and Harrell (2005) reported that Zinc sorption was enhanced by $H_2PO_4^-$ in acid soils. Cristina et al. (2011) reported that in acid soils, the largest Zn sorption was observed with $H_2PO_4^-$. Therefore, greater

Zn amounts were sorbed as a result of the presence of P and moreover even greater in co-localization of phosphorus, enhancing Zn sorption in the section of application.

Zinc coated urea experiment

For Zn-coated urea treatments, similar trends were observed for soil water Ca (Figure S5.11) and soil water Fe (Figure S5.13). Soil water Ca concentrations were significantly greater for $ZnSO_4$-U treatment as compared to other Zn treatments. Similar reasons are expected behind this response as mentioned above in Zn-only sources. Soil water Fe was observed to be significantly different for ZnEDTA-U treatment. Surprisingly, soil water Zn was significantly greater for ZnO-U and $ZnSO_4$- U treatments than all other treatments (Figure 5.6). As discussed previously, Zn-coated urea seems to work via a synergistic mechanism, i.e., benefiting each other. Zn coating might be helping urea to release slowly, and acidification caused by urea might be helping Zn to become more available and more soluble. At the end of the fifth week, among all Zn-coated urea treatments, soil water Zn was significantly greater for ZnO-U and ZnEDTA-U than Zn mix-U, ZnP-U, urea, and control treatment (Figure 5.6). Zinc mix-U seems to undergo adsorption and precipitation reactions making Zn less soluble. Low soil water Zn for ZnP-U can be due to its Zn species. As per XANES results from a previous study done for calcareous soil, hopeite was the dominant Zn species when ZnP was applied as ZnP coated urea. Water solubility of hopeite is very low which leads to low soil water Zn observed. Other reasons can be increased Zn sorption due to presence of P, discussed in detail above.

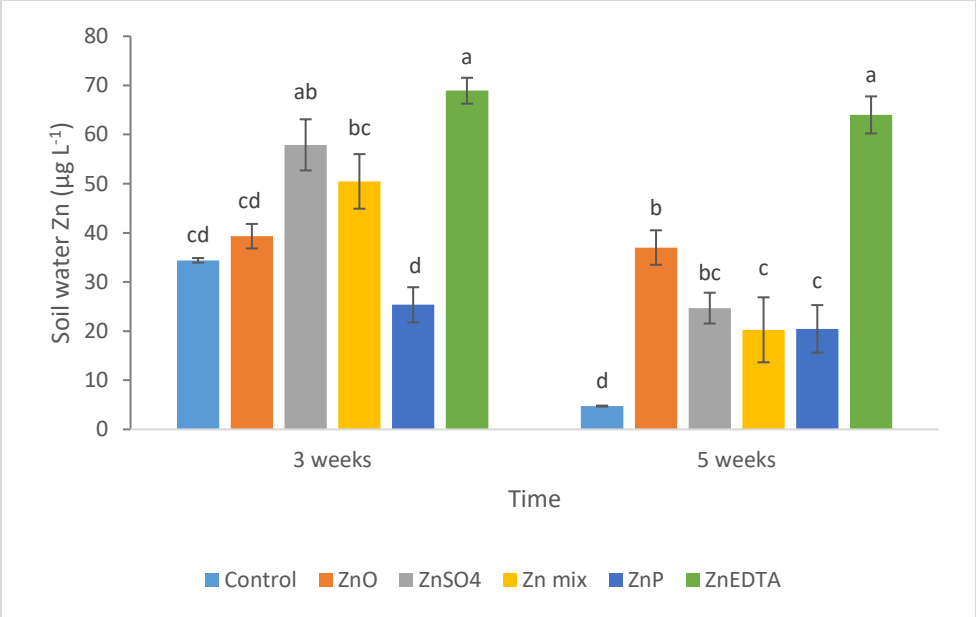


**Figure 5.5 Soil water Zn for Zn only treatments for the acid soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a time period containing the same letter are not significantly different at $P \leq 0.05$ according to Tukey pairwise method. Error bars represent the standard error of three replicates.**

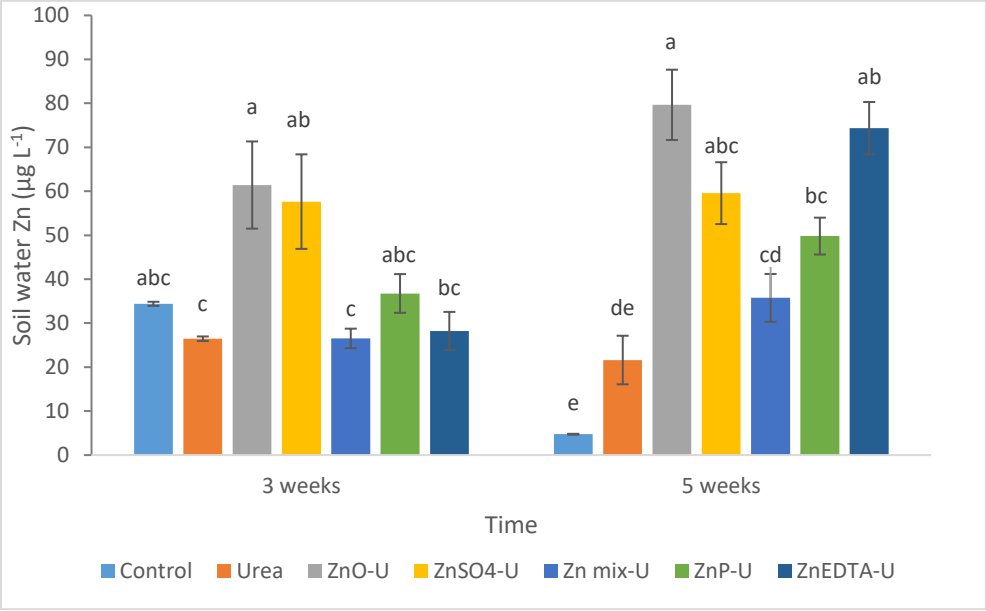


**Figure 5.6 Soil water Zn for Zn-coated urea treatments for acid soil greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U=**

**zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a time period containing the same letter are not significantly different at $P \leq 0.05$ according to Tukey pairwise method. Error bars represent the standard error of three replicates.**

#### *5.3.1.2.3. Soil pH*

Zinc-only experiment

The effect of pH on the activity of Zn in solution in acidic soils was observed to decrease with increasing pH (Saeed and Fox, 1977). A 30-fold decrease in Zn concentration was reported for each unit increase in pH between 5 and 7 in acidic soil (McBride and Blasiak, 1979). The gradual decrease in Zn activity as pH increases was related to increasing CEC. In the present study, soil pH was significantly greater for ZnO treatment than ZnEDTA treatment in the section of Zn application (Figure S5.8). Both treatments were not significantly different from control. Still, these pH changes are known to affect the behavior and availability of Zn in the soil, as mentioned above. The possible reasons for the increase in soil pH for ZnO are discussed in section 5.3.3.2. Also, soil pH for ZnEDTA treatment was significantly different from control for section 4. This pH decrease in ZnEDTA is due to the presence of EDTA as the complexing agent. The reason behind EDTA addition reducing the pH is its low pka values- 0, 1.5, 2, 2.66, because of the deprotonation of four carboxyl groups (Coleman, 2008). The addition of EDTA leads to the deprotonation of all four carboxyl groups, releasing 4 protons, thereby decreasing soil pH.

Zinc coated urea experiment

For Zn-coated urea, no significant difference between treatments and both controls (control and urea) were observed for section 1 (Figure S5.9). For section 2, urea, ZnO-U, and ZnP-U treatments were significantly different from the control treatment. This can be due to two

reasons. First is urea hydrolysis and conversion of ammonia to ammonium. The conversion reaction involves taking two protons from the soil and thereby increasing soil pH.

$CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2$

$2NH_3 + 2H^+ \rightarrow 2NH_4^+$

Second, when urea is added to soil, it leads to the formation of bicarbonate and ammonium-N. The bicarbonate could react with $H^+$ ions in the soil solution, which reduces the acidity and increases pH. The greater pH in the case of ZnP-U might be due to the ZnP dissolution, consumption of H+, or release of $OH^-$ ions which increase soil pH. (Su et al., 2019)

$Zn_3(PO_4)_2.4H_2O \rightarrow 3Zn^{2+} + 2HPO_4^{2-} + 2OH^-$

The reason for greater pH for ZnO-U can be due to urea hydrolysis as mentioned above and ZnO dissociation as discussed in section 5.3.3.2.

***5.3.1.2.4. Zinc diffusion***

Zinc only experiment

The Zn movement was more rapid in the acid soil than in the calcareous soil. The total concentration of Zn registered in each section of the container reflected a great difference between the behaviors of the Zn fertilizers in this soil. When Zn was applied as ZnO, Zn mix, and ZnP, most of the added Zn remained in the section of application, although Zn concentrations in the first and third sections also slightly increased (Figure S5.14). The application of these fertilizers resulted in an accumulation of Zn in the application section. Neither of the ZnO, Zn mix, and ZnP treatments resulted in sufficient Zn mobility to distribute Zn throughout the sections and thereby did not transport it to the sections farther away from the point of application. This behavior might be due to the retention of Zn by the solid phase of the soil in the second section where these were applied or might be due to their low solubilities.

Lower solubility of ZnP, can be due to the presence of phosphorous in the fertilizer. Adding ZnP, enhanced sorption due to co-localization of P and therefore increased Zn adsorption of Zn in the section of application can negate the positive effects of ZnP. In acid soil, the presence of phosphate can either increase (McBride, 1985) or decrease (Clark and McBride, 1984) Zn mobility depending on its affect over pH (Barrow, 1987). In the present study, although not significant but ZnP was observed to increase the soil pH upon application, therefore resulted in reduced Zn mobility (section 5.3.2.3). Zinc applied as ZnEDTA and $ZnSO_4$ moved through sections 1, 3, and 4, although the differences were not significant for the third section for $ZnSO_4$ and the fourth section for both ZnEDTA and $ZnSO_4$. Similar to extractable Zn results, Zn mobility was greatest when ZnEDTA was applied. Adriano (2001) observed that high clay content causes Zn immobilization via the formation of hydroxides and Zn adsorption on clays. The acidic pH-induced greater solubilities to the applied Zn fertilizers probably played a role in retaining metals in the soil layers.

Zinc coated urea experiment

Zinc diffusion results complemented the plant biomass results. Out of all the treatments, ZnEDTA-U treatment permeated out to a larger soil volume (Figure S5.16). The percent Zn recovered from the point of application (section 2) was significantly lower for ZnEDTA-U treatment, followed by $ZnSO_4$-U treatment which refers to the movement of ZnEDTA-U to other sections of the container. A significantly greater percent Zn added was recovered from section 3 for ZnEDTA-U treatment than all other treatments. This explains the greater effectiveness of ZnEDTA-U treatment. A considerable amount of Zn was observed in section 4 for ZnEDTA-U, $ZnSO_4$-U, and Zn mix-U, although only ZnEDTA-U was significantly different from ZnO-U and ZnP-U. Reasons for ZnP-U reducing Zn movement are discussed above.

#### *5.3.1.2.5. Extractable Zn*

In general, the DTPA-Zn was reduced for this soil as compared to its initial DTPA Zn value. The decrease of Zn availability has been reported by several studies (Das and Mandal, 1988; Dutta et al., 1989). Soluble $Zn^{2+}$ can get adsorbed onto oxide minerals, organic matter, and clay minerals.

Zinc only experiment

For Zn-only treatments, all Zn treatments were significantly greater than the control for section 2 (Figure S5.18). For section 1, a significantly greater DTPA Zn was observed for ZnEDTA treatment for all treatments except for $ZnSO_4$. For ZnEDTA treatment, DTPA Zn was significantly greater than all other treatments for section 3. The results suggest greater availability of Zn for ZnEDTA treatment followed by $ZnSO_4$. Mehdi et al. (1990) observed that the relative effectiveness of Zn sources was in the order- ZnEDTA > Zn $(NO_3)_2$ > $(NH_4)_2ZnO_2$ > $ZnSO_4$ > $ZnCl_2$. The results might also be because chelated Zn and $ZnSO_4$ have greater water solubility and are readily available, making their effects visible in the DTPA-extractable Zn content in the soil. In contrast, ZnO, Zn mix (60% ZnO), and ZnP are sparingly soluble and is not readily available; confirmed by soil water results (Figure 5.5). The water solubility of Zn sources is considered an essential criterion for Zn availability (Slaton et al., 2005; Shaver et al., 2007; Shivay et al., 2008).

Zinc coated urea experiment

For Zn-coated urea treatments, The DTPA Zn for urea treatment was significantly lower than all the treatments for sections 1, 2, 3, and 4 (Figure S5.19). Greater pH was recorded for urea treatment might have limited the availability of native Zn. Stahl and James (1991) reported that Zn activity decreases gradually with an increase in soil pH. The ZnEDTA-U and $ZnSO_4$-U

showed significantly greater DTPA Zn for section 1 compared to all other treatments. For section 2, DTPA Zn was significantly lower for ZnEDTA-U treatment suggesting its movement to other sections of the container; also confirmed by total Zn results (Figure S5.17). For section 3, the ZnEDTA-U treatment had significantly greater DTPA extractable Zn than ZnO-U, Zn mix-U, ZnP-U, urea, and control. For this section, pH recorded for ZnEDTA-U was significantly lower as compared to ZnO-U and Urea (Figure S5.9). McBride and Blasiak (1979) observed that Zn concentrations were decreased by 97% for each unit increase in soil pH between 5 and 7. For section 4, DTPA Zn for ZnEDTA-U treatment was statistically greater than all other treatments, suggesting greater Zn diffusion and availability for this treatment. In the present study, the available Zn fraction diminished as we moved away from the application point. The decreasing level of DTPA extractable Zn for ZnEDTA-U for father sections can be because of the plant uptake. Plant uptake of Zn resulted in lower DTPA Zn concentrations in soil. The decreasing trend was prominent in ZnEDTA-U and can be attributed to ZnEDTA being highly water-soluble and has zero charges on it. Therefore, most of it remained in labile form, making it most mobile and available. Other interesting results were observed for Zn mix treatment, no significant differences were observed for Zn mix-U and $ZnSO_4$-U for section 3 (close to the plant) (Figure S5.19). This confirms our hypothesis that $ZnSO_4$ species of Zn shows dominance when Zn mix applied as Zn mix coated urea. This was also confirmed by XANES results from a previous study done using calcareous soil.

### 5.3.2. Incubation study- Calcareous soil

#### 5.3.2.1. Visualization

Zinc diffusion can be observed for four visualization times- for days 1, 14, 21, and 35 (Figure 5.7). The pink zone was not perfectly symmetrical around the application point.

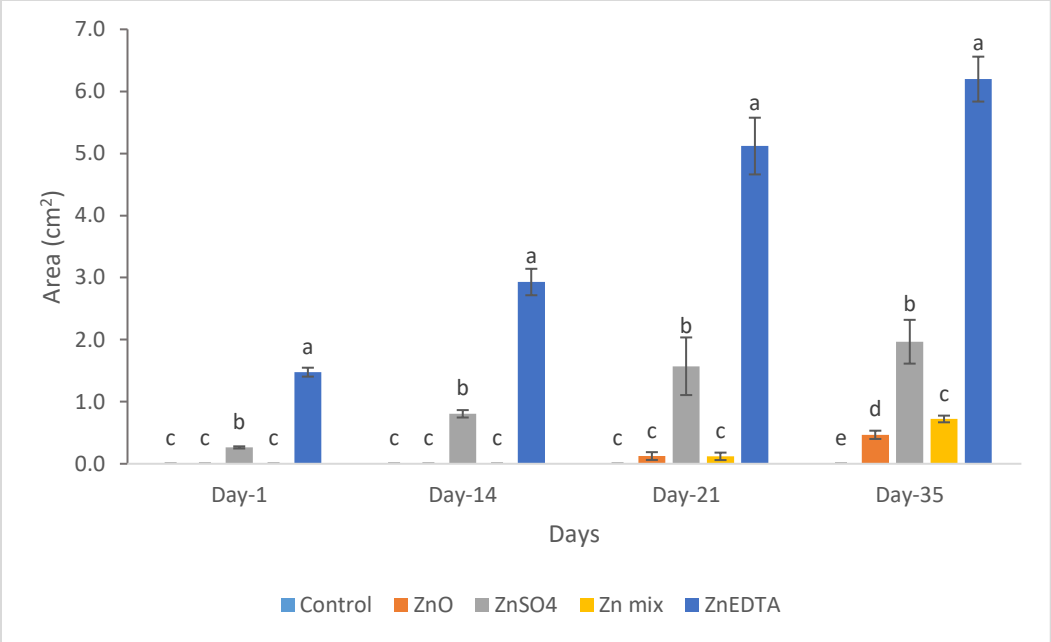


**Figure 5.7 Area quantified for the Zn zone using GIMP 2.10 software at day 1, 7, 21, and 35. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means within a time period for each treatment containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

This asymmetry can be due to the differences in pore distribution or heterogeneity in soil mineral distribution. Data analyzed using GIMP 2.10.20 showed that Zn in the case of ZnEDTA diffused farther from the point of application than other treatments (Figure S5.20). Zinc diffusion was in order ZnEDTA > $ZnSO_4$ > Zn mix > ZnO (Figure 5.7). Due to greater water solubility and availability of ZnEDTA (Figure 5.9), greater portion of Zn was available to react with $CaCO_3$ impregnated paper, which gave a pink color zone highlighting the Zn movement in this soil. The water solubility of $ZnSO_4$ is 99.9% at pH 6.3 to 7.4 (Westfall et al., 1999). Greater water solubility helped Zn to diffuse farther from the application point for $ZnSO_4$ treatment. Low Zn movement in ZnO and Zn mix treatments can be due to the lower water solubility and greater soil pH. The resultant soil pH for these treatments was more elevated than $ZnSO_4$ and ZnEDTA

(Figure 5.8), which might have contributed to their slow dissolution as well as increased sorption (Alloway, 2009; Rashid and Ryan, 2004).

#### 5.3.2.2 Soil pH

Soil pH was consistently lower for $ZnSO_4$ treatment and greater for ZnO and Zn mix treatments (Figure 5.8). However, these differences were not significant except in section 3, in which the $ZnSO_4$ treatment was significantly different from all other Zn treatments and the control soil. The lower pH in $ZnSO_4$ is because when $ZnSO_4$ comes in contact with water, being highly soluble, it is hydrolyzed to form sulfuric acid and zinc hydroxide. Sulfuric acid is a strong acid, while zinc hydroxide is a weak base; hence, the solution is acidic or it can be due to the remaining sulfuric acid (Wang and Dreisinger, 1998)

$$ZnSO_4 + H_2O \rightarrow H_2SO_4 + Zn(OH)_2$$

The reason behind ZnO and Zn mix having greater soil pH is the dissociation of ZnO releasing $OH^-$ ions. Although Zn mix is a mixture of ZnO, $ZnSO_4$, and ZnEDTA but ZnO being the major component (60%) shows dominance in this treatment. Zinc oxide, on its dissociation, releases $OH^-$ ions which result in an increase in soil pH (Su et al., 2019)

$$ZnO + H_2O \rightarrow Zn^{2+} + OH^-$$

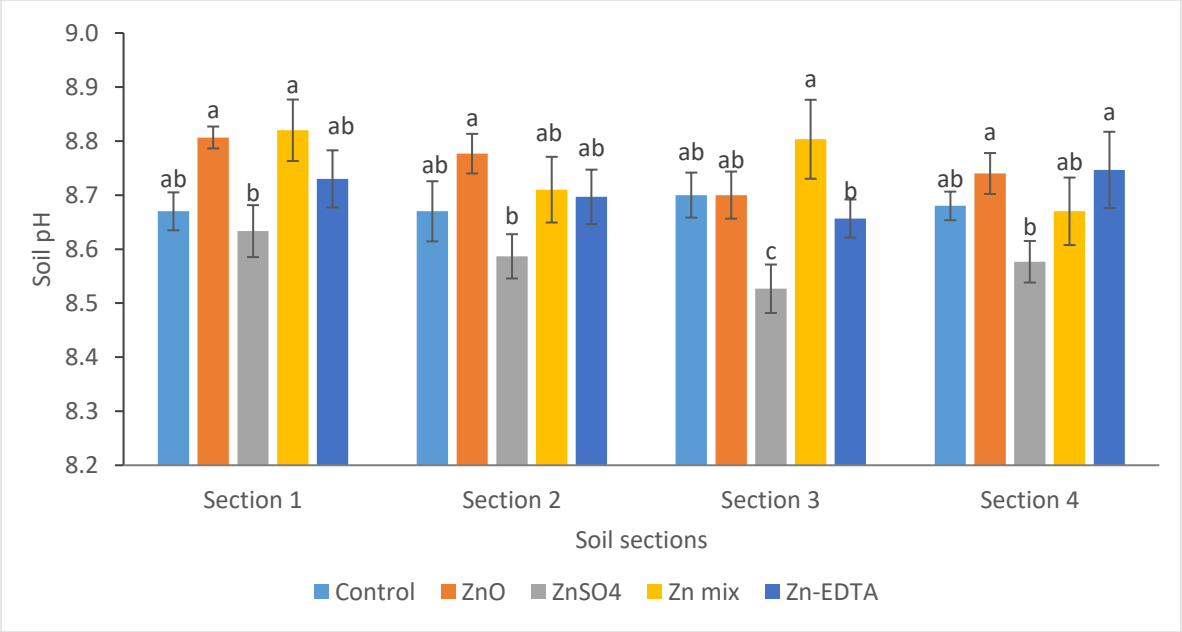


**Figure 5.8 Soil pH for the calcareous soil incubation study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

#### 5.3.2.3 Water extractable Zn

Water-extractable Zn was significantly greater for ZnO and Zn mix treatments than ZnEDTA, $ZnSO_4$, and control in the application section (section 1) (Figure 5.9). This implies that although Zn diffusion was low in ZnO and Zn mix as observed by visualization studies, yet Zn in the center section remained water extractable. Overall ZnEDTA treatment showed the greatest water extractability and water-extractable Zn was significantly greater for sections 2, 3, and 4 than all other Zn treatments and the control.

