Improving crop quality: Investigations on soil selenium and zinc transfer and bioavailability

by

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B.S., King Saud University, 2002
M.S., King Saud University, 2009

AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

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Abstract

Management of beneficial and/or essential trace elements, such as Se and Zn, is challenging, and it is complicated by the fact that the margin of safety between the levels that will cause dietary deficiency, and those that result in toxicity, is narrow. This research focused on the ability of the plant system to pretreat wastewaters rich in potentially toxic trace elements and nutrients and enhancing phytoavailability of Zn in Zn-deficient calcareous soils.

Plant systems may possess a significant capacity to remediate marginal waters through several phytoremediation processes, including uptake, accumulation, and assisting with biotransformation of inorganic and organic compounds. The aim of the first study was to determine the ability of the halophyte, *salicornia europaea*, to grow in wastewater or brackish waters and to remove excess trace elements, nutrients, and salts in these highly saline wastewaters. Greenhouse and growth chamber studies were conducted to examine the ability of *salicornia europaea* to grow and remediate marginal waters. *Salicornia europaea* showed the ability to remove excess trace elements (Se and B) and salts (Na), indicating *salicornia europaea* has the potential to be used for precleaning the highly saline wastewaters. Enhanced biomass showed that it can also produce valuable stock for biofuel and bio-based products from marginal waters.

Agronomic biofortification is an effective way to increase micronutrient concentrations in grain crops. Formation of dissolved micronutrient-organic C complexations can enhance the solubility of micronutrients. The aims of the second study were to investigate the effectiveness of various Zn sources (organic and inorganic) with and without organic C-based fertilizer co-additives on biofortification of wheat with Zn in a mildly-calcareous soil and to determine distribution (stems/leaves, whole grain, bran and flour) and bioavailability of Zn in different

plant parts (bran and flour). A greenhouse experiment was conducted to study wheat grown under different Zn sources. Application of Zn significantly increased grain yield, grain Zn concentration, and Zn bioavailability in white flour. Less soluble ZnO showed more promising results compared to soluble $ZnSO_4$. Co-additives did not improve the soil Zn extractability or the Zn uptake by wheat.

Understanding the interactions and speciation of Zn is very important to gain more insights into the fate of added Zn in calcareous soil and also for the efficient management of soil for optimum crop production and environmental conservation. The objectives of the third study were to investigate and understand differences in mobility, extractability, and fractionation of Zn from different sources of granular and liquid Zn, with and without co-additives, in two mildly calcareous soils. A 5-wk long incubation study allowed for spatial evaluation of Zn fate and transport in two soils. Diffusion of Zn was limited to a 0 to 7.5 mm section for all treatments with or without co-additives. The energy dispersive X-ray analysis results were in agreement and revealed that the remaining Zn-incorporated monoammonium phosphate granules, after incubation in soil, contained significant amounts of P and Zn. This study also showed that the liquid Zn sources with no P were better than the co-granulated Zn-P fertilizers.

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Dedication

To My Parents

Mathkar A Almutari

Gamra A Almutairi

My Wife

Zakiah N Almutairi

My children

Yazan M Almutari

Wasan M Almutari

To my brothers and sisters

Hamdan M Almutairi

Hamdah M Almutairi

Abdullah A Almutairi

Marim M Almutairi

Sarah M Almutairi

Abdurhman M Almutairi

Hamoodh M Almutairi

Maram M Almutairi

Abdulaziz M Almutairi

Abdulmajed M Almutairi

Majedah M Almutairi

To all of my nephews and nieces

To my friends that have supported me in all my endeavors over the years.

Thank you.