High Zn solubility also explains its greater migration/ movement to other sections. Zinc-EDTA can increase water-extractable Zn. Also, ZnEDTA can increase soil Zn concentrations in solution by converting solid-phase labile Zn into soluble Zn (Hamza and Sadnandan, 2005; Gupta and Deb, 1984; Parsad et al., 1975; Alvarez and Gonzlez, 2006).

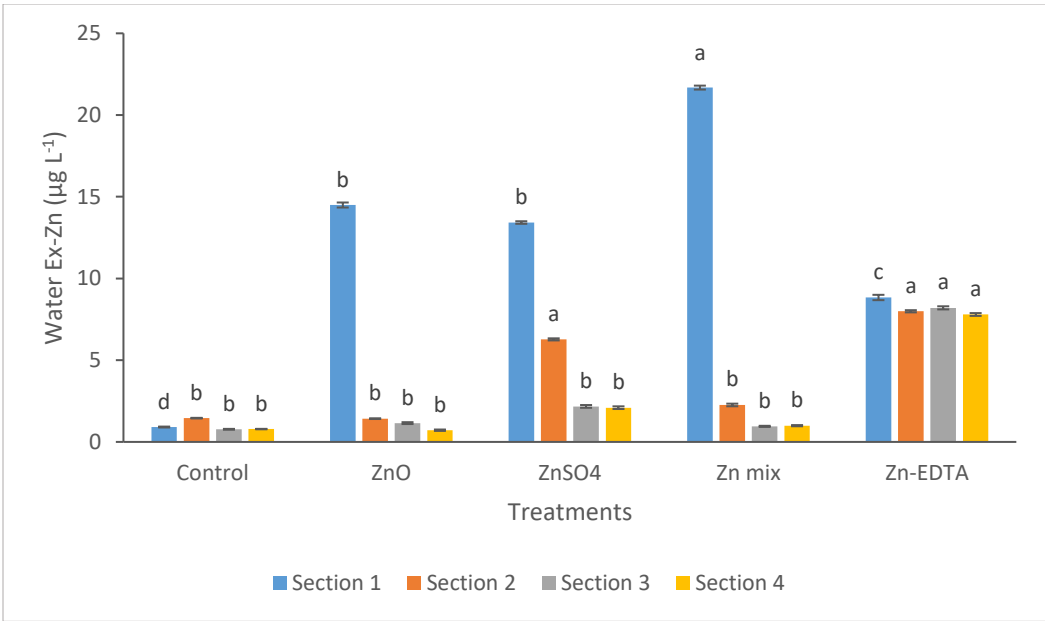


**Figure 5.9 Water extractable Zn for the calcareous soil incubation study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

#### 5.3.2.4 Zinc diffusion

For percent (%) total Zn, a significant part of the applied Zn remained in the application section. More than 70% of the added Zn for ZnO and Zn mix treatment recovered from the point of application, signifying the accumulation of Zn in this section (Figure 5.10). Zinc diffusion for the ZnO and Zn mix treatments was very low compared to $ZnSO_4$ and ZnEDTA. This response was due to the low solubility of ZnO and Zn mix (60% ZnO). In contrast, ZnEDTA treatment showed more Zn mobility. For ZnEDTA treatment, a significantly greater percentage of Zn was recovered from sections- second, third, and fourth for incubation studies, implying Zn moved through these sections in this soil. Thus, the product with the greatest Zn diffusion through this calcareous soil is ZnEDTA.

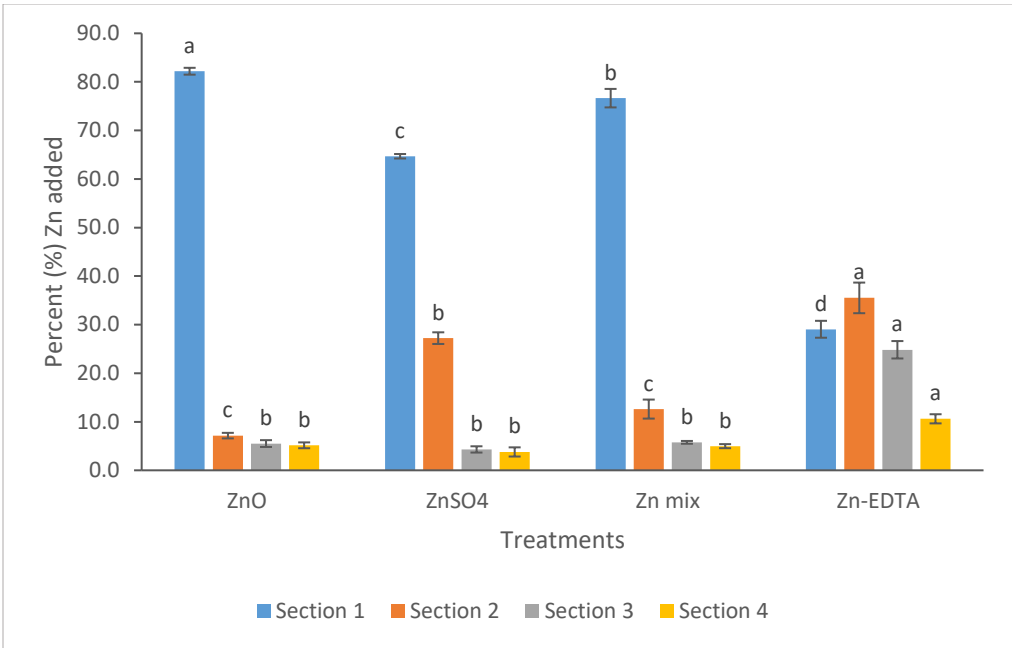


**Figure 5.10 Percent total Zn recovered from each section for the calcareous soil incubation study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

Several authors have noted the greater migration capacity of ZnEDTA fertilizer in different soils than other inorganic Zn sources such as ZnO and $ZnSO_4$. Increased mobility or increased shuttling of Zn by ZnEDTA was due to reduction of sorption reaction of Zn with soil colloids (Alvarez and Gonzalez, 2006). These results reflected in water-extractable Zn (Figure 5.9). Concerning $ZnSO_4$ treatment, Zn recovered was significantly greater than ZnO and Zn mix for section 2, but no significant differences were seen for sections 3 and 4. This shows that the Zn in $ZnSO_4$ migrates better than ZnO and Zn mix through the soil, but the concentration decreases with distance. This is not surprising as soil pH was not significantly reduced in section 4 (Figure 5.8), leading to cationic Zn adsorption and precipitation as $Zn(OH)_2$ or $Zn(CO)_3$, which are insoluble Zn species. The inverse relationship between pH and Zn

adsorption/precipitation also explains why a much greater percentage of Zn was recovered from a section of the application and reduced by increasing distance.

## 5.4 Conclusions

Zinc fertilizers added to different soils behave differently. Several adsorption and precipitation reactions control Zn fate in calcareous and acid soils. Zinc availability is hindered by formation of $ZnCO_3$ precipitates in calcareous soil (Wang and Harrell, 2005); Zn-Fe precipitates in both acid and calcareous soils (Scheinost et al., 2002); and Zn-phosphate precipitates in acid soil (Pérez-Novo et al., 2011). Fertilizers that can avoid the above-mentioned reactions are efficient Zn sources in these soils. According to our research, ZincEDTA and ZnO seem to be the best treatments that can efficiently mitigate Zn-deficiency in both the calcareous and acid soil tested. The tendency of ZnEDTA to stay in solution imparting increased available Zn and increased mobility helps Zn reach plant roots and thus, results in increased Zn uptake. On the other hand, zinc oxide seems to impart slow-release (in calcareous and acid soil) and more soluble when applied as coated onto urea (in acid soil). These properties of ZnO helps it to provide the required Zn amount over time, avoiding Zn adsorption and precipitation losses. Zinc rate used for Zn-coated urea treatments was half the rate used for Zn only sources, still soil water Zn and plant uptake values were quite comparable for Zn treatments for both forms due to the released $NH^{4+}$ ions that decreased Zn sorption and converted more adsorbed Zn to labile pool (Wang and Harell, 2005). In order to mitigate Zn-deficiencies by addition of Zn fertilizers, it is very important to wisely choose the most effective and efficient Zn source particular to soil type to enhance wheat yield and grain quality and hence profitability in a sustainable agriculture system.

## 5.5 References

Adriano, D. C. 2001. Trace Elements in the Terrestrial Environment, Springer-Verlag, NY.

Alloway, B.J. 2008b. Zinc in soils and crop nutrition. International Zinc Association & International Fertilizer Industry Association, Brussels, Belgium and Paris, France.

Alloway, B.J. 2009. Soil factors associated with zinc deficiency in crops and humans Environ. Geochem. Health. 31:537-548.

Allison, L. and C. Moodie. 1965. p. 149-157. Cation-exchange capacity. In A.G. Norman (ed.) Methods of Soil Analysis. Part-2. Chemical and Microbiological Properties. SSSA, Madison, WI.

Alvarez, J.M., M. I. Rico, and A. Obrador. 1997. Leachability and distribution of zinc applied to an acid soil as controlled-release zinc chelates. Comm. Soil Sci. Plant Anal. 28:17-18, 1579-1590.

Alvarez, J.M., and D. Gonzales. 2006. Zinc Transformations in Neutral Soil and Zinc Efficiency in Maize Fertilization. J. Agric. Food Chem., 54: 9488-9495.

Amrani M., D.G. Westfall, and G.A. Peterson. 1999. Influence of water solubility of granular zinc fertilizers on plant uptake and growth. J. Plant Nutr. 22:1815-1827.

Attanayake, C., G.M. Hettiarachchi, S. Martin, G. Pierzynski. 2015. Potential Bioavailability of Lead, Arsenic, and Polycyclic Aromatic Hydrocarbons in Compost-Amended Urban Soils. J. Environ. Qual. 44: 930-944.

Arai, Y., and D.L. Sparks. 2007. p. 135-179. Phosphate reaction dynamics in soils and soil components: a multiscale approach. D. Sparks (Ed.), Advances in Agronomy, Vol. 14, Academic Press, San Diego, CA.

Barrow, N.J., 1987. The effects of phosphate on zinc sorption by a soil. Journal of Soil Science. 38:453-459.

Buzea, C., I. Pacheco, and K. Robbie. 2008. Nanomaterials and Nanoparticles: Sources and Toxicity. Biointerphases 2. MR17-MR71.

Boawn, L. C. 1973. Comparison of zinc sulphate and Zn EDTA as zinc fertilizer sources. Soil Sci. Soc. Am. Proc. 37:111−115.

Brennan, R. and M. Bolland. 2006. Zinc sulfate is more effective at producing wheat shoots than zinc oxide in an alkaline soil but both sources are equally effective in an acid soil. Aus. J. Exp. Agr. 46.

Coleman, W.F. 2008. Molecular Models of EDTA and Other Chelating Agents. J. Chem. Edu. 85:1296.

Clark, C.J., and M.B. McBride, M.B. 1984. Adsorption of Cu (II) by allophane as affected by phosphate. Soil Sci. 38:412-421.

Das, D.K., and L.N. Mandal. 1988. Effect of puddling and different times of organic matter application before puddling on the availability of applied iron, manganese, copper and zinc in rice soils. Agrochimica. 32:327-336.

Degryse, F., R. Baird, and M. McLaughlin. 2015. Diffusion and solubility control of fertilizer-applied zinc: chemical assessment and visualization. Plant and Soil. 386.

Dong, Y., M. He, Z.L. Wang, W.F. Chen, J. Hou, X.K. Qiu, J.W. Zhang. 2016. Effects of new coated release fertilizer on the growth of maize. J. Soil Sci. Plant Nutr. 16.

Dutta, D., B. Mandal, L.N. Mandal. 1989. Decrease in availability of zinc and copper in acidic to near neutral soils on submergence. Soil Sci. 147: 187-195.

Espitia, P.J.P., N.F.F. Soares, and J.S.R. Coimbra. 2012. Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications. Food Bioprocess Technol. 5:1447-1464.

Giordano, P.M., and J.J. Mortvedt. 1973. Zinc Sources and Methods of Application for Rice. Agron. J. 65: 51-53.

García-Gómez, C., A. Obrador, D. González, M. Babín, and M.D. Fernández, M. D. 2017. Comparative effect of ZnO NPs, ZnO bulk and ZnSO4 in the antioxidant defenses of two plant species growing in two agricultural soils under greenhouse conditions. Sci. Total Environ. 589:11-24.

Gupta G.N. and D.L. Deb. 1984. Effect of chelating agents on zinc diffusion in two soils. J. Plant Nutr. Soil Sci. 147:533-539

Gonzalez, D., J. Novillo, M. I. Rico, and J. M. Alvarez. 2008. Leaching and Efficiency of Six Organic Zinc Fertilizers Applied to Navy Bean Crop Grown in a Weakly Acidic Soil of Spain. J. Agri. Food Chem. 56:3214-3221.

Hamon, R.E., M.J. Melauglin and G. Cozens. 2002. Mechanisms of attenuation of metal availability in in-situ remediation treatment. Environ. Sci. Technol. 36:3991-3996.

Hamza, S. and A.K. Sadanandan. 2005. Effect of source and method of application of zinc on yield and quality of black pepper (Piper nigrum L.). J. Spices Aromatic Crops. 14:117-121.

Haslett, B.S., R.J. Reid, and Z. Rengel. 2001. Zinc Mobility in Wheat: Uptake and Distribution of Zinc Applied to Leaves or Roots. Ann. Bot. 3:379-86.

Hao, H.L., Y.Z. Wei, X.E. Yang, Y. Feng, and C.Y. Wu. 2007. Effects of different nitrogen fertilizer levels on Fe, Mn, Cu and Zn concentrations in shoot and grain quality in rice (Oryza sativa). Rice Sci. 14:289-294.

Hodgson, J. F. 1963. Chemistry of micronutrient elements in soils. Adv. Agron. 15: 119-159.

Hinsinger, P., C. Plassard, and C. Tang. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant and Soil. 248:43-59.

Jadon, P., R. Selladurai, S.S. Yadav, M.V. Coumar, M.L. Dotaniya, and A.K. Singh. 2018. Volatilization and leaching losses of nitrogen from different coated urea fertilizers. J. Soil Sci. Plant Nutr. 18:1036-1047.

Jenkinson, D., and D. Powlson. 1976. The effects of biocidal treatments on metabolism in soil V: A method for measuring soil biomass. Soil Biol. Biochem. 8:209-213.

Karak, T., U.K. Singh, S. Das, D.K. Das, and Y. Kuzyakov. 2005. Comparative efficacy of $ZnSO_4$ and ZnEDTA application for fertilization of rice (*Oryza sativa* L.). Arch. Agron. Soil Sci. 51:253-264.

Kaya, C., D. Higgs, and A. Burton. 1999. Foliar application of iron as a remedy for zinc toxic tomato plants. J. Plant Nutr. 22:1829-1837.

Lindsay, W.L. 1979. Chemical Equilibrium in Soils. John Wiley & Sons, NY.

Lindsay, W.L. and W.A. Norvell. 1969. Equilibrium Relationships of $Zn^{2+}$, $Fe^{3+}$, $Ca^{2+}$, and $H^{+}$ with EDTA and DTPA in Soils. Soil Sci. Soc. Am. J. 33: 62-68.

Lopez-Valdivia, L.M., M.D. Fernandez, A. Obrador, and J.M. Alvarez. 2002. Zinc transformations in acidic soil and zinc efficiency on maize by adding six organic zinc complexes. J. Agric. Food Chem. 50:1455-1460.

Martín-Ortiz, D., L. Hernández-Apaolaza, and A. Gárate. 2009. Efficiency of a NPK fertilizer with adhered zinc lignosulfonate as a zinc source for maize (Zea mays L.). J. Agric. Food Chem. 57:9071-9078.

McBride, M.B. 1985. Sorption of copper(II) on aluminum hydroxide as affected by phosphate. Soil Sci. Soc. Am. J. 49:843-846.

McBride, M.B. and J.J. Blasiak. 1979. Zinc and Copper Solubility as a Function of pH in an Acid Soil. Soil Sci. Soc. Am. J. 43: 866-870.

McBeath, T. M., and M.J. McLaughlin. 2014. Efficacy of Zn oxides as fertilizers. Plant Soil. 374:843-855.

Mehdi, S.M., A.M. Ranjha, and T. Hussian. 1990. Relative efficiency of various sources of zinc. Sarhad J. Agric. 6:103-106.

Montalvo, D., F. Degryse, R.C. da Silva, R. Baird, and M.J. McLaughlin. 2016. Agronomic effectiveness of zinc sources as micronutrient fertilizer. Adv. Agron. 139:215-267.

Nair S., A. Sasidharan, V.V. Divya Rani, D. Menon, S. Nair, K. Manzoor, and S. Raina. 2009. Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. J. Mater Sci. Mater. Med. 20:235-241.

Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. U.S. Government Printing Office, Washington D.C.

Ortiz, E.M.E. and O.A. Garcia. 1998. Process of sorption of zinc in three soils of Colombia cultivated with rice (Oryza sativa) of the dry land variety Llano-5. In: Proceedings of the VIII Congress of Soil Sciences, Santa Marta, Colombia. Suelos-Ecuatoriales. 28:112-117.

Pérez-Novo, C., A. Bermúdez-Couso, J. López-Periago, D. Fernández-Calviño, and M. Arias-Estévez. 2011. Zinc adsorption in acid soils: Influence of phosphate. Geoderma. 162.358-364.

Prasad, B., M.K. Sinha, and N.S. Randhawa. 1975. Effect of mobile chelating agents on diffusion of zinc in soils. Soil Sci. 122:260-266.

Raghupathi, K., R. Koodali, A. Manna. 2011. Size-Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles. Langmuir: the ACS journal of surfaces and colloids. 27:4020-8.

Rashid, A., and J. Ryan. 2004. Micronutrient constraints to crop production in soils with Mediterranean-type characteristics: A review. J. Plant Nutr. 27: 959−975.

Rico, M.I., J.M. Alvarez, and J.I. Mingot. 1996. Efficiency of zinc ethylenediamine tetraacetate and zinc lignosulfonate soluble and coated fertilizers for maize in calcareous soil. J. Agric. Food Chem. 44: 3219-3223.

Saeed, M. and R.L. Fox. 1977. Relation between suspension pH and zinc solubility in acid and calcareous soils. Soil Sci. 124:199-204.

Santos, W.O., P.M. de Aquino, R.L.C. Cuesta, R. L. C., I.M.H. Saldanha, E.M. Mattiello, and L. Vergütz. 2019. Zinc diffusion and availability affected by different sources in soils of contrasting textures. J. Agri. Sci. 11:916-9752.

Scheinost, A. C., R. Kretzschmar and S. Pfister. 2002. Combining selective sequential extraction, X-ray absorption spectroscopy, and principal component analysis for quantitative Zn speciation in soil. Environ. Sci. Technol. 36:5021-5028.

Shaver, T., D. Westfall, and M. Ronaghi. 2007. Zinc Fertilizer Solubility and Its Effects on Zinc Bioailability Over Time. J. Plant Nutr. 30:123-133.

Shivay, Y.S., D. Kumar, and R. Prasad. 2008. Effect of zinc-enriched urea on productivity, zinc uptake and efficiency of an aromatic rice-wheat cropping system. Nutr. Cycl. Agroecosys. 81:229- 243.

Singh, P., and A. Nanda. 2013. Antimicrobial and antifungal potential of zinc oxide nanoparticles in comparison to conventional zinc oxide particles. J. Chem. Pharm. Res. 5:457-463.

Slaton, N.A., R.J. Norman, and C.E. Wilson. 2005. Effect of zinc source and application time on zinc uptake and grain yield of flood-irrigated rice. Agron. J. 97:272−278.

Soil Survey Staff. 2011. Burt, R. (Ed.). Soil Survey Laboratory Information Manual. Soil Survey Investigations. Report No. 45, Version 2.0. U.S. Department of Agriculture, Natural Resources Conservation Service. Washington, DC.

Srivastava, P., D. Ghosh, D., and V. Singh. 1999. Evaluation of different zinc sources for lowland rice production. Biol. Fertil. Soils. 30:168-172.

Stahl, R., and B. James. 1991. Zinc Sorption by B Horizon Soils as a Function of pH. Soil Sci. Soc. Am. J. 55.

Su, Y., J. Gao, Y. Qin, Y. Zheng, and D. Zhu. 2019. Interfacial Zinc Phosphate is the Key to Controlling Biocompatibility of Metallic Zinc Implants. Adv. Sci. 6.

Tang, C., and Z. Rengel. 2002. p. 71-96. Role of plant cation/anion uptake ratio in soil acidification. In Handbook of Soil Acidity. Ed. Z Rengel. Marcel Dekker, NY.

Viets, F. G. 1962. Chemistry and availability of micronutrients in soils. J. Agric. Food Chem. 10: 174−178.

Wallace, A. and E.M. Romney. 1970. The effect of zinc sources on micro-nutrient contents of golden cross bantam corn. Soil Sci. 109:66-67.

Wang, J.J., and D.L. Harrell. 2005. Effect of ammonium, potassium, and sodium cations and phosphate, nitrate, and chloride anions on Zinc sorption and lability in selected acid and calcareous soils. Soil Sci. Soc. Am. J. 69:1036-1046.

Wang, W., Breisinger, D.B. 1998. The acid-base behavior of zinc sulfate electrolytes: The temperature effect. Metall. Mater. Trans. B. 29:1157-1166.

Watson, M.E., and J.R. Brown. 1998. p. 13-17. pH and lime requirement. In J. R. Brown (Ed.), Recommended chemical soil test procedures for the North Central Region Missouri. Agricultural Experiment Station, University of Missouri, Columbia, MO.

Westfall, D.G., M. Amrani, and G.A. Peterson. 1999. Water-Solubility of Zinc Fertilizer, *Does It Matter?*

Westfall, D. G., W.J. Gangloff, G.A. Peterson, G. A., and J.J. Mortvedt. 2000. Organic and inorganic fertilizers: Relative availability. Technical Bulletin, TB01-1; Colorado Agricultural Experimental Station, CO.

Westfall, D., J. Mortvedt, G. Peterson, and W. Gangloff. 2005. Efficient and Environmentally Safe Use of Micronutrients in Agriculture. Commun. Soil Sci. Plant Anal. 36:169-182.

Wright, A. and J. Bailey. 2001. Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 Dry Combustion Analyzer. Commun. Soil Sci. Plant Anal. 32:3243-3258.

Zarcinas, B.A., M.J. McLaughlin, and M.K. Smart. 1996. The effect of acid digestion technique on the performance of nebulization systems used in inductively coupled plasma spectrometry. C. Soil Sci. Plant Anal. 27:1331-1354.

Zhang, S.G., Y.C. Yang, B. Gao, Y.C. Li, and Z.G. Liu. 2017. Superhydrophobic controlled-release fertilizers coated with bio-based polymers with organosilicon and nano-silica modifications. Journal Mater. Chem. A. 5:19943-19953.

## 5.6 Supplementary Information

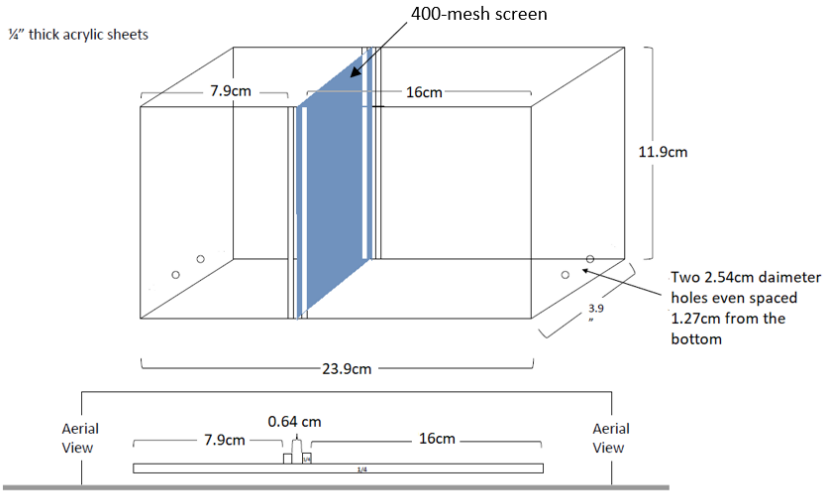


**Figure S5.1 - Acrylic containers used for the greenhouse study**

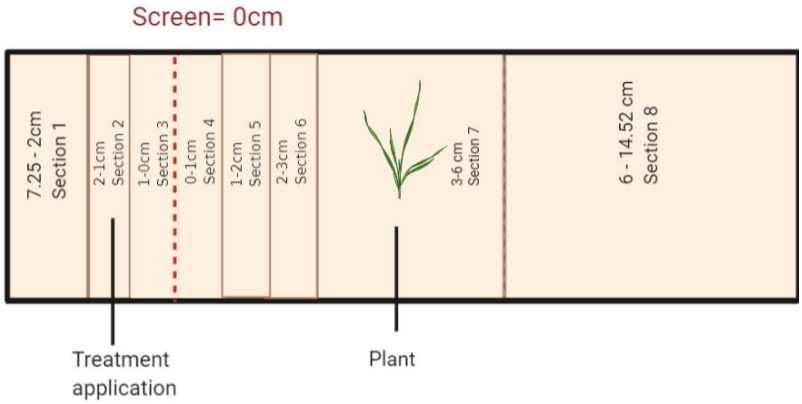


**Figure S5.2 Soil sectioning done for soil 1- calcareous soil**

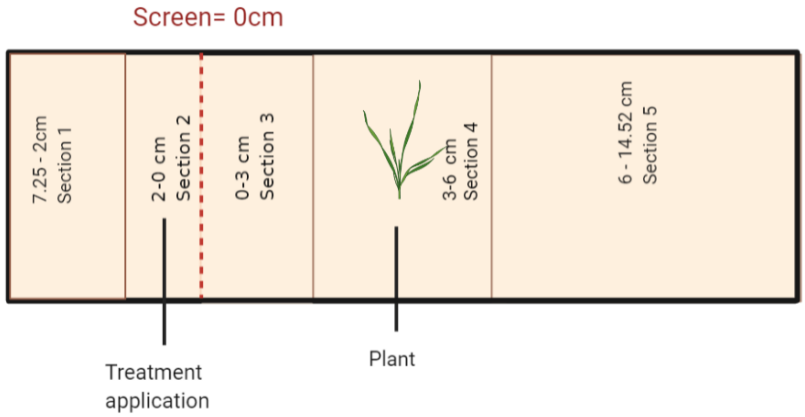


**Figure S5.3 Soil sectioning done for soil 2- acid soil**

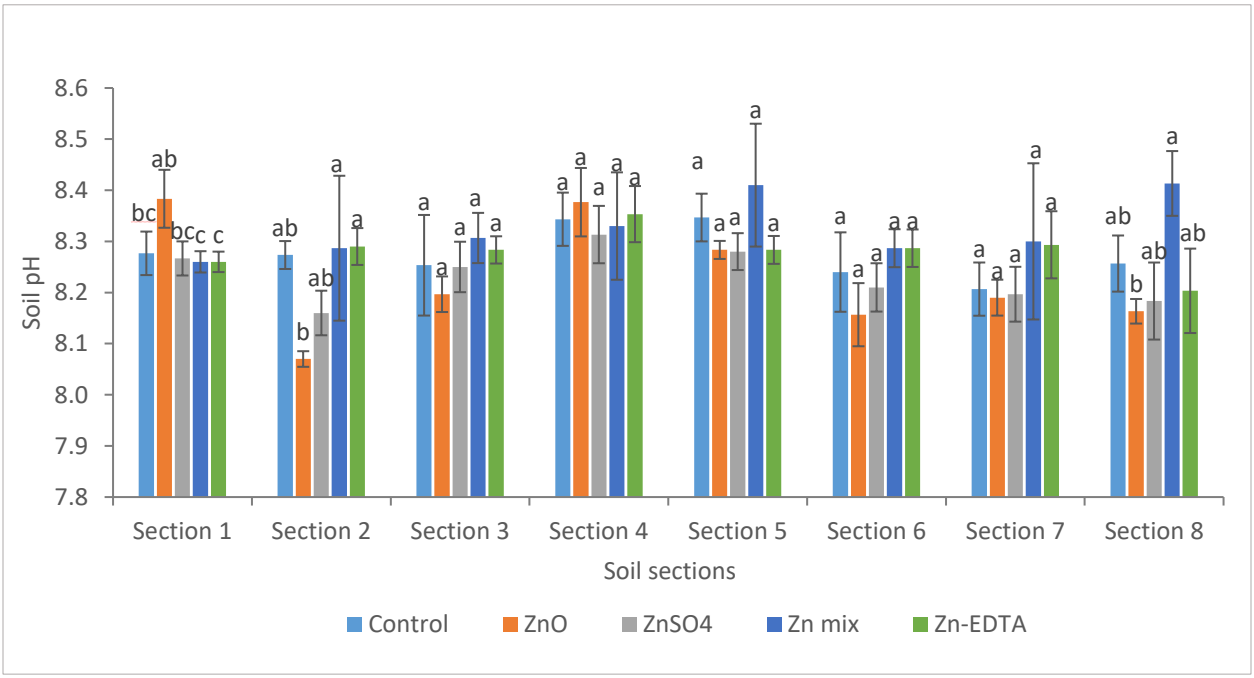


**Figure S5.4 Soil pH for the calcareous soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

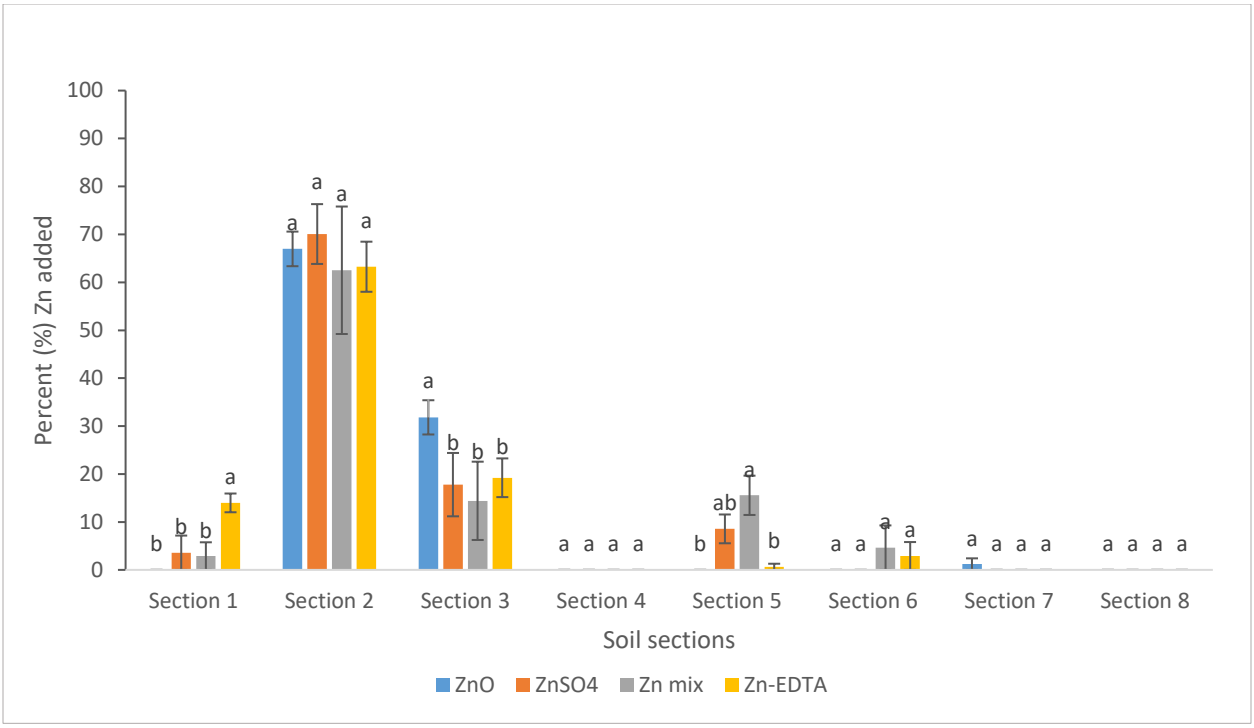


**Figure S5.5 Percent total Zn recovered from each section for the calcareous soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

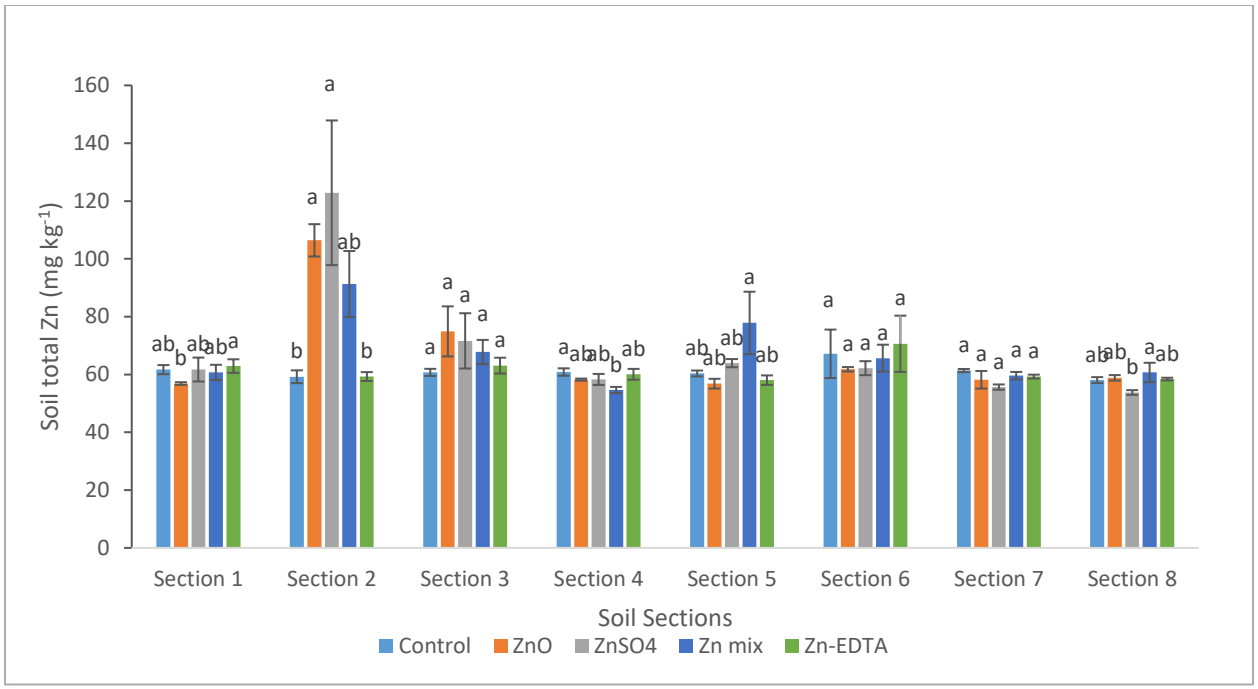


**Figure S5.6 Soil total Zn for the calcareous soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

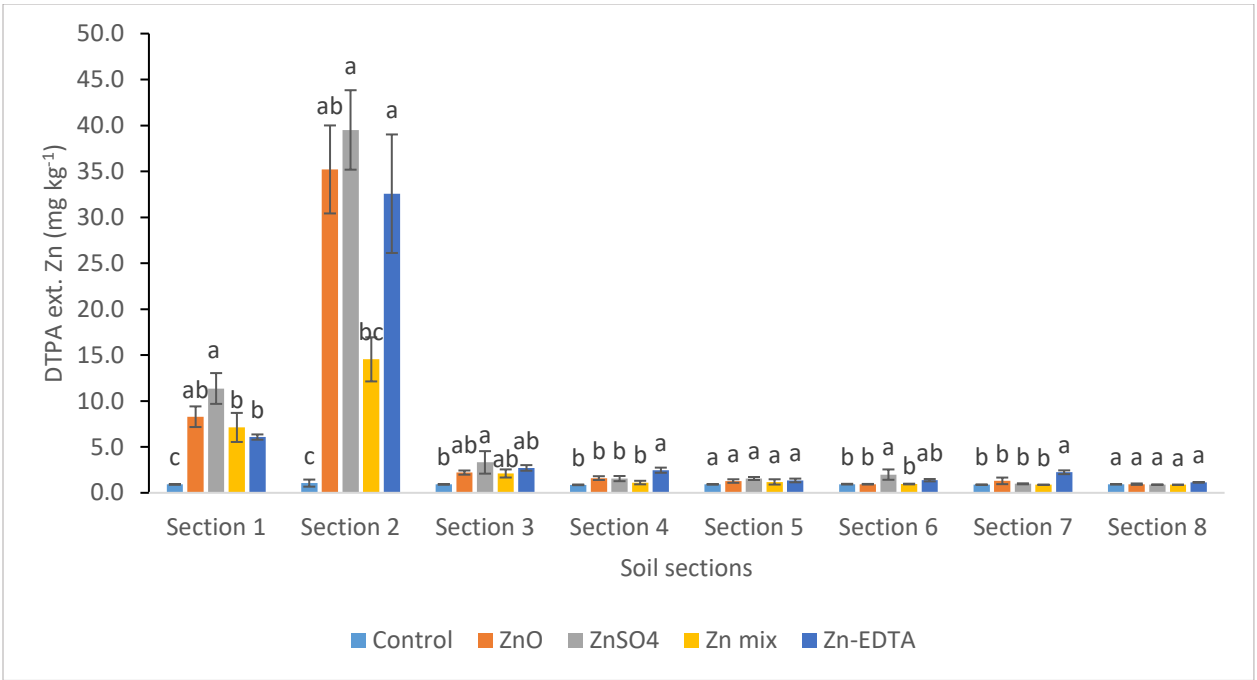


**Figure S5.7 DTPA extractable Zn for the calcareous soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

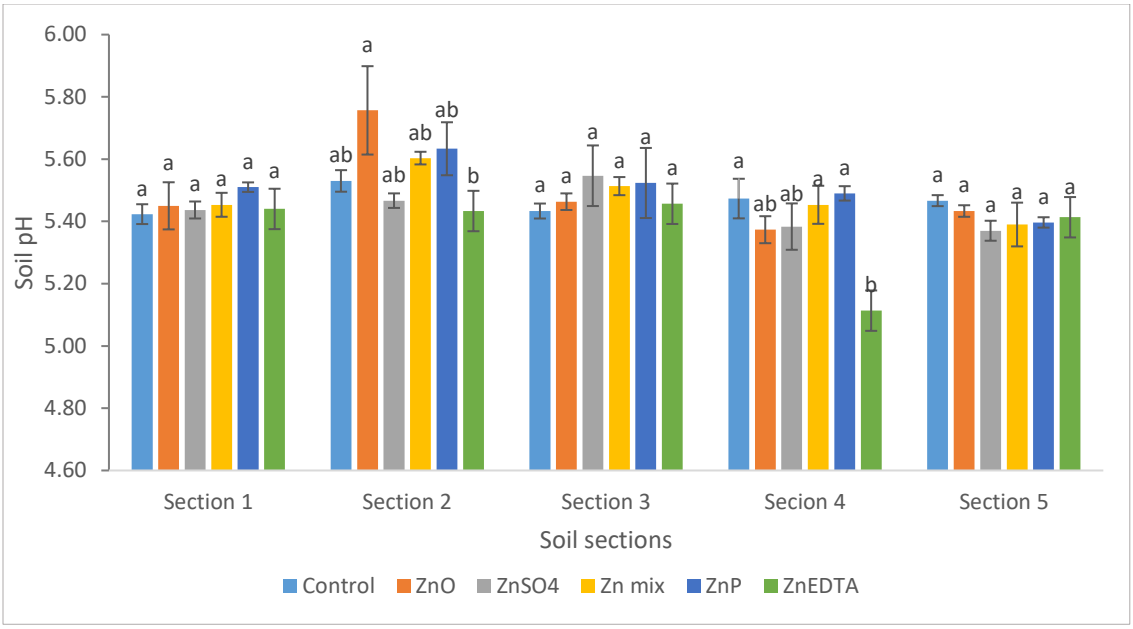


**Figure S5.8 Soil pH for Zn only treatments in acid soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

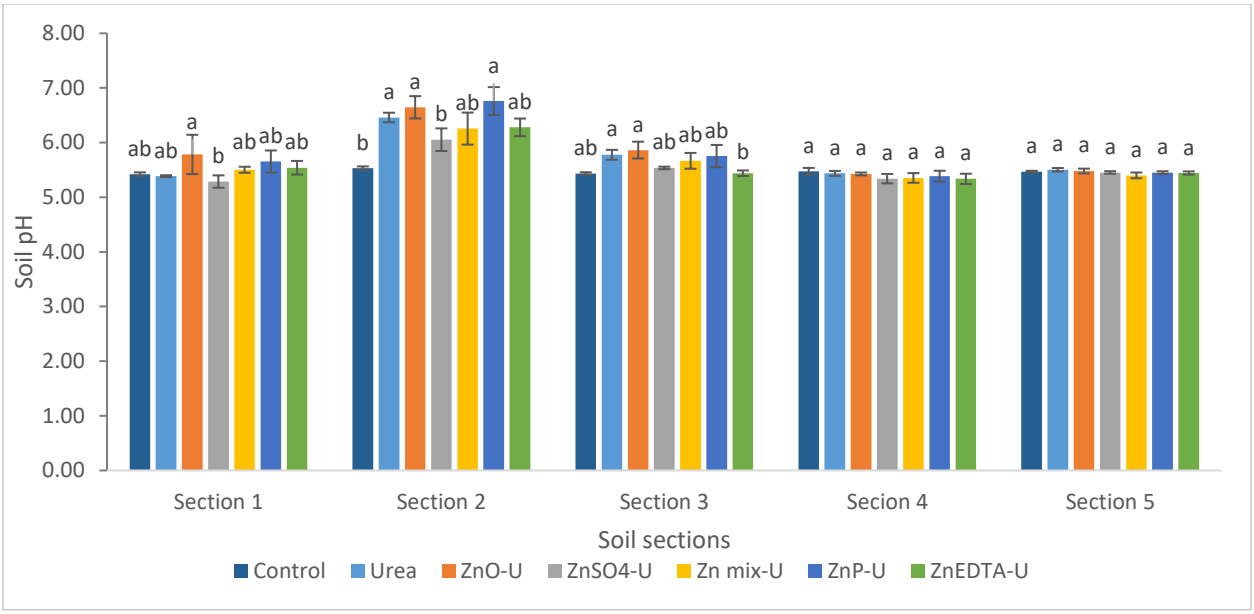


**Figure S5.9 Soil pH for Zn-coated urea treatments in acid soil greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