Chapter 1 - Introduction

In an effort to reduce the amount of sulfur dioxide (SO_2) released into the atmosphere, as regulated by the Clean Air Act of (1963) and the Clean Air Act Amendments of 1990, fossil-fueled power plants have installed flue-gas desulfurization (FGD) or scrubber systems. While FGD systems are effective in decreasing sulfur dioxide emissions, this process results in wastewater containing high concentrations of mercury (Hg), selenium (Se), boron (B), and arsenic (As) (Electric Power Research Institute, 1999). This wastewater must thereafter be treated to eliminate these contaminants in order to achieve discharge limitations established under the National Pollution Discharge Elimination System (NPDES) and Clean Water Act (CWA). Constructed wetland treatment systems (CWTS) have considerable potential to remove Hg, Se, and As from FGD wastewater (Sundberg et al., 2006). Earlier studies reported that high salinity of the FGD wastewater did inhibit Se sorption. These studies provided useful information to improve the performance, and longevity of a full-scale CWTS designed for FGD wastewaters. They also suggested that pre-treatment of FGD wastewaters is desirable to remove dissolved solids such as trace elements, salts, and nutrients (Paredez et al., 2017; Galkaduwa et al., 2017). In addition, Paredez et al. (2017) suggested that the high salinity of the FGD wastewater may have influenced B sequestration.

Phytoremediation exploits a plant's innate biological mechanisms for human benefit. Four subsets of this technology, as applicable to potentially toxic metal(loid) remediation from soil and water, are (i) phytoextraction - use of metal(loid) -accumulating plants to remove metal(loid)s from soil (Jadia and Fulekar, 2009), (ii) phytovolatilization - evaporation of certain metals(loid) from aerial parts of the plant (US EPA, 1998), (iii) phytostabilization - use of plants to eliminate the bioavailability of metal(loid)s in soils (Blaylock, et al., 1995), and (iv) rhizofiltration - use of plant roots to remove metal(loid)s from polluted waters (Long et al., 2002).

In phytoextraction, technology refers to the process in which plants absorb metal(loid)s from soil and translocate, or further accumulate in shoots that are subsequently harvested to remove contaminants from soils (Salt et al., 1995). In phytovolatilization, the plants uptake contaminants from the soil and transform them into volatile forms for further release into the atmosphere. Phytovolatilization of Se has been reported by Bañuelos et al., (2000), where Se is transformed into dimethyl selenide and dimethyl diselenide in the soil and, released into the atmosphere.

Halophytes are naturally salt tolerant plants and thus, have found application in agronomy-engineering projects such as recycling of agricultural and industrial brines (Jordan et al., 2009), revegetation of salt-affected region (Ruan et al., 2008), urban landscaping irrigated with saline water sources (Glenn et al., 2009), treatment of aquaculture effluent (Brown et al., 1999), and phytoremediation of contaminated soils (Lin and Mendelssohn, 2009; McKeon et al., 2005). Studies on biofiltration of aquaculture effluent by various halophyte plants have been reviewed recently by Buhmann and Papenbrock (2013). Halophytes may have ability to pretreat the highly saline wastewaters such as FGD wastewaters. Information on the ability of halophytes to take up, translocate, and accumulate salts in FGD wastewater is limited.

Wheat is one of the three leading cereal crops worldwide and is the dominant crop used for human food. Wheat is responsible up to 50% of daily calorie intake of the population living in rural regions and an important source for zinc (Zn) for human beings living in the developing world (Cakmak 2008). Zinc is an essential micronutrient for plants, humans, animals, and microorganisms. Zinc deficiency occurs in a wide range of the major soil types in many parts of the world that are frequently associated with Zn deficiency. These include calcareous soil (calcisols), sandy soil (arenosols), weathered tropical soil (ferralsols), saline soil, waterlogged soil (gleysols) and heavy cracking clay soil (Cakmak and Kutman, 2018). Graham and Welch (1996) reported that it is estimated that approximately 50% of soils utilized for cereal production in the world have a low standard of plant-available Zn. Calcareous soils are among the most micronutrient-deficient soils in the world (Malakouti, 2008).

Major factors affecting Zn uptake, as well as reducing solubility and mobility, are high soil pH, low organic matter content, low soil moisture, and high calcium carbonate. According to Lindsay (1972), soils most commonly associated with Zn deficiency problems frequently have one or more of these characteristics. Calcareous soils, generally with $pH > 7.4$, have relatively little available Zn because the solubility of Zn decreases with increasing pH. More often, total Zn content of calcareous soils is similar to that in soils of other types, or even higher, but Zn availability is low. Adsorption of Zn onto the $CaCO_3$ is also a contributory factor. Precipitation of Zn with P can also be significant in calcareous soils, especially when Zn is added with P fertilizers (Hettiarachchi et al., 2008).