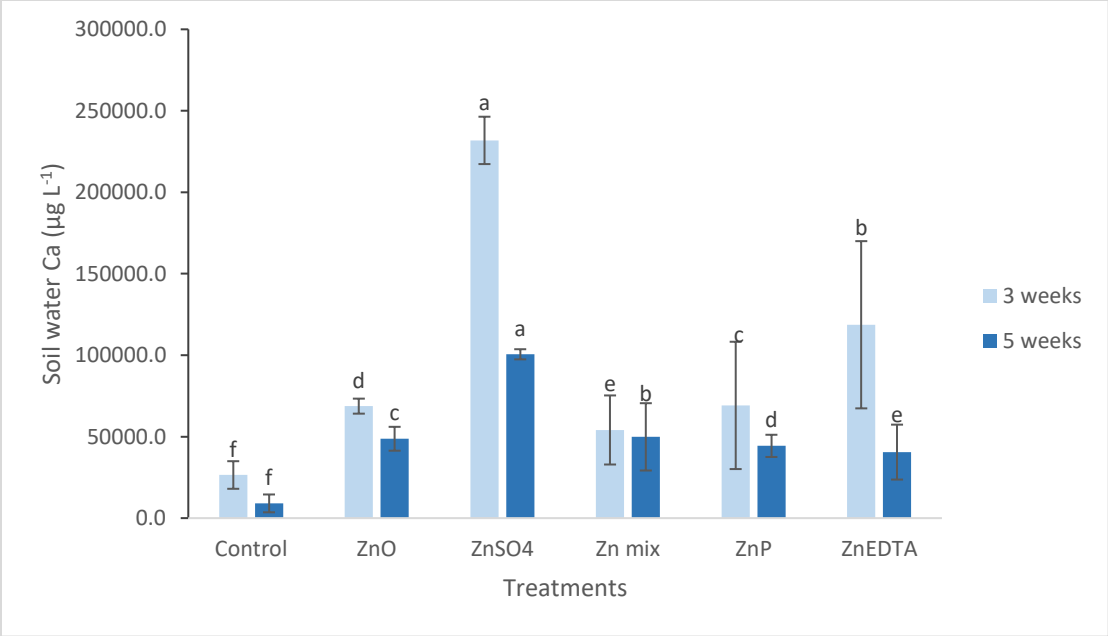


**Figure S5.10 Soil water Ca for Zn only treatments for acid soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

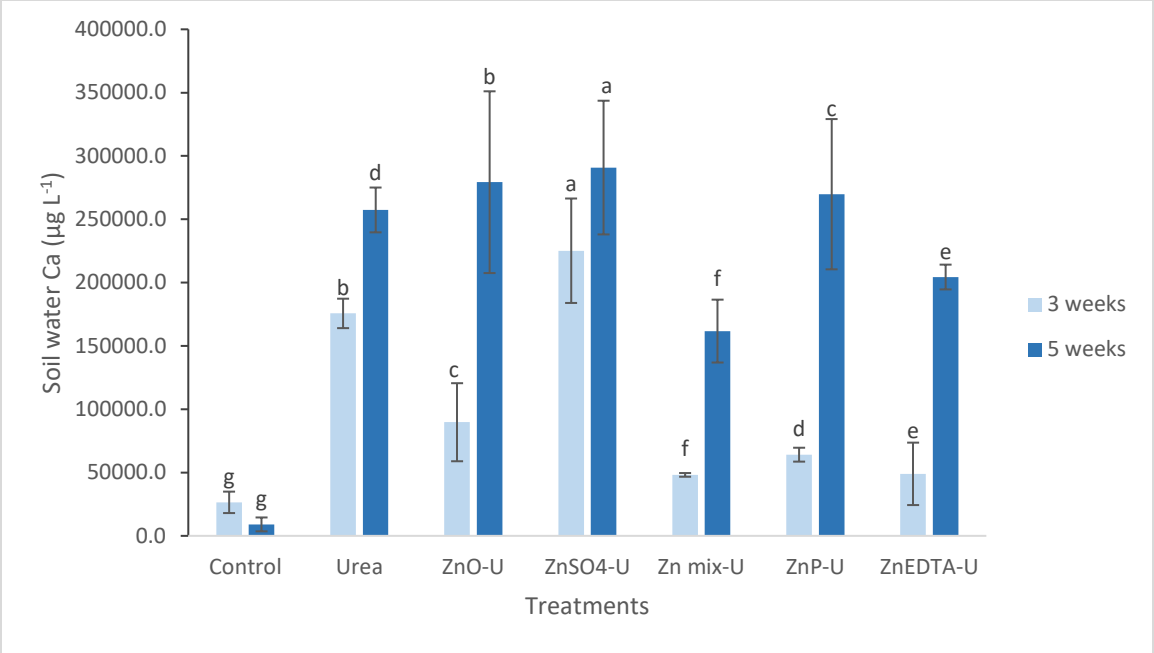


**Figure S5.11 Soil water Ca for Zn-coated urea treatments for acid soil greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

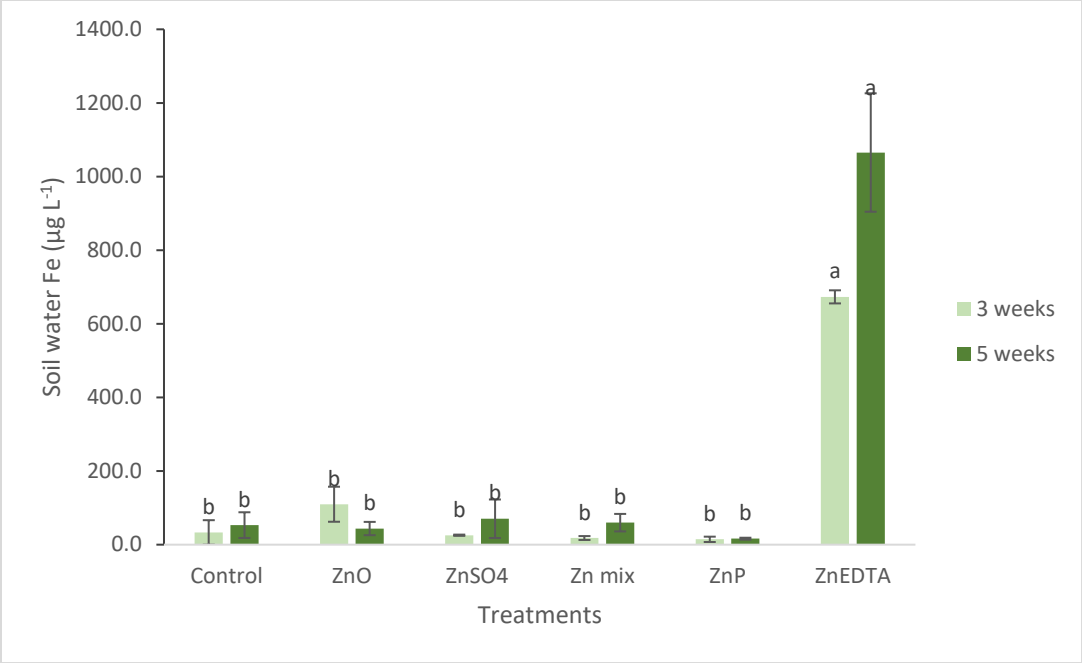


**Figure S5.12 Soil water Fe for Zn only treatments in acid soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

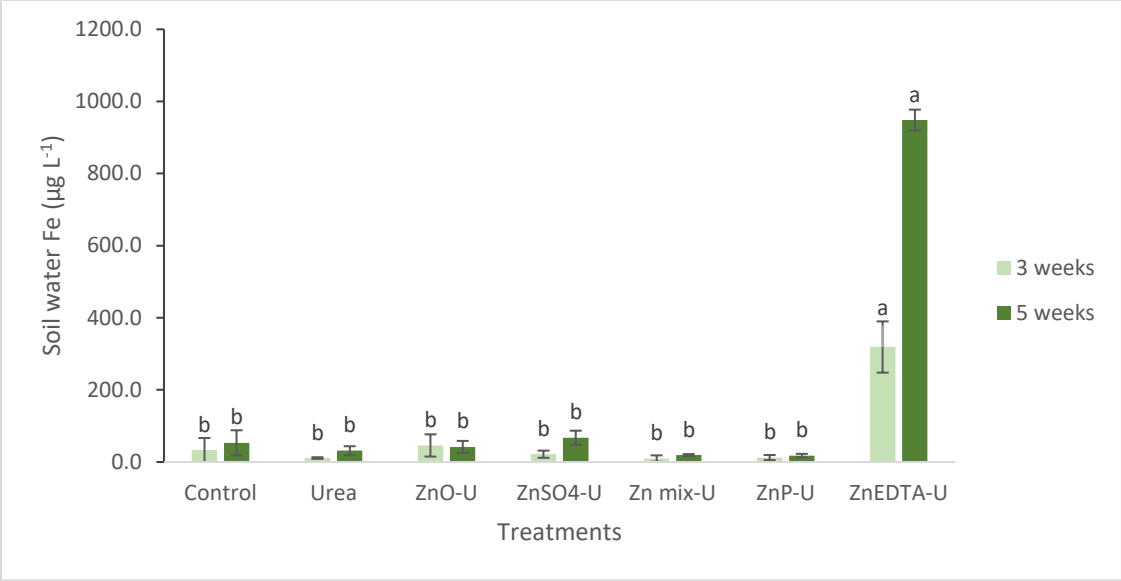


**Figure S5.13 Soil water Fe for Zn-coated urea treatments in acid soil greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

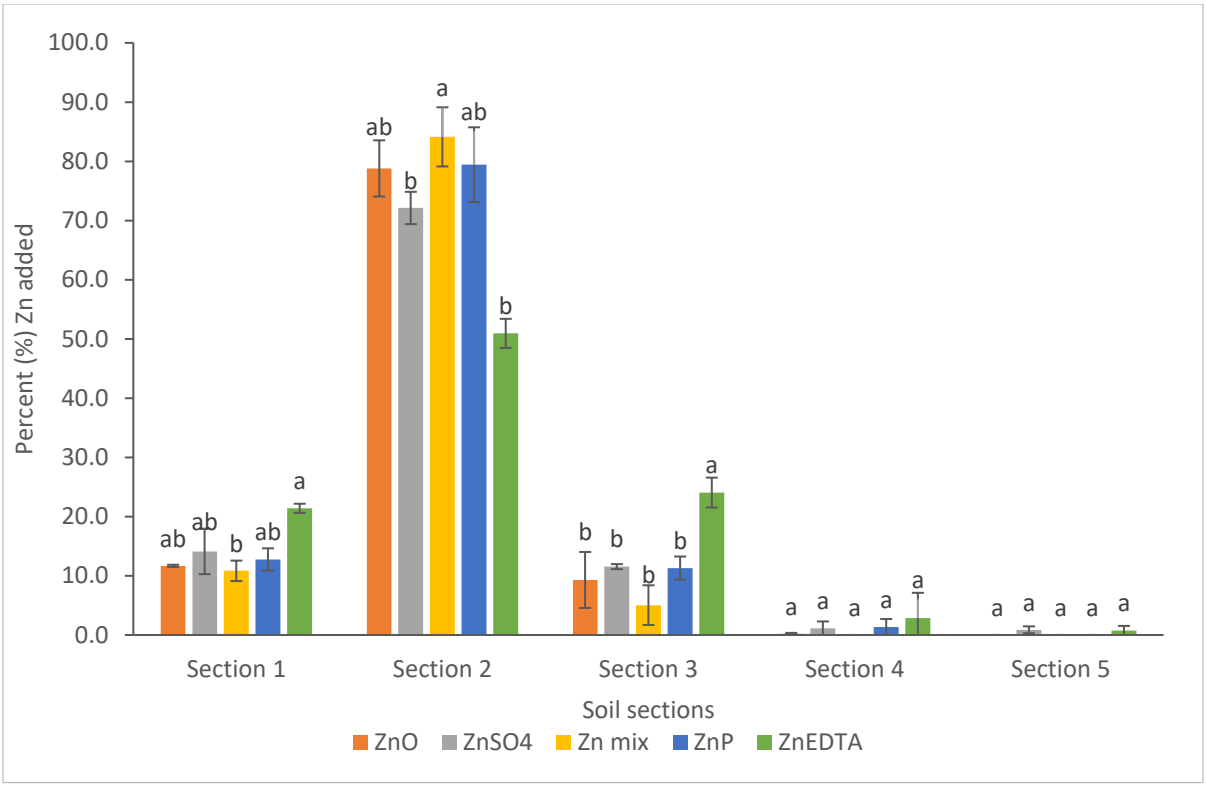


**Figure S5.14 Percent (%) Zn added recovered from each soil section for acid soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

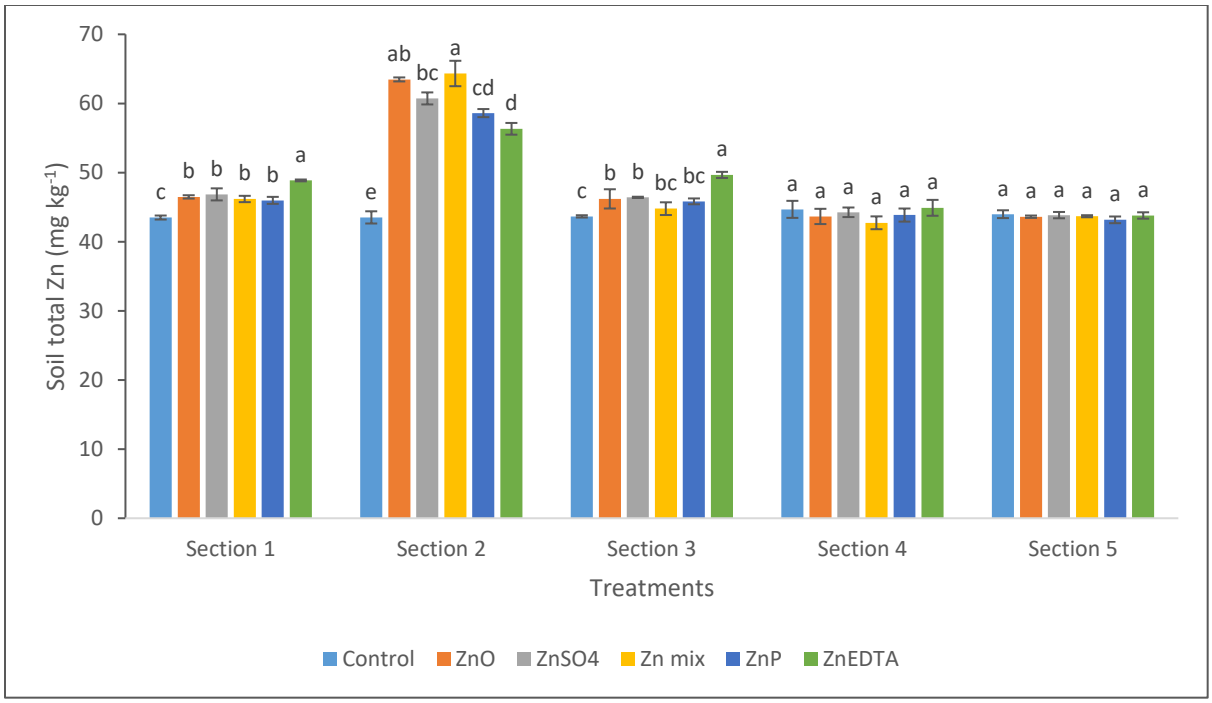


**Figure S5.15 Soil total Zn for acid soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

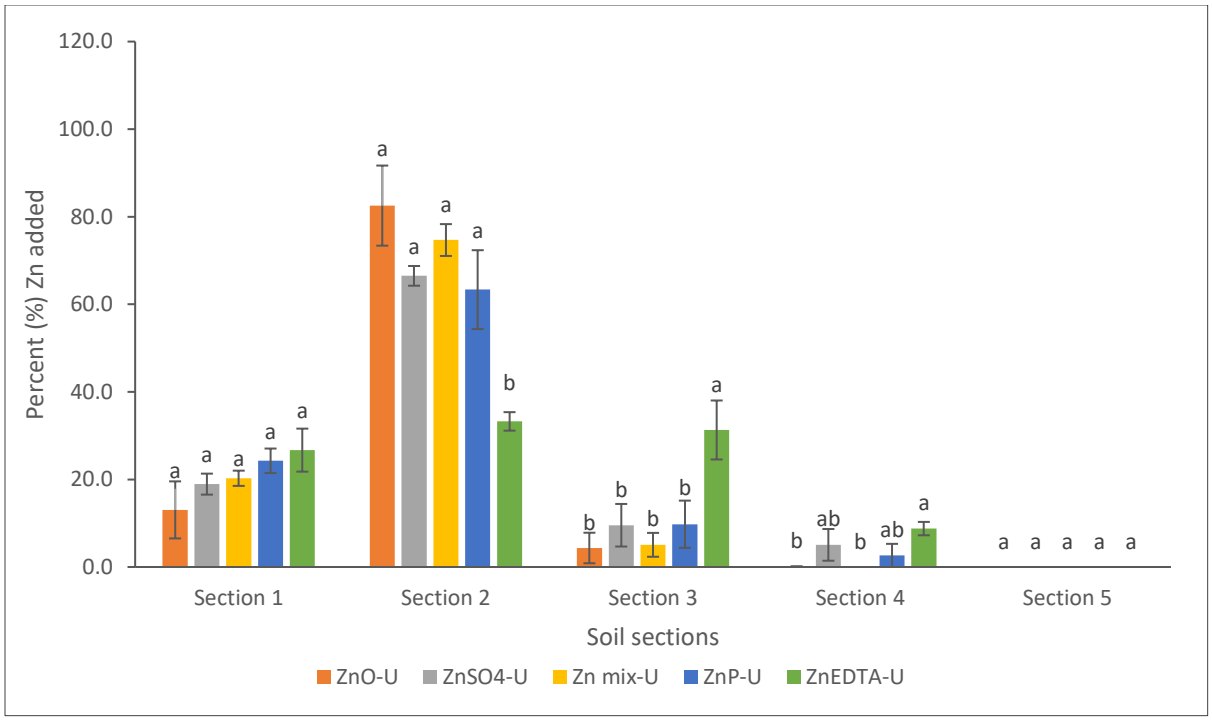


**Figure S5.16 Percent (%) Zn added recovered from each section for acid soil greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

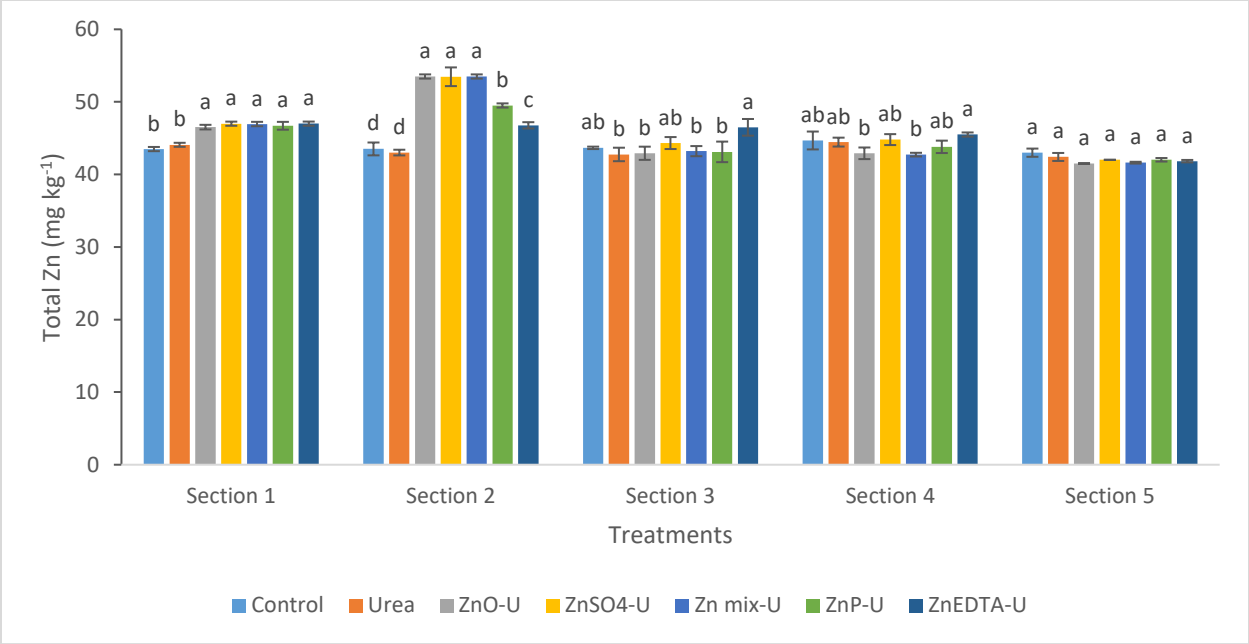


**Figure S5.17 Soil total Zn for acid soil greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

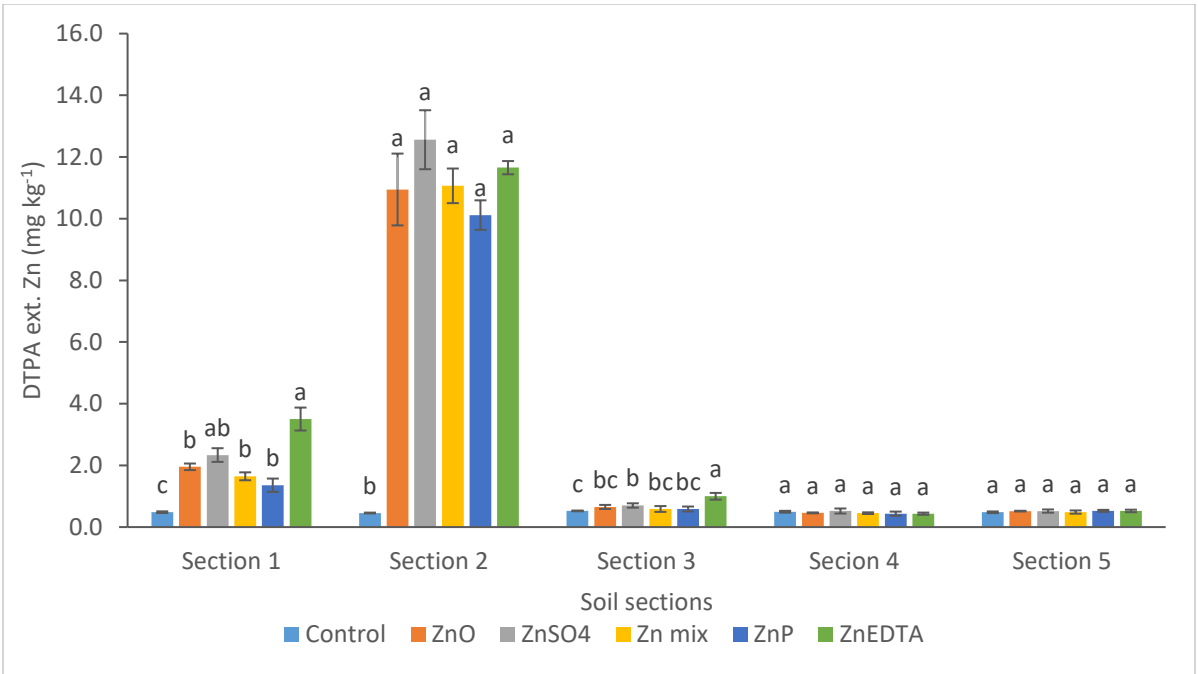


**Figure S5.18 DTPA extractable Zn in each section for acid soil greenhouse study. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnP= zinc phosphate; ZnEDTA= zinc-ethylenediamine tetra acetic acid. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