Biofortification of crops with Zn may offer a sustainable option for alleviating Zn deficiency globally. Application of fertilizers (agronomic biofortification) is the fastest route to alleviate Zn level in diets. However, the most commonly used inorganic Zn fertilizers rapidly become ineffective in calcareous and alkaline soils as zinc reacts with soil minerals and organic matter. Inorganic sources of Zn such as zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO) are commonly used as Zn fertilizer to correct Zn deficiency. Zinc sulfate ($ZnSO_4{:}7H_2O$) is the most widely applied inorganic Zn fertilizer, because of its high solubility (Cakmak et al., 2010). When $ZnSO_4$ is added to calcareous soil, Zn is generally get fixed to a large extent. This is because of the presence of active $CaCO_3$ in calcareous soil, which induces the dissolved Zn immobilization via the adsorption of Zn by carbonates leading to the precipitation of Zn hydroxide or carbonate, and or the formation of insoluble calcium zincate (Montalvo et al., 2016). Zinc oxide is an inorganic compound nearly insoluble in water but soluble in acids (Montalvo et al., 2016) and therefore, in alkaline soils ZnO may act as a slow-releasing Zn fertilizer in calcareous soils.

The release and dissolution rates of water-soluble fertilizers may depend on the coating materials such as co-additive AVAIL. The AVAIL® (Specialty Fertilizer Products, Leawood, KS, USA) , is designed to sequester antagonistic metals in the soil surrounding the fertilizer granule, thereby reduce tie-up of phosphate and make phosphate more available to the plant (Doydora et al., 2017). The proposed mode of action claims that high cation exchange capacity of AVAIL facilitates sequesteration of Ca, Mg, Al, Fe, Mn, and other multivalent cations, thus reducing their interaction with P in soil solution (Chien et al., 2014). Co-additives such as AVAIL may provide benefit for Zn nutrition by preventing (or reducing) Zn sorption in calcareous soils via complexation.

The slow-releasing fertilizer and controlled release fertilizers are made to release their nutrient contents gradually and to coincide with the nutrient requirement of a plant (Ge et al., 2002; Shavit et al., 2003). There are several benefits of slow-release Zn in fertilizers. They provided low precipitation or fixation. Since, the nutrients are released for an extended period of time, the efficiency is greater for slow-releasing fertilizer than conventional fertilizers (Dou and Alva, 1998). Therefore, nutrient uptake efficiency is greater for slow-release fertilizer products as compared to readily available forms of fertilizers (Riandy and Sofyan, 2015).

Biosolids not only release free Zn into soil solutions by decomposition but also may alter the solubility and mobilization of soil Zn through formation of Zn organic complexes or ligand-promoted dissolution (Smith, 2009). In addition, organic amendments and biosolids were shown to change the distribution of Zn precipitated by calcium carbonate, which is assumed to be a major factor in the loss of Zn availability in calcareous soil (Jalali and Khanlari 2008). The influence of organic matter on the availability of soil Zn depends on the maturity and ability of organic amendments. The availability of Zn is small wherever mature organic materials are present due to the formation of stable organic complexes with organic matter such as humic acid (Smith, 2009). According to operational definitions, humin is the insoluble fraction of humic substances; humic acid (HA), is the fraction soluble under alkaline conditions but not acidic conditions (generally $pH < 2$); and fulvic acid (FA), is the fraction soluble under all pH conditions. Previous studies have suggested some beneficial effect of HA on Zn uptake (Xu et al., 2017; Boguta and Sokołowska, 2016; Katkat et al., 2009). The ability of metal–humic complexes to provide the metal complexes to plant roots is supported by their efficiency in soil cultivated plants, which heavily depends on the solubility-mobility in soil solution (Chen and Aviad 1990; Tan 2003; Garcia 2006). Therefore, it is logical to predict the beneficial effects of metal–humic complexes in micronutrient root uptake which in turn varies depending on the soil solution properties (pH, I, elemental composition, etc.). Previous study has also shown that the functional complementarity between plant nutritional properties in different soil types does also affect both stability and solubility of metal (Fe, Zn, Cu) humic complexes, which are higher for those pH ranges that favor metal precipitation and potential bio-unavailability (Erro et al., 2016).