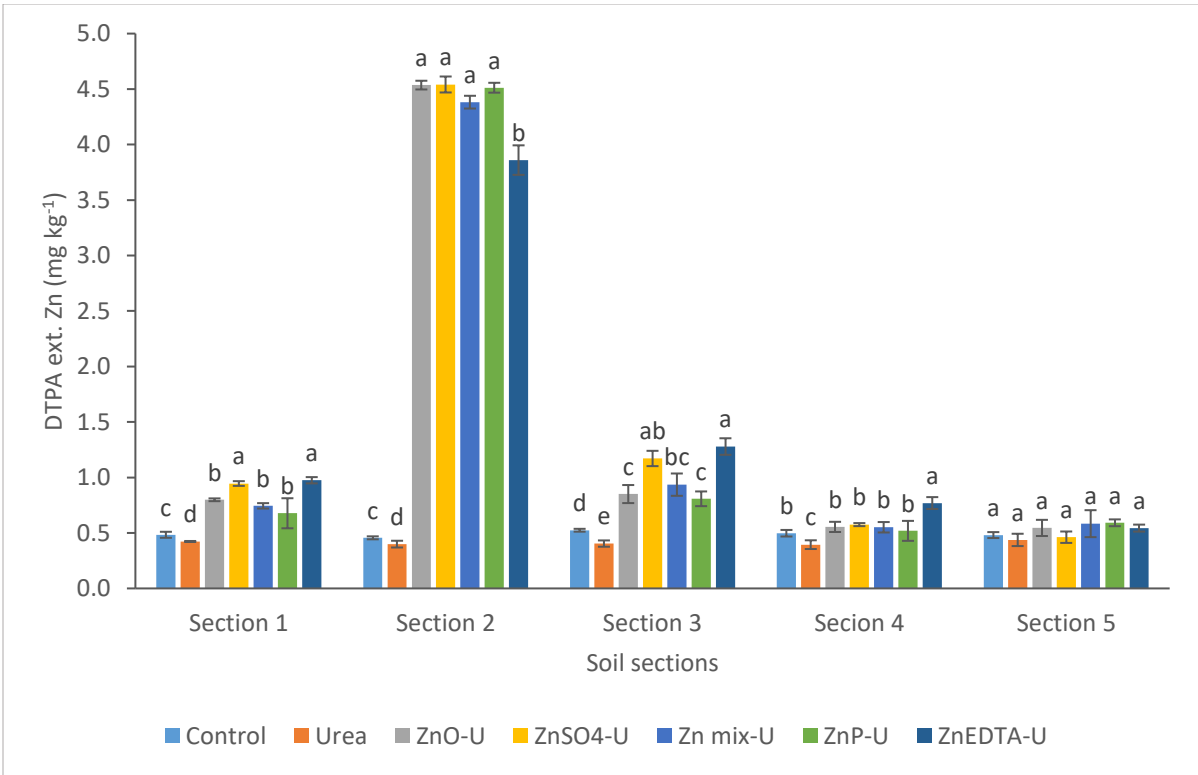


**Figure S5.19 DTPA extractable Zn in each section for acid soil greenhouse study. Control= no-Zn; Urea = urea control containing urea and no-Zn; ZnO-U= zinc oxide coated urea; $ZnSO_4$-U= zinc sulfate coated urea; Zn mix-U= zinc mix coated urea; ZnP-U= zinc phosphate coated urea; ZnEDTA-U= zinc-ethylenediamine tetra acetic acid coated urea. Means for each treatment within a soil section containing the same letter are not significantly different at $P \leq 0.05$ according to LSD pairwise method. Error bars represent the standard error of three replicates.**

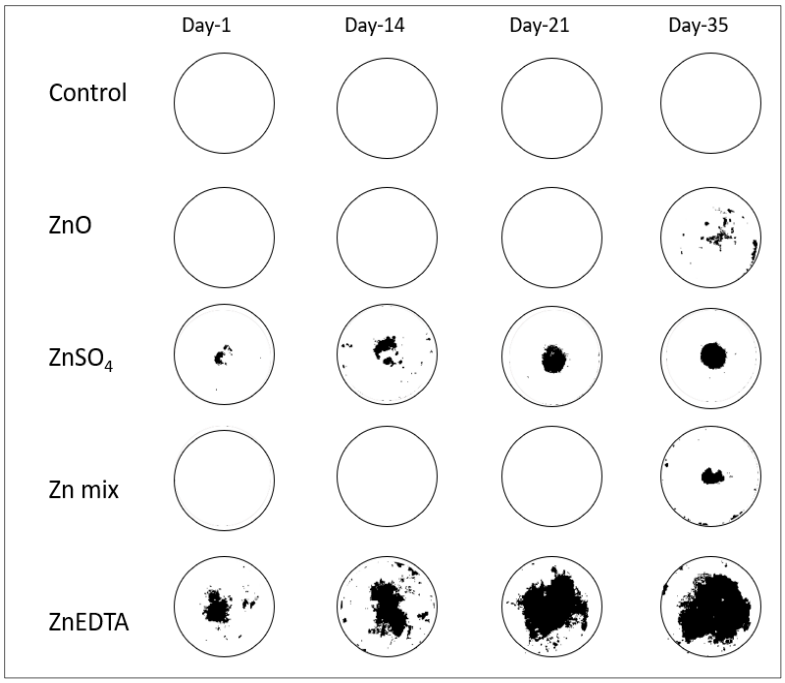


**Figure S5.20 Images collected from visualization study and analyzed using GIMP software for day 1, 7, 21, and 35 for calcareous soil. Control= no-Zn; ZnO= zinc oxide; $ZnSO_4$= zinc sulfate; Zn mix= zinc mix; ZnEDTA= zinc-ethylenediamine tetra acetic acid.**

# Appendix A - Chapter 3

Visualization- Zn only

1= control

2=ZnO

3=$ZnSO_4$

4=EDTA

5=Zn mix

6=ZnP

7=ZnEDTA

Day 1

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0.01350 | 0.01566 | 12 | 0.86 | 0.4054 |
| **Rep** | 2 | -0.01451 | 0.01566 | 12 | -0.93 | 0.3723 |
| **Rep** | 3 | 0.001011 | 0.01566 | 12 | 0.06 | 0.9496 |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 6 | 12 | 389.97 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0 | . | . | . | . |
| **Rep** | 2 | 0 | . | . | . | . |
| **Rep** | 3 | 0 | . | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 6 | 12 | 536.63 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | -222E-18 | 0.03043 | 12 | -0.00 | 1.0000 |
| **Trt** | 2 | 0 | 0.03043 | 12 | 0.00 | 1.0000 |
| **Trt** | 3 | 1.1099 | 0.03043 | 12 | 36.47 | <.0001 |
| **Trt** | 4 | 0 | 0.03043 | 12 | 0.00 | 1.0000 |
| **Trt** | 5 | -444E-18 | 0.03043 | 12 | -0.00 | 1.0000 |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| Effect | Trt | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
| Trt | 6 | -222E-18 | 0.03043 | 12 | -0.00 | 1.0000 |
| Trt | 7 | 1.1791 | 0.03043 | 12 | 38.74 | <.0001 |

Week 3

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| Effect | Rep | Estimate | Std Err Pred | DF | t Value | Pr > \|t\| |
| Rep | 1 | 0.02713 | 0.03983 | 12 | 0.68 | 0.5087 |
| Rep | 2 | -0.01714 | 0.03983 | 12 | -0.43 | 0.6746 |
| Rep | 3 | -0.00999 | 0.03983 | 12 | -0.25 | 0.8062 |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Time | 0 | . | . | . |
| Trt | 6 | 12 | 273.26 | <.0001 |
| Time*Trt | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | -888E-18 | 0.1095 | 12 | -0.00 | 1.0000 |
| **Trt** | 2 | 0.09188 | 0.1095 | 12 | 0.84 | 0.4179 |
| **Trt** | 3 | 1.5435 | 0.1095 | 12 | 14.09 | <.0001 |
| **Trt** | 4 | -178E-17 | 0.1095 | 12 | -0.00 | 1.0000 |
| **Trt** | 5 | 0.1256 | 0.1095 | 12 | 1.15 | 0.2740 |
| **Trt** | 6 | 0.06615 | 0.1095 | 12 | 0.60 | 0.5571 |
| **Trt** | 7 | 4.7234 | 0.1095 | 12 | 43.13 | <.0001 |

Week 5

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0.01793 | 0.02801 | 12 | 0.64 | 0.5341 |
| **Rep** | 2 | -0.00597 | 0.02801 | 12 | -0.21 | 0.8348 |
| **Rep** | 3 | -0.01196 | 0.02801 | 12 | -0.43 | 0.6769 |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 6 | 12 | 1615.44 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 0 | 0.08087 | 12 | 0.00 | 1.0000 |
| **Trt** | 2 | 0.2756 | 0.08087 | 12 | 3.41 | 0.0052 |
| **Trt** | 3 | 2.2345 | 0.08087 | 12 | 27.63 | <.0001 |
| **Trt** | 4 | 0 | 0.08087 | 12 | 0.00 | 1.0000 |
| **Trt** | 5 | 0.1632 | 0.08087 | 12 | 2.02 | 0.0666 |
| **Trt** | 6 | 0 | 0.08087 | 12 | 0.00 | 1.0000 |
| **Trt** | 7 | 8.5582 | 0.08087 | 12 | 105.82 | <.0001 |

Visualization- Zn coated Urea

1=Urea

2=ZnO-U

3-$ZnSO_4$-U

4=ZnP-U

5=Zn mix-U

6=ZnEDTA-U

Week 1

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | -0.00382 | 0.005529 | 10 | -0.69 | 0.5058 |
| **Rep** | 2 | 0.000525 | 0.005529 | 10 | 0.09 | 0.9262 |
| **Rep** | 3 | 0.003292 | 0.005529 | 10 | 0.60 | 0.5649 |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 97.29 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 0 | 0.01317 | 10 | 0.00 | 1.0000 |
| **Trt** | 2 | 0.06925 | 0.01317 | 10 | 5.26 | 0.0004 |
| **Trt** | 3 | 0.09005 | 0.01317 | 10 | 6.84 | <.0001 |
| **Trt** | 4 | 5.55E-17 | 0.01317 | 10 | 0.00 | 1.0000 |
| **Trt** | 5 | 0.2319 | 0.01317 | 10 | 17.61 | <.0001 |
| **Trt** | 6 | 0.3025 | 0.01317 | 10 | 22.97 | <.0001 |

Week 3

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0.001059 | 0.004242 | 10 | 0.25 | 0.8079 |
| **Rep** | 2 | -0.00076 | 0.004242 | 10 | -0.18 | 0.8621 |
| **Rep** | 3 | -0.00030 | 0.004242 | 10 | -0.07 | 0.9446 |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 58.11 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 0 | 0.02727 | 10 | 0.00 | 1.0000 |
| **Trt** | 2 | 0.2930 | 0.02727 | 10 | 10.75 | <.0001 |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 3 | 0.3630 | 0.02727 | 10 | 13.31 | <.0001 |
| **Trt** | 4 | 0.6428 | 0.02727 | 10 | 23.57 | <.0001 |
| **Trt** | 5 | 0.2836 | 0.02727 | 10 | 10.40 | <.0001 |
| **Trt** | 6 | 0.3838 | 0.02727 | 10 | 14.08 | <.0001 |

Week 5

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0 | . | . | . | . |
| **Rep** | 2 | 0 | . | . | . | . |
| **Rep** | 3 | 0 | . | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 197.59 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 1.11E-16 | 0.02340 | 10 | 0.00 | 1.0000 |
| **Trt** | 2 | 0.4726 | 0.02340 | 10 | 20.20 | <.0001 |
| **Trt** | 3 | 0.6995 | 0.02340 | 10 | 29.90 | <.0001 |
| **Trt** | 4 | 0.8318 | 0.02340 | 10 | 35.55 | <.0001 |
| **Trt** | 5 | 0.6995 | 0.02340 | 10 | 29.90 | <.0001 |
| **Trt** | 6 | 0.9018 | 0.02340 | 10 | 38.54 | <.0001 |

Soil pH- Incubation study

Zn only

1= Control

2=ZnO

3=ZnSO4

4=EDTA

5=Zn mix

6=ZnEDTA

7=ZnP

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 7 | 1 2 3 4 5 6 7 |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 4 | 1 2 3 4 |

| | |
|---|---|
| **Number of Observations Read** | 84 |
| **Number of Observations Used** | 84 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 41 | 0.70881190 | 0.01728810 | 5.21 | <.0001 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Error** | 42 | 0.13948333 | 0.00332103 | | |
| **Corrected Total** | 83 | 0.84829524 | | | |

| R-Square | Coeff Var | Root MSE | pH Mean |
|---|---|---|---|
| 0.835572 | 0.635741 | 0.057628 | 9.064762 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 0.14432857 | 0.02405476 | 7.24 | <.0001 |
| **REPS(TREATS)** | 14 | 0.04311667 | 0.00307976 | 0.93 | 0.5386 |
| **SUBPLOTS** | 3 | 0.15009524 | 0.05003175 | 15.07 | <.0001 |
| **TREATS*SUBPLOTS** | 18 | 0.37127143 | 0.02062619 | 6.21 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 0.14432857 | 0.02405476 | 7.24 | <.0001 |
| **REPS(TREATS)** | 14 | 0.04311667 | 0.00307976 | 0.93 | 0.5386 |
| **SUBPLOTS** | 3 | 0.15009524 | 0.05003175 | 15.07 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS*SUBPLOTS** | 18 | 0.37127143 | 0.02062619 | 6.21 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 6 | 0.14432857 | 0.02405476 | 7.81 | 0.0008 |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 7 | 1 2 3 4 5 6 7 |
| **REPS** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 21 |
| **Number of Observations Used** | 21 |

Soil pH- Zn coated Urea

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U Znmix-U |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 4 | 1 2 3 4 |

| | |
|---|---|
| **Number of Observations Read** | 72 |
| **Number of Observations Used** | 72 |

| **Source** | **DF** | **Sum of Squares** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| **Model** | 35 | 0.28446111 | 0.00812746 | 6.75 | <.0001 |
| **Error** | 36 | 0.04331667 | 0.00120324 | | |
| **Corrected Total** | 71 | 0.32777778 | | | |

| **R-Square** | **Coeff Var** | **Root MSE** | **totalzn Mean** |
|---|---|---|---|
| 0.867847 | 0.418710 | 0.034688 | 8.284444 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 0.01567778 | 0.00313556 | 2.61 | 0.0413 |
| **REPS(TREATS)** | 12 | 0.01975000 | 0.00164583 | 1.37 | 0.2260 |
| **SUBPLOTS** | 3 | 0.14102222 | 0.04700741 | 39.07 | <.0001 |
| **TREATS*SUBPLOTS** | 15 | 0.10801111 | 0.00720074 | 5.98 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 0.01567778 | 0.00313556 | 2.61 | 0.0413 |
| **REPS(TREATS)** | 12 | 0.01975000 | 0.00164583 | 1.37 | 0.2260 |
| **SUBPLOTS** | 3 | 0.14102222 | 0.04700741 | 39.07 | <.0001 |
| **TREATS*SUBPLOTS** | 15 | 0.10801111 | 0.00720074 | 5.98 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 0.01567778 | 0.00313556 | 1.91 | 0.1671 |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U Znmix-U |
| **REPS** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 18 |

Water extractable Zn in soil- Zn coated Urea

| **Class Level Information** | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | DDP-U Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 4 | 1 2 3 4 |

| | |
|---|---|
| **Number of Observations Read** | 72 |
| **Number of Observations Used** | 72 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 35 | 14891.94583 | 425.48417 | 27.26 | <.0001 |
| **Error** | 36 | 561.97573 | 15.61044 | | |
| **Corrected Total** | 71 | 15453.92157 | | | |

| R-Square | Coeff Var | Root MSE | waterextzn Mean |
|---|---|---|---|
| 0.963635 | 32.99569 | 3.951005 | 11.97431 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 7280.012624 | 1456.002525 | 93.27 | <.0001 |
| **REPS(TREATS)** | 12 | 57.801467 | 4.816789 | 0.31 | 0.9835 |
| **SUBPLOTS** | 3 | 3011.058215 | 1003.686072 | 64.30 | <.0001 |
| **TREATS*SUBPLOTS** | 15 | 4543.073526 | 302.871568 | 19.40 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 7280.012624 | 1456.002525 | 93.27 | <.0001 |
| **REPS(TREATS)** | 12 | 57.801467 | 4.816789 | 0.31 | 0.9835 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| SUBPLOTS | 3 | 3011.058215 | 1003.686072 | 64.30 | <.0001 |
| TREATS*SUBPLOTS | 15 | 4543.073526 | 302.871568 | 19.40 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
| TREATS | 5 | 7280.012624 | 1456.002525 | 302.28 | <.0001 |

Total Zn- Percentage Zn recovered as of added Zn- Zn only

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| TREATS | 5 | ZnEDTA ZnO ZnP ZnSO4 Znmix |
| REPS | 3 | 1 2 3 |
| SUBPLOTS | 4 | 1 2 3 4 |

| | |
|---|---|
| Number of Observations Read | 60 |
| Number of Observations Used | 60 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 29 | 68366.53602 | 2357.46676 | 542.76 | <.0001 |
| **Error** | 30 | 130.30412 | 4.34347 | | |
| **Corrected Total** | 59 | 68496.84013 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.998098 | 8.358688 | 2.084099 | 24.93333 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 0.40000 | 0.10000 | 0.02 | 0.9989 |
| **REPS(TREATS)** | 10 | 1.33333 | 0.13333 | 0.03 | 1.0000 |
| **SUBPLOTS** | 3 | 60757.58627 | 20252.52876 | 4662.75 | <.0001 |
| **TREATS*SUBPLOTS** | 12 | 7607.21641 | 633.93470 | 145.95 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 0.40000 | 0.10000 | 0.02 | 0.9989 |
| **REPS(TREATS)** | 10 | 1.33333 | 0.13333 | 0.03 | 1.0000 |
| **SUBPLOTS** | 3 | 60757.58627 | 20252.52876 | 4662.75 | <.0001 |
| **TREATS*SUBPLOTS** | 12 | 7607.21641 | 633.93470 | 145.95 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 4 | 0.40000000 | 0.10000000 | 0.75 | 0.5801 |

Total Zn- Percentage Zn recovered as of added Zn- Zn coated Urea

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 5 | ZnEDTA ZnO ZnP ZnSO Znmix |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 4 | 1 2 3 4 |

| | |
|---|---|
| **Number of Observations Read** | 60 |
| **Number of Observations Used** | 60 |

| **Source** | **DF** | **Sum of Squares** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| **Model** | 29 | 78649.39195 | 2712.04800 | 191.67 | <.0001 |
| **Error** | 30 | 424.47791 | 14.14926 | | |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Corrected Total | 59 | 79073.86986 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.994632 | 14.81291 | 3.761551 | 25.39374 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TREATS | 4 | 8.45948 | 2.11487 | 0.15 | 0.9618 |
| REPS(TREATS) | 10 | 35.52276 | 3.55228 | 0.25 | 0.9873 |
| SUBPLOTS | 3 | 75522.57332 | 25174.19111 | 1779.19 | <.0001 |
| TREATS*SUBPLOTS | 12 | 3082.83639 | 256.90303 | 18.16 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TREATS | 4 | 8.45948 | 2.11487 | 0.15 | 0.9618 |
| REPS(TREATS) | 10 | 35.52276 | 3.55228 | 0.25 | 0.9873 |
| SUBPLOTS | 3 | 75522.57332 | 25174.19111 | 1779.19 | <.0001 |
| TREATS*SUBPLOTS | 12 | 3082.83639 | 256.90303 | 18.16 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 4 | 8.45947725 | 2.11486931 | 0.60 | 0.6742 |

Greenhouse study

Soil pH- Zn only

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Control ZnEDTA ZnO ZnP ZnSO4 Znmix |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 90 |
| **Number of Observations Used** | 90 |

| **Source** | **DF** | **Sum of Squares** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| **Model** | 41 | 0.77138222 | 0.01881420 | 3.23 | <.0001 |
| **Error** | 48 | 0.27954667 | 0.00582389 | | |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Corrected Total | 89 | 1.05092889 | | | |

| R-Square | Coeff Var | Root MSE | pH Mean |
|---|---|---|---|
| 0.734000 | 0.845204 | 0.076314 | 9.029111 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TREATS | 5 | 0.04308889 | 0.00861778 | 1.48 | 0.2140 |
| REPS(TREATS) | 12 | 0.16992000 | 0.01416000 | 2.43 | 0.0147 |
| SUBPLOTS | 4 | 0.38889556 | 0.09722389 | 16.69 | <.0001 |
| TREATS*SUBPLOTS | 20 | 0.16947778 | 0.00847389 | 1.46 | 0.1437 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TREATS | 5 | 0.04308889 | 0.00861778 | 1.48 | 0.2140 |
| REPS(TREATS) | 12 | 0.16992000 | 0.01416000 | 2.43 | 0.0147 |
| SUBPLOTS | 4 | 0.38889556 | 0.09722389 | 16.69 | <.0001 |
| TREATS*SUBPLOTS | 20 | 0.16947778 | 0.00847389 | 1.46 | 0.1437 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 0.04308889 | 0.00861778 | 0.61 | 0.6954 |

Soil pH- Zn coated Urea

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 7 | Control Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U Znmix-U |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 105 |
| **Number of Observations Used** | 105 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 48 | 6.81845143 | 0.14205107 | 17.60 | <.0001 |
| **Error** | 56 | 0.45205333 | 0.00807238 | | |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Corrected Total** | 104 | 7.27050476 | | | |

| R-Square | Coeff Var | Root MSE | pH Mean |
|---|---|---|---|
| 0.937824 | 1.025756 | 0.089846 | 8.759048 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 1.59270476 | 0.26545079 | 32.88 | <.0001 |
| **REPS(TREATS)** | 14 | 0.18548000 | 0.01324857 | 1.64 | 0.0963 |
| **SUBPLOTS** | 4 | 3.32120952 | 0.83030238 | 102.86 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 1.71905714 | 0.07162738 | 8.87 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 1.59270476 | 0.26545079 | 32.88 | <.0001 |
| **REPS(TREATS)** | 14 | 0.18548000 | 0.01324857 | 1.64 | 0.0963 |
| **SUBPLOTS** | 4 | 3.32120952 | 0.83030238 | 102.86 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 1.71905714 | 0.07162738 | 8.87 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 6 | 1.59270476 | 0.26545079 | 20.04 | <.0001 |

Total Zn- Zn only

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Control Zn mix ZnEDTA ZnO ZnP ZnSO4 |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 90 |
| **Number of Observations Used** | 90 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 31 | 5237.375875 | 168.947609 | 137.17 | <.0001 |
| **Error** | 58 | 71.438988 | 1.231707 | | |
| **Corrected Total** | 89 | 5308.814863 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.986543 | 2.399105 | 1.109823 | 46.25986 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 456.440127 | 91.288025 | 74.12 | <.0001 |
| **REPS(TREATS)** | 2 | 0.060469 | 0.030235 | 0.02 | 0.9758 |
| **SUBPLOTS** | 4 | 3882.874083 | 970.718521 | 788.11 | <.0001 |
| **TREATS*SUBPLOTS** | 20 | 898.001196 | 44.900060 | 36.45 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 456.440127 | 91.288025 | 74.12 | <.0001 |
| **REPS(TREATS)** | 2 | 0.060469 | 0.030235 | 0.02 | 0.9758 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **SUBPLOTS** | 4 | 3882.874083 | 970.718521 | 788.11 | <.0001 |
| **TREATS*SUBPLOTS** | 20 | 898.001196 | 44.900060 | 36.45 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 456.4401271 | 91.2880254 | 3019.31 | 0.0003 |

Total Zn- Zn coated Urea

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 7 | Control Znmix-U ZnEDTA-U ZnO-U ZnP-U ZnSO4-U urea |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 105 |

| Number of Observations Used | 105 |
|---|---|

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 36 | 1341.393879 | 37.260941 | 37.48 | <.0001 |
| Error | 68 | 67.597234 | 0.994077 | | |
| Corrected Total | 104 | 1408.991112 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.952024 | 2.303064 | 0.997034 | 43.29163 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TREATS | 6 | 193.2668995 | 32.2111499 | 32.40 | <.0001 |
| REPS(TREATS) | 2 | 0.0604694 | 0.0302347 | 0.03 | 0.9701 |
| SUBPLOTS | 4 | 651.3633806 | 162.8408451 | 163.81 | <.0001 |
| TREATS*SUBPLOTS | 24 | 496.7031293 | 20.6959637 | 20.82 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 193.2668995 | 32.2111499 | 32.40 | <.0001 |
| **REPS(TREATS)** | 2 | 0.0604694 | 0.0302347 | 0.03 | 0.9701 |
| **SUBPLOTS** | 4 | 651.3633806 | 162.8408451 | 163.81 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 496.7031293 | 20.6959637 | 20.82 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 6 | 193.2668995 | 32.2111499 | 1065.37 | 0.0009 |

DTPA Zn- Zn only

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Control Zn mix ZnEDTA ZnO ZnP ZnSO4 |
| **REPS** | 3 | 1 2 3 |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| SUBPLOTS | 5 | 1 2 3 4 5 |

| | |
|---|---|
| Number of Observations Read | 90 |
| Number of Observations Used | 90 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 41 | 3592.416933 | 87.619925 | 16.64 | <.0001 |
| Error | 48 | 252.676540 | 5.264095 | | |
| Corrected Total | 89 | 3845.093474 | | | |

| R-Square | Coeff Var | Root MSE | DTPAZn Mean |
|---|---|---|---|
| 0.934286 | 65.33658 | 2.294361 | 3.511603 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TREATS | 5 | 186.501588 | 37.300318 | 7.09 | <.0001 |
| REPS(TREATS) | 12 | 79.457055 | 6.621421 | 1.26 | 0.2741 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **SUBPLOTS** | 4 | 2551.212728 | 637.803182 | 121.16 | <.0001 |
| **TREATS*SUBPLOTS** | 20 | 775.245562 | 38.762278 | 7.36 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 186.501588 | 37.300318 | 7.09 | <.0001 |
| **REPS(TREATS)** | 12 | 79.457055 | 6.621421 | 1.26 | 0.2741 |
| **SUBPLOTS** | 4 | 2551.212728 | 637.803182 | 121.16 | <.0001 |
| **TREATS*SUBPLOTS** | 20 | 775.245562 | 38.762278 | 7.36 | <.0001 |

| **Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term** | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 186.5015883 | 37.3003177 | 5.63 | 0.0067 |

DTPA Zn- Zn coated Urea

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 7 | Control Znmix-U ZnEDTA-U ZnO-U ZnP-U ZnSO4-U urea |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 105 |
| **Number of Observations Used** | 105 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 48 | 552.5005939 | 11.5104290 | 28.12 | <.0001 |
| **Error** | 56 | 22.9200052 | 0.4092858 | | |
| **Corrected Total** | 104 | 575.4205991 | | | |

| R-Square | Coeff Var | Root MSE | DTPAZn Mean |
|---|---|---|---|
| 0.960168 | 35.68699 | 0.639754 | 1.792683 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 75.8927166 | 12.6487861 | 30.90 | <.0001 |
| **REPS(TREATS)** | 14 | 2.9166917 | 0.2083351 | 0.51 | 0.9180 |
| **SUBPLOTS** | 4 | 303.7127948 | 75.9281987 | 185.51 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 169.9783908 | 7.0824330 | 17.30 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 75.8927166 | 12.6487861 | 30.90 | <.0001 |
| **REPS(TREATS)** | 14 | 2.9166917 | 0.2083351 | 0.51 | 0.9180 |
| **SUBPLOTS** | 4 | 303.7127948 | 75.9281987 | 185.51 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 169.9783908 | 7.0824330 | 17.30 | <.0001 |

| **Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term** | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 6 | 75.8927166 1 | 12.64878610 | 60.71 | <.0001 |

Soil water Zn- Zn only

1-Control

3-ZnO

4-$ZnSO_4$

5-Zn mix

6-ZnP

7-ZnEDTA

| Model Information | |
|---|---|
| **Data Set** | WORK.SOILWAT |
| **Dependent Variable** | soilwt |
| **Covariance Structures** | Variance Components, Ante-dependence |
| **Subject Effect** | Rep(Trt) |
| **Estimation Method** | REML |
| **Residual Variance Method** | None |
| **Fixed Effects SE Method** | Model-Based |
| **Degrees of Freedom Method** | Containment |

| Class Level Information | |
|---|---|
| **Levels** | **Values** |
| 1 | wk3 |
| 6 | 1 2 3 4 5 6 |
| 3 | 1 2 3 |

| Iteration History | | |
|---|---|---|
| **Evaluations** | **-2 Res Log Like** | **Criterion** |
| 1 | 85.19351787 | |
| 2 | 85.19454432 | 0.00003196 |
| 1 | 85.19351853 | 0.00000002 |

| Class Level Information | | |
|---|---|---|
| Levels | Values | |
| 1 | 85.19351787 | 0.00000000 |

Week 3

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Time | 0 | . | . | . |
| Trt | 5 | 10 | 41.79 | <.0001 |
| Time*Trt | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| Effect | Trt | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
| Trt | 1 | 2.2553 | 3.6945 | 10 | 0.61 | 0.5552 |
| Trt | 2 | 5.8190 | 3.6945 | 10 | 1.58 | 0.1463 |
| Trt | 3 | 6.9253 | 3.6945 | 10 | 1.87 | 0.0903 |
| Trt | 4 | 3.4727 | 3.6945 | 10 | 0.94 | 0.3694 |
| Trt | 5 | 4.9923 | 3.6945 | 10 | 1.35 | 0.2064 |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 6 | 63.0500 | 3.6945 | 10 | 17.07 | <.0001 |

Week 5

| Type 3 Tests of Fixed Effects | | | |
|---|---|---|---|
| **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| 0 | . | . | . |
| 5 | 10 | 27.10 | <.0001 |
| 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 2.3567 | 5.1575 | 10 | 0.46 | 0.6575 |
| **Trt** | 2 | 6.1053 | 5.1575 | 10 | 1.18 | 0.2639 |
| **Trt** | 3 | 2.0520 | 5.1575 | 10 | 0.40 | 0.6991 |
| **Trt** | 4 | 5.6333 | 5.1575 | 10 | 1.09 | 0.3003 |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 5 | 4.0633 | 5.1575 | 10 | 0.79 | 0.4491 |
| **Trt** | 6 | 69.6900 | 5.1575 | 10 | 13.51 | <.0001 |

Soil water Zn- Zn coated Urea

1-Control

2-Urea

3-ZnO-U

4-$ZnSO_4$-U

5-Zn mix-U

6-ZnP-U

7-ZnEDTA-U

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Time** | 1 | wk3 |
| **Trt** | 7 | 1 2 3 4 5 6 7 |
| **Rep** | 3 | 1 2 3 |

| Type 3 Tests of Fixed Effects | | |
|---|---|---|
| **Den DF** | **F Value** | **Pr > F** |
| . | . | . |

| Type 3 Tests of Fixed Effects | | |
|---|---|---|
| **Den DF** | **F Value** | **Pr > F** |
| 12 | 29.21 | <.0001 |
| . | . | . |

| Least Squares Means | | | | |
|---|---|---|---|---|
| **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| 2.2553 | 2.0552 | 12 | 1.10 | 0.2940 |
| 4.8213 | 2.0552 | 12 | 2.35 | 0.0370 |
| 7.4733 | 2.0552 | 12 | 3.64 | 0.0034 |
| 9.7513 | 2.0552 | 12 | 4.74 | 0.0005 |
| 5.8487 | 2.0552 | 12 | 2.85 | 0.0147 |
| 6.0380 | 2.0552 | 12 | 2.94 | 0.0124 |
| 34.7833 | 2.0552 | 12 | 16.92 | <.0001 |

Week 5

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 6 | 12 | 35.11 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 2.3567 | 1.7561 | 12 | 1.34 | 0.2044 |
| **Trt** | 2 | 4.4840 | 1.7561 | 12 | 2.55 | 0.0253 |
| **Trt** | 3 | 6.0700 | 1.7561 | 12 | 3.46 | 0.0047 |
| **Trt** | 4 | 5.2993 | 1.7561 | 12 | 3.02 | 0.0107 |
| **Trt** | 5 | 4.3827 | 1.7561 | 12 | 2.50 | 0.0281 |
| **Trt** | 6 | 3.9627 | 1.7561 | 12 | 2.26 | 0.0435 |
| **Trt** | 7 | 29.8700 | 1.7561 | 12 | 17.01 | <.0001 |

Plant results- Zn only

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | biomass |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | Control Znmix ZnEDTA ZnO ZnP ZnSO4 |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 18 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 7 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 18 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 7 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |

| Optimization Information | |
|---|---|
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | -19.13 |
| **AIC (smaller is better)** | -5.13 |
| **AICC (smaller is better)** | 22.87 |
| **BIC (smaller is better)** | -1.73 |
| **CAIC (smaller is better)** | 5.27 |
| **HQIC (smaller is better)** | -6.38 |
| **Pearson Chi-Square** | 0.08 |
| **Pearson Chi-Square / DF** | 0.01 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 5 | 12 | 3.73 | 0.0285 |

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | PlantZnconc. |

| Model Information | |
|---|---|
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | Control Znmix ZnEDTA ZnO ZnP ZnSO4 |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 18 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 7 |
| **Columns in Z** | 0 |

| Dimensions | |
|---|---|
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 18 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 7 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 86.04 |
| **AIC (smaller is better)** | 100.04 |
| **AICC (smaller is better)** | 128.04 |
| **BIC (smaller is better)** | 103.43 |
| **CAIC (smaller is better)** | 110.43 |
| **HQIC (smaller is better)** | 98.78 |
| **Pearson Chi-Square** | 527.30 |

| Fit Statistics | |
|---|---|
| **Pearson Chi-Square / DF** | 43.94 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 5 | 12 | 5.89 | 0.0056 |

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | PlantZnUptake |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | Control Znmix ZnEDTA ZnO ZnP ZnSO4 |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 18 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 7 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 18 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 7 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 72.28 |
| **AIC (smaller is better)** | 86.28 |
| **AICC (smaller is better)** | 114.28 |
| **BIC (smaller is better)** | 89.67 |
| **CAIC (smaller is better)** | 96.67 |
| **HQIC (smaller is better)** | 85.02 |
| **Pearson Chi-Square** | 167.46 |
| **Pearson Chi-Square / DF** | 13.95 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 5 | 12 | 10.43 | 0.0005 |

Plant results- Zn coated Urea

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | biomass |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |

| Model Information | |
|---|---|
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | Control Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 21 |
| **Number of Observations Used** | 17 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 7 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 17 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 7 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | -15.33 |
| **AIC (smaller is better)** | -1.33 |
| **AICC (smaller is better)** | 36.00 |
| **BIC (smaller is better)** | 1.45 |
| **CAIC (smaller is better)** | 8.45 |
| **HQIC (smaller is better)** | -3.09 |
| **Pearson Chi-Square** | 0.09 |
| **Pearson Chi-Square / DF** | 0.01 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| Effect | Num DF | Den DF | F Value | Pr > F |
| Treatments | 5 | 11 | 2.53 | 0.0923 |

| Model Information | |
|---|---|
| Data Set | WORK.IN |
| Response Variable | PlantZnconc. |
| Response Distribution | Gaussian |
| Link Function | Identity |
| Variance Function | Default |
| Variance Matrix | Diagonal |
| Estimation Technique | Restricted Maximum Likelihood |
| Degrees of Freedom Method | Residual |

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| Treatments | 7 | ZNMIX-U Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U control |
| Replications | 3 | 1 2 3 |

| | |
|---|---|
| Number of Observations Read | 21 |

| Number of Observations Used | 20 |
|---|---|

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 8 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 20 |

| Optimization Information | |
|---|---|
| Optimization Technique | None |
| Parameters | 8 |
| Lower Boundaries | 1 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

| Fit Statistics | |
|---|---|
| -2 Res Log Likelihood | 89.91 |
| AIC (smaller is better) | 105.91 |
| AICC (smaller is better) | 141.91 |

| Fit Statistics | |
|---|---|
| **BIC (smaller is better)** | 110.43 |
| **CAIC (smaller is better)** | 118.43 |
| **HQIC (smaller is better)** | 104.98 |
| **Pearson Chi-Square** | 438.21 |
| **Pearson Chi-Square / DF** | 33.71 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 6 | 13 | 5.63 | 0.0045 |

# Appendix B - Chapter-4

Visualization

1-Control

2-ZnO

3-ZnSO4

4-EDTA

5-Znmix

6-ZnEDTA

Day-1

| Model Information | |
|---|---|
| **Data Set** | WORK.DIFFUSION |
| **Dependent Variable** | Radius |
| **Covariance Structures** | Variance Components, Ante-dependence |
| **Subject Effect** | Rep(Trt) |
| **Estimation Method** | REML |
| **Residual Variance Method** | None |
| **Fixed Effects SE Method** | Model-Based |
| **Degrees of Freedom Method** | Containment |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Time** | 1 | Day1 |
| **Trt** | 6 | 1 2 3 4 5 6 |
| **Rep** | 3 | 1 2 3 |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 2 |
| **Columns in X** | 14 |
| **Columns in Z** | 3 |
| **Subjects** | 1 |
| **Max Obs per Subject** | 18 |

| Number of Observations | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 18 |
| **Number of Observations Not Used** | 0 |

**Type 3 Tests of Fixed Effects**

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 27.16 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 0.1867 | 0.1536 | 10 | 1.21 | 0.2523 |
| **Trt** | 2 | 0.2733 | 0.1536 | 10 | 1.78 | 0.1056 |
| **Trt** | 3 | 1.6367 | 0.1536 | 10 | 10.65 | <.0001 |
| **Trt** | 4 | 0.06667 | 0.1536 | 10 | 0.43 | 0.6736 |
| **Trt** | 5 | 0.2967 | 0.1536 | 10 | 1.93 | 0.0823 |
| **Trt** | 6 | 1.6667 | 0.1536 | 10 | 10.85 | <.0001 |

Week 1

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 47.27 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 0.1567 | 0.1011 | 10 | 1.55 | 0.1522 |
| **Trt** | 2 | 0.3600 | 0.1011 | 10 | 3.56 | 0.0052 |
| **Trt** | 3 | 1.2200 | 0.1011 | 10 | 12.07 | <.0001 |
| **Trt** | 4 | 0.05667 | 0.1011 | 10 | 0.56 | 0.5874 |
| **Trt** | 5 | 0.7000 | 0.1011 | 10 | 6.93 | <.0001 |
| **Trt** | 6 | 1.8500 | 0.1011 | 10 | 18.30 | <.0001 |

Week 3

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 382.90 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 0.08333 | 0.07659 | 10 | 1.09 | 0.3021 |
| **Trt** | 2 | 0.5733 | 0.07659 | 10 | 7.49 | <.0001 |
| **Trt** | 3 | 2.7033 | 0.07659 | 10 | 35.29 | <.0001 |
| **Trt** | 4 | 0.07333 | 0.07659 | 10 | 0.96 | 0.3609 |
| **Trt** | 5 | 0.6433 | 0.07659 | 10 | 8.40 | <.0001 |
| **Trt** | 6 | 2.6867 | 0.07659 | 10 | 35.08 | <.0001 |

Week 5

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 207.70 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 0.2200 | 0.1158 | 10 | 1.90 | 0.0867 |
| **Trt** | 2 | 0.9233 | 0.1158 | 10 | 7.97 | <.0001 |
| **Trt** | 3 | 3.4467 | 0.1158 | 10 | 29.75 | <.0001 |
| **Trt** | 4 | 0.07000 | 0.1158 | 10 | 0.60 | 0.5591 |
| **Trt** | 5 | 0.7467 | 0.1158 | 10 | 6.45 | <.0001 |
| **Trt** | 6 | 3.3467 | 0.1158 | 10 | 28.89 | <.0001 |

Soil pH

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Control EDTA ZnEDTA ZnO ZnSO4 Znmix |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 4 | 1 2 3 4 |

| | |
|---|---|
| **Number of Observations Read** | 72 |
| **Number of Observations Used** | 72 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 35 | 7.34798194 | 0.20994234 | 7.48 | <.0001 |
| **Error** | 36 | 1.01101667 | 0.02808380 | | |
| **Corrected Total** | 71 | 8.35899861 | | | |

| R-Square | Coeff Var | Root MSE | SoilpH Mean |
|---|---|---|---|
| 0.879051 | 2.683522 | 0.167582 | 6.244861 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 1.16270694 | 0.23254139 | 8.28 | <.0001 |
| **REPS(TREATS)** | 12 | 0.57771667 | 0.04814306 | 1.71 | 0.1046 |
| **SUBPLOTS** | 3 | 3.19363750 | 1.06454583 | 37.91 | <.0001 |
| **TREATS*SUBPLOTS** | 15 | 2.41392083 | 0.16092806 | 5.73 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 1.16270694 | 0.23254139 | 8.28 | <.0001 |
| **REPS(TREATS)** | 12 | 0.57771667 | 0.04814306 | 1.71 | 0.1046 |
| **SUBPLOTS** | 3 | 3.19363750 | 1.06454583 | 37.91 | <.0001 |
| **TREATS*SUBPLOTS** | 15 | 2.41392083 | 0.16092806 | 5.73 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 1.16270694 | 0.23254139 | 4.83 | 0.0119 |

Total Zn- Percentage Zn recovered as of added Zn

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 4 | ZnEDTA ZnO ZnSO4 Znmix |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 4 | 1 2 3 4 |

| | |
|---|---|
| **Number of Observations Read** | 48 |
| **Number of Observations Used** | 48 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 23 | 37010.49630 | 1609.15201 | 2370.15 | <.0001 |
| **Error** | 24 | 16.29420 | 0.67893 | | |
| **Corrected Total** | 47 | 37026.79050 | | | |

| R-Square | Coeff Var | Root MSE | %Zn Mean |
|---|---|---|---|
| 0.999560 | 3.295849 | 0.823969 | 25.00021 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 3 | 0.00009 | 0.00003 | 0.00 | 1.0000 |
| **REPS(TREATS)** | 8 | 0.00013 | 0.00002 | 0.00 | 1.0000 |
| **SUBPLOTS** | 3 | 26325.39712 | 8775.13237 | 12925.0 | <.0001 |
| **TREATS*SUBPLOTS** | 9 | 10685.09895 | 1187.23322 | 1748.70 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 3 | 0.00009 | 0.00003 | 0.00 | 1.0000 |
| **REPS(TREATS)** | 8 | 0.00013 | 0.00002 | 0.00 | 1.0000 |
| **SUBPLOTS** | 3 | 26325.39712 | 8775.13237 | 12925.0 | <.0001 |
| **TREATS*SUBPLOTS** | 9 | 10685.09895 | 1187.23322 | 1748.70 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 3 | 0.00008958 | 0.00002986 | 1.79 | 0.2265 |

Greenhouse study

Soil pH

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Control EDTA ZnEDTA ZnO ZnSO4 Znmix |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 8 | 1 2 3 4 5 6 7 8 |

| | |
|---|---|
| **Number of Observations Read** | 144 |
| **Number of Observations Used** | 144 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 59 | 7.27973889 | 0.12338540 | 3.43 | <.0001 |
| **Error** | 84 | 3.01759167 | 0.03592371 | | |
| **Corrected Total** | 143 | 10.29733056 | | | |

| R-Square | Coeff Var | Root MSE | pH Mean |
|---|---|---|---|
| 0.706954 | 3.179163 | 0.189536 | 5.961806 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 0.28793056 | 0.05758611 | 1.60 | 0.1682 |
| **REPS(TREATS)** | 12 | 1.44627500 | 0.12052292 | 3.35 | 0.0005 |
| **SUBPLOTS** | 7 | 3.21696389 | 0.45956627 | 12.79 | <.0001 |
| **TREATS*SUBPLOTS** | 35 | 2.32856944 | 0.06653056 | 1.85 | 0.0115 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 0.28793056 | 0.05758611 | 1.60 | 0.1682 |
| **REPS(TREATS)** | 12 | 1.44627500 | 0.12052292 | 3.35 | 0.0005 |
| **SUBPLOTS** | 7 | 3.21696389 | 0.45956627 | 12.79 | <.0001 |
| **TREATS*SUBPLOTS** | 35 | 2.32856944 | 0.06653056 | 1.85 | 0.0115 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 0.28793056 | 0.05758611 | 0.48 | 0.7862 |

Soil water Zn

1=control

2=ZnO

3=ZnSO4

4=Znmix

5=EDTA

6=ZnEDTA

Week 3

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Time** | 1 | w3 |
| **Trt** | 6 | 1 2 3 4 5 6 |
| **Rep** | 3 | 1 2 3 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 2 |
| **Columns in X** | 14 |
| **Columns in Z** | 3 |
| **Subjects** | 1 |
| **Max Obs per Subject** | 18 |

| Number of Observations | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 18 |
| **Number of Observations Not Used** | 0 |

| Iteration History | | | |
|---|---|---|---|
| **Iteration** | **Evaluations** | **-2 Res Log Like** | **Criterion** |
| **0** | 1 | 128.74575857 | |
| **1** | 2 | 128.79141273 | 0.0007683 2 |
| **2** | 1 | 128.74671839 | 0.0000177 0 |
| **3** | 1 | 128.74575915 | 0.0000000 1 |
| **4** | 1 | 128.74575857 | 0.0000000 0 |

Convergence criteria met.