This dissertation consists of three studies: the first study involves using phytoextraction of contaminants while the second and third studies focus on agronomic biofortification of Zn. Specific objectives are given below:

1. The objective of the first study, *Evaluating Soil- and Plant-based Treatment Options for Marginal Waters* (Chapter 3), was to determine the ability of *salicornia europaea* to remove excess salts in FGD wastewater and brackish waters.
2. The objectives of the second study, *Agronomic biofortification of wheat with Zn through co-addition of organic C with Zn fertilizers* (Chapter 4), were to study the effectiveness of various Zn sources (inorganic as well as organic) with and without co-additives on biofortification of wheat with Zn; and to determine distribution of Zn in (stems/leaves, whole grain, bran and flour) and bioavailability of Zn (bran and flour) in different plant parts.
3. The objective of the third study, *understanding reaction pathways of fertilizer Zn with and without co-additives in two calcareous soils* (Chapter 5), was conducted to understand differences in mobility, extractability and reaction products of Zn from different Zn sources with and without co-additives using wet chemical and microscopic techniques.

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Chapter 2 - Literature Review

Flue-gas desulfurization process and flue-gas desulfurization wastewater

According to the information on the United States provided by the U.S. Energy Information Administration (USEIA) in 2014, about 39% of the total electricity was generated by coal. Coal is one of the energy sources that are used for electricity generation because of its affordable price, availability, high energy production upon combustion, and the easiness to burn. Coal-fired power plants are a type of power plant that makes use of the combustion of coal in order to generate electricity. Coal-fired power plants release many pollutants and also emit environmentally harmful gases that include sulfur (SO_x), oxides of nitrogen (NO_x), carbon dioxide (CO_2), methane (CH_4) - and particulate matter, which are known to contribute to global warming and climate change. Coal combustion wastes generated at thermoelectric, coal-burning power plants contain numerous trace elements such as arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pd), mercury (Hg), molybdenum (Mo), selenium (Se), and zinc (Zn) (Cherry and Guthrie, 1977; Walia and Mehra, 1998; Smith, 2009). Severe physiological effects on organisms or species may be caused by exposure to these trace elements when they are present above tolerable concentrations (Chang, 1996). These power plants have now significantly decreased air emissions of sulfur dioxide (SO_2) by installing flue-gas desulfurization (FGD) scrubbers (US Department of Energy, 2000).

The most commonly used FGD scrubber is referred to as a “wet scrubber”. This uses lime- or limestone-calcium carbonate ($CaCO_3$), calcium sulfite ($CaSO_3$) saturated water to solubilize gaseous SO_2 and oxidize and precipitate sulfur compounds like calcium sulfite ($CaSO_3xH_2O$) or calcium sulfate ($CaSO_4$). This scrubbing process occurs by direct contact with the flue gases after combustion and oxidization of the coal with a scrubbing solution. It oxygenates the gases by forced-air injections in the collection basin. The resulting byproduct, water, is typically referred to as FGD wastewater. At each power plant, the composition of FGD water is a result of several chemical and physical variables that include the coal source and composition; burner, FGD scrubber design and operation; post-scrubber treatment processes, plus initial constituents in the local water supply (Miller, 1995)

Flue-gas desulfurization is a process where SO_2 is removed from the exhaust flue gases before they are released into the atmosphere. This process also tends to remove sulfur from other processes in the power plant system before it is also released. Different scrubbers are used in the plants for the removal of slurry. These scrubbers help to keep the different system components dry (Zawislanski at el., 2001). For example, the characteristics of the parent coal, specifically the S content influences the pH of the material. Also, using a dolomitic lime as the extractant often confers higher concentrations of Mg to the final by-product (Miller, 1995).