***Estimated G matrix is not positive definite.***

| Covariance Parameter Estimates | | |
|---|---|---|
| **Cov Parm** | **Subject** | **Estimate** |
| **Rep** | | 0 |
| **Var(1)** | Rep(Trt) | 1543.23 |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 128.7 |
| **AIC (Smaller is Better)** | 130.7 |
| **AICC (Smaller is Better)** | 131.1 |
| **BIC (Smaller is Better)** | 129.8 |

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0 | . | . | . | . |
| **Rep** | 2 | 0 | . | . | . | . |
| **Rep** | 3 | 0 | . | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 17.61 | 0.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 49.1833 | 22.6806 | 10 | 2.17 | 0.0553 |
| **Trt** | 2 | 144.05 | 22.6806 | 10 | 6.35 | <.0001 |
| **Trt** | 3 | 70.1300 | 22.6806 | 10 | 3.09 | 0.0114 |
| **Trt** | 4 | 126.01 | 22.6806 | 10 | 5.56 | 0.0002 |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 5 | 50.7733 | 22.6806 | 10 | 2.24 | 0.0491 |
| **Trt** | 6 | 300.18 | 22.6806 | 10 | 13.24 | <.0001 |

Week 5

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | -3.9997 | 4.8604 | 10 | -0.82 | 0.4297 |
| **Rep** | 2 | 5.1298 | 4.8604 | 10 | 1.06 | 0.3161 |
| **Rep** | 3 | -1.1301 | 4.8604 | 10 | -0.23 | 0.8208 |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 13.75 | 0.0003 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 35.2700 | 8.3660 | 10 | 4.22 | 0.0018 |
| **Trt** | 2 | 43.0333 | 8.3660 | 10 | 5.14 | 0.0004 |
| **Trt** | 3 | 60.1967 | 8.3660 | 10 | 7.20 | <.0001 |
| **Trt** | 4 | 46.8600 | 8.3660 | 10 | 5.60 | 0.0002 |
| **Trt** | 5 | 39.6267 | 8.3660 | 10 | 4.74 | 0.0008 |
| **Trt** | 6 | 110.49 | 8.3660 | 10 | 13.21 | <.0001 |

DTPA Zn

1=control

2=ZnO

3=ZnSO4

4=EDTA

5=Zn mix

6=ZnEDTA

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | 1 2 3 4 5 6 |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 8 | 1 2 3 4 5 6 7 8 |

| | |
|---|---|
| **Number of Observations Read** | 144 |
| **Number of Observations Used** | 144 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 59 | 1481.885695 | 25.116707 | 53.74 | <.0001 |
| **Error** | 84 | 39.258404 | 0.467362 | | |
| **Corrected Total** | 143 | 1521.144099 | | | |

| R-Square | Coeff Var | Root MSE | dtpazn Mean |
|---|---|---|---|
| 0.974192 | 26.57616 | 0.683639 | 2.572376 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 227.5382962 | 45.5076592 | 97.37 | <.0001 |
| **REPS(TREATS)** | 12 | 3.9539979 | 0.3294998 | 0.71 | 0.7424 |
| **SUBPLOTS** | 7 | 802.6096242 | 114.6585177 | 245.33 | <.0001 |
| **TREATS*SUBPLOTS** | 35 | 447.7837768 | 12.7938222 | 27.37 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 227.5382962 | 45.5076592 | 97.37 | <.0001 |
| **REPS(TREATS)** | 12 | 3.9539979 | 0.3294998 | 0.71 | 0.7424 |
| **SUBPLOTS** | 7 | 802.6096242 | 114.6585177 | 245.33 | <.0001 |
| **TREATS*SUBPLOTS** | 35 | 447.7837768 | 12.7938222 | 27.37 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 227.5382962 | 45.5076592 | 138.11 | <.0001 |

Total Zn

1=control

2=ZnO

3=ZnSO4

4=EDTA

5=Znmix

6=ZnEDTA

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | 1 2 3 4 5 6 |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 8 | 1 2 3 4 5 6 7 8 |

| | |
|---|---|
| **Number of Observations Read** | 144 |
| **Number of Observations Used** | 144 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 59 | 29368.34424 | 497.76855 | 291.25 | <.0001 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Error** | 84 | 143.56197 | 1.70907 | | |
| **Corrected Total** | 143 | 29511.90622 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.995135 | 4.729254 | 1.307314 | 27.64315 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 1810.99240 | 362.19848 | 211.93 | <.0001 |
| **REPS(TREATS)** | 12 | 32.12040 | 2.67670 | 1.57 | 0.1175 |
| **SUBPLOTS** | 7 | 17354.12223 | 2479.16032 | 1450.59 | <.0001 |
| **TREATS*SUBPLOTS** | 35 | 10171.10922 | 290.60312 | 170.04 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 1810.99240 | 362.19848 | 211.93 | <.0001 |
| **REPS(TREATS)** | 12 | 32.12040 | 2.67670 | 1.57 | 0.1175 |
| **SUBPLOTS** | 7 | 17354.12223 | 2479.16032 | 1450.59 | <.0001 |
| **TREATS*SUBPLOTS** | 35 | 10171.10922 | 290.60312 | 170.04 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 1810.992401 | 362.198480 | 135.32 | <.0001 |

Plant results

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | Biomass |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | EDTA ZnEDTA ZnO ZnSO4 Znmix control |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 17 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 7 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 17 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 7 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | -3.31 |
| **AIC (smaller is better)** | 10.69 |
| **AICC (smaller is better)** | 48.03 |
| **BIC (smaller is better)** | 13.48 |
| **CAIC (smaller is better)** | 20.48 |
| **HQIC (smaller is better)** | 8.94 |
| **Pearson Chi-Square** | 0.27 |
| **Pearson Chi-Square / DF** | 0.02 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 5 | 11 | 2.75 | 0.0753 |

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | PlantZnconc. |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | EDTA ZnEDTA ZnO ZnSO4 Znmix control |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 17 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 7 |
| **Columns in Z** | 0 |

| Dimensions | |
|---|---|
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 17 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 7 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 55.19 |
| **AIC (smaller is better)** | 69.19 |
| **AICC (smaller is better)** | 106.53 |
| **BIC (smaller is better)** | 71.98 |
| **CAIC (smaller is better)** | 78.98 |
| **HQIC (smaller is better)** | 67.44 |
| **Pearson Chi-Square** | 55.43 |
| **Pearson Chi-Square / DF** | 5.04 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 5 | 11 | 8.38 | 0.0017 |

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | PlantZnuptake |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | EDTA ZnEDTA ZnO ZnSO4 Znmix control |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 17 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 7 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 17 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 7 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 68.68 |
| **AIC (smaller is better)** | 82.68 |
| **AICC (smaller is better)** | 120.01 |
| **BIC (smaller is better)** | 85.46 |
| **CAIC (smaller is better)** | 92.46 |
| **HQIC (smaller is better)** | 80.92 |
| **Pearson Chi-Square** | 188.86 |
| **Pearson Chi-Square / DF** | 17.17 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 5 | 11 | 7.05 | 0.0035 |

# Appendix C - Chapter 5

Chapter 5

Greenhouse study

Calcareous soil

Soil pH

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 5 | Control ZnO ZnSO4 Znmix ZnEDTA |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 8 | 1 2 3 4 5 6 7 8 |

| | |
|---|---|
| **Number of Observations Read** | 120 |
| **Number of Observations Used** | 120 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 49 | 1.25323750 | 0.02557628 | 3.47 | <.0001 |
| **Error** | 70 | 0.51587500 | 0.00736964 | | |
| **Corrected Total** | 119 | 1.76911250 | | | |

| R-Square | Coeff Var | Root MSE | pH Mean |
|---|---|---|---|
| 0.708399 | 1.037892 | 0.085847 | 8.271250 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 0.16228333 | 0.04057083 | 5.51 | 0.0007 |
| **REPS(TREATS)** | 10 | 0.56039167 | 0.05603917 | 7.60 | <.0001 |
| **SUBPLOTS** | 7 | 0.27300583 | 0.03900083 | 5.29 | <.0001 |
| **TREATS*SUBPLOTS** | 28 | 0.25755667 | 0.00919845 | 1.25 | 0.2257 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 0.16228333 | 0.04057083 | 5.51 | 0.0007 |
| **REPS(TREATS)** | 10 | 0.56039167 | 0.05603917 | 7.60 | <.0001 |
| **SUBPLOTS** | 7 | 0.27300583 | 0.03900083 | 5.29 | <.0001 |
| **TREATS*SUBPLOTS** | 28 | 0.25755667 | 0.00919845 | 1.25 | 0.2257 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 4 | 0.16228333 | 0.04057083 | 0.72 | 0.5952 |

Soil water Zn

Week 3

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 4 | 8 | 4.35 | 0.0369 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 27.3133 | 4.0240 | 8 | 6.79 | 0.0001 |
| **Trt** | 2 | 22.6400 | 4.0240 | 8 | 5.63 | 0.0005 |
| **Trt** | 3 | 25.5733 | 4.0240 | 8 | 6.36 | 0.0002 |
| **Trt** | 4 | 27.8333 | 4.0240 | 8 | 6.92 | 0.0001 |
| **Trt** | 5 | 43.7133 | 4.0240 | 8 | 10.86 | <.0001 |

Week 5

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 4 | 8 | 0.28 | 0.8846 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 131.22 | 8.8449 | 8 | 14.84 | <.0001 |
| **Trt** | 2 | 140.58 | 8.8449 | 8 | 15.89 | <.0001 |
| **Trt** | 3 | 134.61 | 8.8449 | 8 | 15.22 | <.0001 |
| **Trt** | 4 | 138.12 | 8.8449 | 8 | 15.62 | <.0001 |
| **Trt** | 5 | 133.02 | 8.8449 | 8 | 15.04 | <.0001 |

Total Zn

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 5 | Control ZnO ZnSO4 Znmix ZnEDTA |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 8 | 1 2 3 4 5 6 7 8 |

| | |
|---|---|
| **Number of Observations Read** | 120 |
| **Number of Observations Used** | 120 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 49 | 22529.34768 | 459.78261 | 4.42 | <.0001 |
| **Error** | 70 | 7274.69774 | 103.92425 | | |
| **Corrected Total** | 119 | 29804.04542 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.755916 | 15.67742 | 10.19432 | 65.02552 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 1167.19365 | 291.79841 | 2.81 | 0.0320 |
| **REPS(TREATS)** | 10 | 976.21326 | 97.62133 | 0.94 | 0.5033 |
| **SUBPLOTS** | 7 | 10191.75867 | 1455.96552 | 14.01 | <.0001 |
| **TREATS*SUBPLOTS** | 28 | 10194.18209 | 364.07793 | 3.50 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 1167.19365 | 291.79841 | 2.81 | 0.0320 |
| **REPS(TREATS)** | 10 | 976.21326 | 97.62133 | 0.94 | 0.5033 |
| **SUBPLOTS** | 7 | 10191.75867 | 1455.96552 | 14.01 | <.0001 |
| **TREATS*SUBPLOTS** | 28 | 10194.18209 | 364.07793 | 3.50 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 4 | 1167.193652 | 291.798413 | 2.99 | 0.0730 |

DTPA Zn

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 5 | Control ZnO ZnSO4 Znmix ZnEDTA |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 8 | 1 2 3 4 5 6 7 8 |

| | |
|---|---|
| **Number of Observations Read** | 120 |
| **Number of Observations Used** | 120 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 49 | 9377.52875 | 191.37814 | 20.22 | <.0001 |
| **Error** | 70 | 662.59254 | 9.46561 | | |
| **Corrected Total** | 119 | 10040.12129 | | | |

| R-Square | Coeff Var | Root MSE | dtpazn Mean |
|---|---|---|---|
| 0.934006 | 63.82704 | 3.076623 | 4.820250 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 656.862738 | 164.215685 | 17.35 | <.0001 |
| **REPS(TREATS)** | 10 | 71.088592 | 7.108859 | 0.75 | 0.6744 |
| **SUBPLOTS** | 7 | 6253.280333 | 893.325762 | 94.38 | <.0001 |
| **TREATS*SUBPLOTS** | 28 | 2396.297088 | 85.582039 | 9.04 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 656.862738 | 164.215685 | 17.35 | <.0001 |
| **REPS(TREATS)** | 10 | 71.088592 | 7.108859 | 0.75 | 0.6744 |
| **SUBPLOTS** | 7 | 6253.280333 | 893.325762 | 94.38 | <.0001 |
| **TREATS*SUBPLOTS** | 28 | 2396.297088 | 85.582039 | 9.04 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 4 | 656.8627383 | 164.2156846 | 23.10 | <.0001 |

Plant results

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | Biomass |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 5 | Control ZnEDTA ZnO ZnSO4 Znmix |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 15 |
| **Number of Observations Used** | 11 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 6 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 11 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 6 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | -11.54 |
| **AIC (smaller is better)** | 0.46 |
| **AICC (smaller is better)** | 84.46 |
| **BIC (smaller is better)** | -0.79 |
| **CAIC (smaller is better)** | 5.21 |
| **HQIC (smaller is better)** | -4.54 |
| **Pearson Chi-Square** | 0.03 |
| **Pearson Chi-Square / DF** | 0.00 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 4 | 6 | 5.65 | 0.0311 |

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | PlantZnuptake |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 5 | Control ZnEDTA ZnO ZnSO4 Znmix |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 15 |
| **Number of Observations Used** | 11 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 6 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 11 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 6 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 47.56 |
| **AIC (smaller is better)** | 59.56 |
| **AICC (smaller is better)** | 143.56 |
| **BIC (smaller is better)** | 58.31 |
| **CAIC (smaller is better)** | 64.31 |
| **HQIC (smaller is better)** | 54.56 |
| **Pearson Chi-Square** | 510.36 |
| **Pearson Chi-Square / DF** | 85.06 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 4 | 6 | 23.27 | 0.0008 |

Acid soil

Soil pH

Zn only

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Control ZnO ZnSO4 Znmix ZnP ZnEDTA |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 90 |
| **Number of Observations Used** | 90 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 41 | 1.06415667 | 0.02595504 | 1.60 | 0.0590 |
| **Error** | 48 | 0.77885333 | 0.01622611 | | |
| **Corrected Total** | 89 | 1.84301000 | | | |

| R-Square | Coeff Var | Root MSE | pH Mean |
|---|---|---|---|
| 0.577401 | 2.332003 | 0.127382 | 5.462333 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TREATS | 5 | 0.19065000 | 0.03813000 | 2.35 | 0.0549 |
| REPS(TREATS) | 12 | 0.11448000 | 0.00954000 | 0.59 | 0.8410 |
| SUBPLOTS | 4 | 0.40170444 | 0.10042611 | 6.19 | 0.0004 |
| TREATS*SUBPLOTS | 20 | 0.35732222 | 0.01786611 | 1.10 | 0.3793 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TREATS | 5 | 0.19065000 | 0.03813000 | 2.35 | 0.0549 |
| REPS(TREATS) | 12 | 0.11448000 | 0.00954000 | 0.59 | 0.8410 |
| SUBPLOTS | 4 | 0.40170444 | 0.10042611 | 6.19 | 0.0004 |
| TREATS*SUBPLOTS | 20 | 0.35732222 | 0.01786611 | 1.10 | 0.3793 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
| TREATS | 5 | 0.19065000 | 0.03813000 | 4.00 | 0.0229 |

Zn coated Urea

| Class Level Information | | |
|---|---|---|
| Class | Levels | Values |
| TREATS | 7 | Control Urea ZnO-U ZnSO4-U Znmix-U ZnP-U ZnEDTA-U |
| REPS | 3 | 1 2 3 |
| SUBPLOTS | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 105 |
| **Number of Observations Used** | 105 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 48 | 14.73162476 | 0.30690885 | 4.31 | <.0001 |
| **Error** | 56 | 3.98776000 | 0.07121000 | | |
| **Corrected Total** | 104 | 18.71938476 | | | |

| R-Square | Coeff Var | Root MSE | pH Mean |
|---|---|---|---|
| 0.786972 | 4.745763 | 0.266852 | 5.622952 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 1.21326476 | 0.20221079 | 2.84 | 0.0174 |
| **REPS(TREATS)** | 14 | 1.13044000 | 0.08074571 | 1.13 | 0.3507 |
| **SUBPLOTS** | 4 | 8.71854667 | 2.17963667 | 30.61 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 3.66937333 | 0.15289056 | 2.15 | 0.0097 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 1.21326476 | 0.20221079 | 2.84 | 0.0174 |
| **REPS(TREATS)** | 14 | 1.13044000 | 0.08074571 | 1.13 | 0.3507 |
| **SUBPLOTS** | 4 | 8.71854667 | 2.17963667 | 30.61 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 3.66937333 | 0.15289056 | 2.15 | 0.0097 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 6 | 1.21326476 | 0.20221079 | 2.50 | 0.0736 |

Soil water Zn

Zn only
1-control
2-ZnO
3-ZnSO4
4-Znmix
5-ZnP
6-ZnEDTA
Week 3

| Model Information | |
|---|---|
| **Data Set** | WORK.SOILWAT |
| **Dependent Variable** | soilwt |
| **Covariance Structures** | Variance Components, Ante-dependence |
| **Subject Effect** | Rep(Trt) |
| **Estimation Method** | REML |
| **Residual Variance Method** | None |
| **Fixed Effects SE Method** | Model-Based |
| **Degrees of Freedom Method** | Containment |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Time** | 1 | week3 |
| **Trt** | 6 | 1 2 3 4 5 6 |
| **Rep** | 3 | 1 2 3 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 2 |
| **Columns in X** | 14 |
| **Columns in Z** | 3 |
| **Subjects** | 1 |
| **Max Obs per Subject** | 18 |

| Number of Observations | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 18 |
| **Number of Observations Not Used** | 0 |

| Iteration History | | | |
|---|---|---|---|
| **Iteration** | **Evaluations** | **-2 Res Log Like** | **Criterion** |
| **0** | 1 | 85.50690225 | |
| **1** | 2 | 85.50729276 | 0.00001218 |
| **2** | 1 | 85.50690234 | 0.00000000 |

Convergence criteria met.

***Estimated G matrix is not positive definite.***

| Covariance Parameter Estimates | | |
|---|---|---|
| **Cov Parm** | **Subject** | **Estimate** |
| **Rep** | | 0 |
| **Var(1)** | Rep(Trt) | 42.0250 |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 85.5 |
| **AIC (Smaller is Better)** | 87.5 |
| **AICC (Smaller is Better)** | 87.9 |
| **BIC (Smaller is Better)** | 86.6 |

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0 | . | . | . | . |
| **Rep** | 2 | 0 | . | . | . | . |
| **Rep** | 3 | 0 | . | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 18.45 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 34.4033 | 3.7428 | 10 | 9.19 | <.0001 |
| **Trt** | 2 | 39.3167 | 3.7428 | 10 | 10.50 | <.0001 |
| **Trt** | 3 | 57.9000 | 3.7428 | 10 | 15.47 | <.0001 |
| **Trt** | 4 | 50.4600 | 3.7428 | 10 | 13.48 | <.0001 |
| **Trt** | 5 | 25.3567 | 3.7428 | 10 | 6.77 | <.0001 |
| **Trt** | 6 | 68.9200 | 3.7428 | 10 | 18.41 | <.0001 |

Week 5

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0 | . | . | . | . |
| **Rep** | 2 | 0 | . | . | . | . |
| **Rep** | 3 | 0 | . | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 5 | 10 | 23.69 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 4.7423 | 4.1535 | 10 | 1.14 | 0.2802 |
| **Trt** | 2 | 37.0033 | 4.1535 | 10 | 8.91 | <.0001 |
| **Trt** | 3 | 24.6767 | 4.1535 | 10 | 5.94 | 0.0001 |
| **Trt** | 4 | 20.2667 | 4.1535 | 10 | 4.88 | 0.0006 |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 5 | 20.4600 | 4.1535 | 10 | 4.93 | 0.0006 |
| **Trt** | 6 | 63.9967 | 4.1535 | 10 | 15.41 | <.0001 |

Zn coated Urea

Week 3

| Model Information | |
|---|---|
| **Data Set** | WORK.SOILWAT |
| **Dependent Variable** | soilwt |
| **Covariance Structures** | Variance Components, Ante-dependence |
| **Subject Effect** | Rep(Trt) |
| **Estimation Method** | REML |
| **Residual Variance Method** | None |
| **Fixed Effects SE Method** | Model-Based |
| **Degrees of Freedom Method** | Containment |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Time** | 1 | week3 |
| **Trt** | 7 | Control Znmix-U Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U |
| **Rep** | 3 | 1 2 3 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 2 |
| **Columns in X** | 16 |
| **Columns in Z** | 3 |

| Dimensions | |
|---|---|
| **Subjects** | 1 |
| **Max Obs per Subject** | 21 |

| Number of Observations | |
|---|---|
| **Number of Observations Read** | 21 |
| **Number of Observations Used** | 21 |
| **Number of Observations Not Used** | 0 |

| Iteration History | | | |
|---|---|---|---|
| **Iteration** | **Evaluations** | **-2 Res Log Like** | **Criterion** |
| **0** | 1 | 113.28249020 | |
| **1** | 2 | 113.30164325 | 0.00040931 |
| **2** | 1 | 113.28265673 | 0.00000378 |
| **3** | 1 | 113.28249021 | 0.00000000 |

Convergence criteria met.

***Estimated G matrix is not positive definite.***

| Covariance Parameter Estimates | | |
|---|---|---|
| **Cov Parm** | **Subject** | **Estimate** |
| **Rep** | | 0 |
| **Var(1)** | Rep(Trt) | 110.43 |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 113.3 |
| **AIC (Smaller is Better)** | 115.3 |
| **AICC (Smaller is Better)** | 115.6 |
| **BIC (Smaller is Better)** | 114.4 |

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0 | . | . | . | . |
| **Rep** | 2 | 0 | . | . | . | . |
| **Rep** | 3 | 0 | . | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 6 | 12 | 5.91 | 0.0045 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | Control | 34.4033 | 6.0671 | 12 | 5.67 | 0.0001 |
| **Trt** | Znmix-U | 26.5333 | 6.0671 | 12 | 4.37 | 0.0009 |
| **Trt** | Urea | 26.4600 | 6.0671 | 12 | 4.36 | 0.0009 |
| **Trt** | ZnEDTA-U | 28.2200 | 6.0671 | 12 | 4.65 | 0.0006 |
| **Trt** | ZnO-U | 61.4200 | 6.0671 | 12 | 10.12 | <.0001 |
| **Trt** | ZnP-U | 36.7600 | 6.0671 | 12 | 6.06 | <.0001 |
| **Trt** | ZnSO4-U | 57.6533 | 6.0671 | 12 | 9.50 | <.0001 |

Week 5

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0 | . | . | . | . |
| **Rep** | 2 | 0 | . | . | . | . |
| **Rep** | 3 | 0 | . | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 6 | 12 | 23.35 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | Control | 4.7423 | 5.6896 | 12 | 0.83 | 0.4208 |
| **Trt** | Znmix-U | 35.7567 | 5.6896 | 12 | 6.28 | <.0001 |
| **Trt** | Urea | 21.6233 | 5.6896 | 12 | 3.80 | 0.0025 |
| **Trt** | ZnEDTA-U | 74.3433 | 5.6896 | 12 | 13.07 | <.0001 |
| **Trt** | ZnO-U | 79.6567 | 5.6896 | 12 | 14.00 | <.0001 |
| **Trt** | ZnP-U | 49.8067 | 5.6896 | 12 | 8.75 | <.0001 |
| **Trt** | ZnSO4-U | 59.5767 | 5.6896 | 12 | 10.47 | <.0001 |

DTPA Zn

Zn only

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Control ZnO ZnSO4 Znmix ZnP ZnEDTA |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 90 |
| **Number of Observations Used** | 90 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 41 | 1405.788326 | 34.287520 | 100.93 | <.0001 |
| **Error** | 48 | 16.307080 | 0.339731 | | |
| **Corrected Total** | 89 | 1422.095406 | | | |

| R-Square | Coeff Var | Root MSE | DTPA Zn Mean |
|---|---|---|---|
| 0.988533 | 22.42262 | 0.582864 | 2.599448 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 87.298914 | 17.459783 | 51.39 | <.0001 |
| **REPS(TREATS)** | 12 | 2.787571 | 0.232298 | 0.68 | 0.7583 |
| **SUBPLOTS** | 4 | 1085.063755 | 271.265939 | 798.47 | <.0001 |
| **TREATS*SUBPLOTS** | 20 | 230.638085 | 11.531904 | 33.94 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 87.298914 | 17.459783 | 51.39 | <.0001 |
| **REPS(TREATS)** | 12 | 2.787571 | 0.232298 | 0.68 | 0.7583 |
| **SUBPLOTS** | 4 | 1085.063755 | 271.265939 | 798.47 | <.0001 |
| **TREATS*SUBPLOTS** | 20 | 230.638085 | 11.531904 | 33.94 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 87.29891393 | 17.45978279 | 75.16 | <.0001 |

Zn coated Urea

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 7 | Znmix-U Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U control |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 105 |
| **Number of Observations Used** | 105 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 48 | 183.9415076 | 3.8321147 | 394.40 | <.0001 |
| **Error** | 56 | 0.5441091 | 0.0097162 | | |
| **Corrected Total** | 104 | 184.4856167 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.997051 | 8.371664 | 0.098571 | 1.177436 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 22.3930420 | 3.7321737 | 384.12 | <.0001 |
| **REPS(TREATS)** | 14 | 0.2397432 | 0.0171245 | 1.76 | 0.0685 |
| **SUBPLOTS** | 4 | 113.2949650 | 28.3237413 | 2915.09 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 48.0137573 | 2.0005732 | 205.90 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 22.3930420 | 3.7321737 | 384.12 | <.0001 |
| **REPS(TREATS)** | 14 | 0.2397432 | 0.0171245 | 1.76 | 0.0685 |
| **SUBPLOTS** | 4 | 113.2949650 | 28.3237413 | 2915.09 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 48.0137573 | 2.0005732 | 205.90 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 6 | 22.39304200 | 3.73217367 | 217.94 | <.0001 |

Total Zn

Zn only

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 6 | Control ZnO ZnSO4 Znmix ZnP ZnEDTA |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 90 |
| **Number of Observations Used** | 90 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 41 | 3468.540005 | 84.598537 | 45.31 | <.0001 |
| **Error** | 48 | 89.613172 | 1.866941 | | |
| **Corrected Total** | 89 | 3558.153177 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.974815 | 2.871233 | 1.366361 | 47.58794 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 263.589734 | 52.717947 | 28.24 | <.0001 |
| **REPS(TREATS)** | 12 | 18.112074 | 1.509340 | 0.81 | 0.6403 |
| **SUBPLOTS** | 4 | 2462.891216 | 615.722804 | 329.80 | <.0001 |
| **TREATS*SUBPLOTS** | 20 | 723.946981 | 36.197349 | 19.39 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 5 | 263.589734 | 52.717947 | 28.24 | <.0001 |
| **REPS(TREATS)** | 12 | 18.112074 | 1.509340 | 0.81 | 0.6403 |
| **SUBPLOTS** | 4 | 2462.891216 | 615.722804 | 329.80 | <.0001 |
| **TREATS*SUBPLOTS** | 20 | 723.946981 | 36.197349 | 19.39 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 5 | 263.5897336 | 52.7179467 | 34.93 | <.0001 |

Zn coated Urea

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 7 | Znmix-U Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U control |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 5 | 1 2 3 4 5 |

| | |
|---|---|
| **Number of Observations Read** | 105 |
| **Number of Observations Used** | 105 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 48 | 1103.273121 | 22.984857 | 22.75 | <.0001 |
| **Error** | 56 | 56.572176 | 1.010217 | | |
| **Corrected Total** | 104 | 1159.845297 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.951224 | 2.232362 | 1.005096 | 45.02387 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 99.1771904 | 16.5295317 | 16.36 | <.0001 |
| **REPS(TREATS)** | 14 | 31.9750920 | 2.2839351 | 2.26 | 0.0160 |
| **SUBPLOTS** | 4 | 573.8438305 | 143.4609576 | 142.01 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 398.2770085 | 16.5948754 | 16.43 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 6 | 99.1771904 | 16.5295317 | 16.36 | <.0001 |
| **REPS(TREATS)** | 14 | 31.9750920 | 2.2839351 | 2.26 | 0.0160 |
| **SUBPLOTS** | 4 | 573.8438305 | 143.4609576 | 142.01 | <.0001 |
| **TREATS*SUBPLOTS** | 24 | 398.2770085 | 16.5948754 | 16.43 | <.0001 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 6 | 99.17719045 | 16.52953174 | 7.24 | 0.0011 |

Plant results

Zn only

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | LFbiomass |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |

| Model Information | |
|---|---|
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | Control ZnEDTA ZnO ZnP Znmix ZnSO4 |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 15 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 6 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 15 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 6 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 2.95 |
| **AIC (smaller is better)** | 14.95 |
| **AICC (smaller is better)** | 42.95 |
| **BIC (smaller is better)** | 16.76 |
| **CAIC (smaller is better)** | 22.76 |
| **HQIC (smaller is better)** | 12.96 |
| **Pearson Chi-Square** | 0.45 |
| **Pearson Chi-Square / DF** | 0.05 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 4 | 10 | 2.78 | 0.0863 |

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | PlantZnconc. |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | Control ZNMIX ZnEDTA ZnO ZnP ZnSO |
| **Replications** | 6 | 1 2 3 4 5 6 |

| | |
|---|---|
| **Number of Observations Read** | 18 |
| **Number of Observations Used** | 18 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 7 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 18 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 7 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 61.40 |
| **AIC (smaller is better)** | 75.40 |
| **AICC (smaller is better)** | 103.40 |
| **BIC (smaller is better)** | 78.79 |
| **CAIC (smaller is better)** | 85.79 |
| **HQIC (smaller is better)** | 74.14 |
| **Pearson Chi-Square** | 67.63 |
| **Pearson Chi-Square / DF** | 5.64 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 5 | 12 | 5.35 | 0.0082 |

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | PlantZnuptake |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 6 | Control Znmix ZnEDTA ZnO ZnP ZnSO |
| **Replications** | 6 | 1 2 3 4 5 6 |

| Number of Observations Read | 18 |
|---|---|
| Number of Observations Used | 18 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 7 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 18 |

| Optimization Information | |
|---|---|
| Optimization Technique | None |
| Parameters | 7 |
| Lower Boundaries | 1 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

| Fit Statistics | |
|---|---|
| -2 Res Log Likelihood | 80.32 |
| AIC (smaller is better) | 94.32 |
| AICC (smaller is better) | 122.32 |
| BIC (smaller is better) | 97.71 |
| CAIC (smaller is better) | 104.71 |
| HQIC (smaller is better) | 93.06 |

| Fit Statistics | |
|---|---|
| **Pearson Chi-Square** | 327.29 |
| **Pearson Chi-Square / DF** | 27.27 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 5 | 12 | 5.83 | 0.0059 |

Zn coated Urea

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | LFbiomass |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 7 | Urea Zn-EDTA- ZnO-U ZnP-U ZnSO4-U Znmix-U control |
| **Replications** | 3 | 1 2 3 |

| Number of Observations Read | 21 |
|---|---|
| Number of Observations Used | 21 |

| Dimensions | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 8 |
| Columns in Z | 0 |
| Subjects (Blocks in V) | 1 |
| Max Obs per Subject | 21 |

| Optimization Information | |
|---|---|
| Optimization Technique | None |
| Parameters | 8 |
| Lower Boundaries | 1 |
| Upper Boundaries | 0 |
| Fixed Effects | Not Profiled |

| Fit Statistics | |
|---|---|
| -2 Res Log Likelihood | 9.04 |
| AIC (smaller is better) | 25.04 |
| AICC (smaller is better) | 53.84 |
| BIC (smaller is better) | 30.15 |
| CAIC (smaller is better) | 38.15 |
| HQIC (smaller is better) | 24.56 |

| Fit Statistics | |
|---|---|
| **Pearson Chi-Square** | 0.90 |
| **Pearson Chi-Square / DF** | 0.06 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 6 | 14 | 7.39 | 0.0010 |

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | PlantZnconc. |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 7 | Urea Zn-EDTA- ZnO-U ZnP-U ZnSO4-U Znmix-U control |
| **Replications** | 3 | 1 2 3 |

| | |
|---|---|
| **Number of Observations Read** | 21 |
| **Number of Observations Used** | 21 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 8 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 21 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 8 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 65.80 |
| **AIC (smaller is better)** | 81.80 |
| **AICC (smaller is better)** | 110.60 |
| **BIC (smaller is better)** | 86.91 |
| **CAIC (smaller is better)** | 94.91 |
| **HQIC (smaller is better)** | 81.32 |
| **Pearson Chi-Square** | 52.02 |
| **Pearson Chi-Square / DF** | 3.72 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 6 | 14 | 10.96 | 0.0001 |

| Model Information | |
|---|---|
| **Data Set** | WORK.IN |
| **Response Variable** | PlantZnuptake |
| **Response Distribution** | Gaussian |
| **Link Function** | Identity |
| **Variance Function** | Default |
| **Variance Matrix** | Diagonal |
| **Estimation Technique** | Restricted Maximum Likelihood |
| **Degrees of Freedom Method** | Residual |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Treatments** | 7 | ZNMIX-U Urea ZnEDTA-U ZnO-U ZnP-U ZnSO4-U control |
| **Replications** | 7 | 1 2 3 4 5 6 7 |

| | |
|---|---|
| **Number of Observations Read** | 21 |
| **Number of Observations Used** | 21 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 1 |
| **Columns in X** | 8 |
| **Columns in Z** | 0 |
| **Subjects (Blocks in V)** | 1 |
| **Max Obs per Subject** | 21 |

| Optimization Information | |
|---|---|
| **Optimization Technique** | None |
| **Parameters** | 8 |
| **Lower Boundaries** | 1 |
| **Upper Boundaries** | 0 |
| **Fixed Effects** | Not Profiled |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | 94.37 |
| **AIC (smaller is better)** | 110.37 |
| **AICC (smaller is better)** | 139.17 |
| **BIC (smaller is better)** | 115.48 |
| **CAIC (smaller is better)** | 123.48 |
| **HQIC (smaller is better)** | 109.90 |
| **Pearson Chi-Square** | 400.46 |
| **Pearson Chi-Square / DF** | 28.60 |

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Treatments** | 6 | 14 | 15.42 | <.0001 |

Incubation study

Calcareous soil

Visualization
1=control
2=ZnO
3=ZnSO4
4=Znmix
5=ZnEDTA
Week 3

| Model Information | |
|---|---|
| **Data Set** | WORK.DIFFUSION |
| **Dependent Variable** | Radius |
| **Covariance Structures** | Variance Components, Ante-dependence |
| **Subject Effect** | Rep(Trt) |
| **Estimation Method** | REML |
| **Residual Variance Method** | None |
| **Fixed Effects SE Method** | Model-Based |
| **Degrees of Freedom Method** | Containment |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Time** | 1 | Day1 |
| **Trt** | 5 | 1 2 3 4 5 |

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **Rep** | 3 | 1 2 3 |

| Dimensions | |
|---|---|
| **Covariance Parameters** | 2 |
| **Columns in X** | 12 |
| **Columns in Z** | 3 |
| **Subjects** | 1 |
| **Max Obs per Subject** | 15 |

| Number of Observations | |
|---|---|
| **Number of Observations Read** | 15 |
| **Number of Observations Used** | 15 |
| **Number of Observations Not Used** | 0 |

| Iteration History | | | |
|---|---|---|---|
| **Iteration** | **Evaluations** | **-2 Res Log Like** | **Criterion** |
| **0** | 1 | -23.40876827 | |
| **1** | 2 | -23.40679047 | 0.00009223 |
| **2** | 1 | -23.40876542 | 0.00000014 |
| **3** | 1 | -23.40876827 | 0.00000000 |

Convergence criteria met.

***Estimated G matrix is not positive definite.***

| Covariance Parameter Estimates | | |
|---|---|---|
| **Cov Parm** | **Subject** | **Estimate** |
| **Rep** | | 0 |
| **Var(1)** | Rep(Trt) | 0.003253 |

| Fit Statistics | |
|---|---|
| **-2 Res Log Likelihood** | -23.4 |
| **AIC (Smaller is Better)** | -21.4 |
| **AICC (Smaller is Better)** | -20.9 |
| **BIC (Smaller is Better)** | -22.3 |

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0 | . | . | . | . |
| **Rep** | 2 | 0 | . | . | . | . |
| **Rep** | 3 | 0 | . | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Trt** | 4 | 8 | 378.89 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 0 | 0.03293 | 8 | 0.00 | 1.0000 |
| **Trt** | 2 | -222E-18 | 0.03293 | 8 | -0.00 | 1.0000 |
| **Trt** | 3 | 0.2636 | 0.03293 | 8 | 8.01 | <.0001 |
| **Trt** | 4 | -222E-18 | 0.03293 | 8 | -0.00 | 1.0000 |
| **Trt** | 5 | 1.4763 | 0.03293 | 8 | 44.83 | <.0001 |

Week 5

| Solution for Random Effects | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Rep** | **Estimate** | **Std Err Pred** | **DF** | **t Value** | **Pr > \|t\|** |
| **Rep** | 1 | 0 | . | . | . | . |
| **Rep** | 2 | 0 | . | . | . | . |
| **Rep** | 3 | 0 | . | . | . | . |

| Type 3 Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **Time** | 0 | . | . | . |
| **Trt** | 4 | 8 | 164.36 | <.0001 |
| **Time*Trt** | 0 | . | . | . |

| Least Squares Means | | | | | | |
|---|---|---|---|---|---|---|
| **Effect** | **Trt** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > \|t\|** |
| **Trt** | 1 | 0 | 0.09895 | 8 | 0.00 | 1.0000 |
| **Trt** | 2 | 0 | 0.09895 | 8 | 0.00 | 1.0000 |
| **Trt** | 3 | 0.8053 | 0.09895 | 8 | 8.14 | <.0001 |
| **Trt** | 4 | 0 | 0.09895 | 8 | 0.00 | 1.0000 |
| **Trt** | 5 | 2.9287 | 0.09895 | 8 | 29.60 | <.0001 |

Soil pH

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 5 | Control ZnO ZnSO4 Znmix ZnEDTA |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 4 | 1 2 3 4 |

| | |
|---|---|
| **Number of Observations Read** | 60 |
| **Number of Observations Used** | 60 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 29 | 0.44428333 | 0.01532011 | 2.54 | 0.0066 |
| **Error** | 30 | 0.18061667 | 0.00602056 | | |
| **Corrected Total** | 59 | 0.62490000 | | | |

| R-Square | Coeff Var | Root MSE | totalzn Mean |
|---|---|---|---|
| 0.710967 | 0.892378 | 0.077592 | 8.695000 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 0.24280000 | 0.06070000 | 10.08 | <.0001 |
| **REPS(TREATS)** | 10 | 0.10185000 | 0.01018500 | 1.69 | 0.1290 |
| **SUBPLOTS** | 3 | 0.02823333 | 0.00941111 | 1.56 | 0.2188 |
| **TREATS*SUBPLOTS** | 12 | 0.07140000 | 0.00595000 | 0.99 | 0.4819 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 0.24280000 | 0.06070000 | 10.08 | <.0001 |
| **REPS(TREATS)** | 10 | 0.10185000 | 0.01018500 | 1.69 | 0.1290 |
| **SUBPLOTS** | 3 | 0.02823333 | 0.00941111 | 1.56 | 0.2188 |
| **TREATS*SUBPLOTS** | 12 | 0.07140000 | 0.00595000 | 0.99 | 0.4819 |

| Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 4 | 0.24280000 | 0.06070000 | 5.96 | 0.0102 |

Water extractable Zn

| Class Level Information | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 5 | Control ZnO ZnSO4 Znmix ZnEDTA |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 4 | 1 2 3 4 |

| | |
|---|---|
| **Number of Observations Read** | 60 |
| **Number of Observations Used** | 60 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 29 | 196767.4728 | 6785.0853 | 36.97 | <.0001 |
| **Error** | 30 | 5505.2705 | 183.5090 | | |
| **Corrected Total** | 59 | 202272.7433 | | | |

| R-Square | Coeff Var | Root MSE | waterexZn Mean |
|---|---|---|---|
| 0.972783 | 25.95381 | 13.54655 | 52.19483 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 35586.97107 | 8896.74277 | 48.48 | <.0001 |
| **REPS(TREATS)** | 10 | 2058.64850 | 205.86485 | 1.12 | 0.3789 |
| **SUBPLOTS** | 3 | 90197.56175 | 30065.85392 | 163.84 | <.0001 |
| **TREATS*SUBPLOTS** | 12 | 68924.29147 | 5743.69096 | 31.30 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 4 | 35586.97107 | 8896.74277 | 48.48 | <.0001 |
| **REPS(TREATS)** | 10 | 2058.64850 | 205.86485 | 1.12 | 0.3789 |
| **SUBPLOTS** | 3 | 90197.56175 | 30065.85392 | 163.84 | <.0001 |
| **TREATS*SUBPLOTS** | 12 | 68924.29147 | 5743.69096 | 31.30 | <.0001 |

| **Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term** | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 4 | 35586.97107 | 8896.74277 | 43.22 | <.0001 |

Total Zn- percentage of Zn recovered as of added Zn

| **Class Level Information** | | |
|---|---|---|
| **Class** | **Levels** | **Values** |
| **TREATS** | 4 | ZnEDTA ZnO ZnSO Znmix |
| **REPS** | 3 | 1 2 3 |
| **SUBPLOTS** | 4 | 1 2 3 4 |

| | |
|---|---|
| **Number of Observations Read** | 48 |
| **Number of Observations Used** | 48 |

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | 17 | 32243.60914 | 1896.68289 | 322.21 | <.0001 |
| **Error** | 30 | 176.59253 | 5.88642 | | |
| **Corrected Total** | 47 | 32420.20166 | | | |

| R-Square | Coeff Var | Root MSE | %Zn Mean |
|---|---|---|---|
| 0.994553 | 9.704776 | 2.426194 | 25.00000 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 3 | 0.00000 | 0.00000 | 0.00 | 1.0000 |
| **REPS(TREATS)** | 2 | 0.00000 | 0.00000 | 0.00 | 1.0000 |
| **SUBPLOTS** | 3 | 24624.92251 | 8208.30750 | 1394.45 | <.0001 |
| **TREATS*SUBPLOTS** | 9 | 7618.68663 | 846.52074 | 143.81 | <.0001 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **TREATS** | 3 | 0.00000 | 0.00000 | 0.00 | 1.0000 |
| **REPS(TREATS)** | 2 | 0.00000 | 0.00000 | 0.00 | 1.0000 |
| **SUBPLOTS** | 3 | 24624.92251 | 8208.30750 | 1394.45 | <.0001 |
| **TREATS*SUBPLOTS** | 9 | 7618.68663 | 846.52074 | 143.81 | <.0001 |

| **Tests of Hypotheses Using the Type III MS for REPS(TREATS) as an Error Term** | | | | | |
|---|---|---|---|---|---|
| **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
| **TREATS** | 3 | 5.668992E-18 | 1.889664E-18 | 6.98 | 0.1279 |