The main processes of reducing pollutants in power generation is accomplished more effectively by using biofuels. Moreover, using biofuels in the FGD system will have a huge impact on the development of suitable strategies to control air pollution and reducing environmental damage. These strategies are related to the development of biofuels for power generation since they have low sulfur content unlike coal. Biofuels do not pollute when flue gases are generated, then released into the atmosphere and enter the soil. Plus, these biofuels have a large influence on the formulation of FGD processes. They will also have a considerable positive effect on the overall benefits of controlling air pollution. This is through the adoption of salicornia as a biofuel (Li at el., 2009)

The main function of the FGD system is the development of power plants as pollution control centers. This function will create great opportunities for cleaner air by eliminating noxious or toxic gases. Hence, it is necessary to treat these flue gases before they are released into the atmosphere. Also, the proportion of sulfur in the fuel can be reduced. Selecting fuels with low proportions of sulfur and other dangerous elements is very important for environmental and ecological reasons. These dangerous elements include Ba, Cu, Hg, Mo and Zn. These types of fuel are also required in the FGD wastewater process. This is to minimize the harmful impact on the atmosphere and the environment (Hudson and Rochelle, 1982). An extensive amount of sulfur can be removed from the fuel itself before energy production. This removal process will help create flue gases with either considerably lower or no sulfur content at all.

The need for cleaner air and controlling pollution has become an urgent global matter. The use of coal as fuel for power generation greatly contributes to the air pollution problem and damages the environment through the formation of acid rain. Now national governments and power industries are actively seeking alternatives to coal as a source of energy. However, one readily available alternative solution is using biofuels for power generation. These fuels are cleaner burning, derived from renewable resources and produce much less pollution (Houte at el., 1978). One of the most important strategies for creating biofuels is using the plant Halophyte as *salicornia*, this is a species of salt tolerant (or halophyte), fleshy, flowering plants that are native to North America, Europe, South Asia and South Africa. Salicornia is an outstanding ingredient for biofuel admixtures used in power generation.

Previous studies have reported the 1990 amendments to the US Clean Air Act mandate a 9.07 million Mg reduction in SO_2 emissions in the US by the year 2000. As industries bring coal-burning boilers into compliance with these regulations, production of FGD by-products are expected to reach 30 to 35 million Mg annually in the US by the year 2005 (US Energy Information Administration, 1993). Disposal of this waste volume will become increasingly difficult as landfill costs increase and available landfill space decreases. Thus, there are powerful economic incentives for the producers of FGD by-products. These incentives include identifying beneficial uses for these by-products and decrease their disposal costs. However, such beneficial uses must be studied carefully to determine their potential impacts on soil, water, air and plant quality.

Researchers have studied the effect of dry scrubber systems that utilize lime or limestone-based sorbents to react with and trap the SO_2 formed during combustion. The resulting FGD by-products comprise three components varying in proportions and composition: (i) the SO_2 reaction product, which is usually anhydrite ($CaSO_4$), (ii) unspent sorbent, and (iii) coal combustion ash (either bed or fly ash). Because of the unspent sorbent component, dry FGD by-products are usually highly alkaline and have significant neutralization potential (Korcak, 1980; Fowler et al., 1992). Decreasing SO_2 emissions by FGD wastewater systems will result in wastewater containing high concentrations of Se, Hg, and As (US Electric Power Research Institute, 1999).

Phytoremediation techniques have been briefly depicted in many works of literature or articles. The generic term “phytoremediation” consists of the Greek prefix ‘phyto’ (plant), attached to the Latin root word ‘remedium’ (to correct or remove an evil) (Muske et al., 2016; US EPA, 2000). Several researchers have used different definitions for phytoremediation. Specific plant species can absorb and hyperaccumulate metal contaminants and/or excess nutrients in harvestable root and shoot tissue in the growth substrate through the phytoextraction process. (US EPA, 2000; Vara Prasad et al., 2003). This absorption process is for metals, metalloids, nonmetals, radionuclides and organic contaminants in soils, sediments, and sludge mediums.

Phytoremediation is a treatment technology which uses different plant species and their associated microbiological communities to remove contaminants from soil and water. Phytoextraction is the process of using a plant species to take up contaminants from the soil, and water and concentrate the contaminants in the roots, shoots, and leaves of the plant (US Environmental Protection Agency, 2000).

Pollution of agricultural soil by heavy metals is of considerable concern with respect to health risks, phytotoxicity to plants, long-term effects on soil fertility, and depreciation of land values and agricultural production capability. Elevated heavy metal concentrations in the soil could lead to enhanced crop uptake of these toxic elements. High heavy metal concentrations in the soil can negatively affect crop growth (Kayser et al., 2000). There are three factors which lead to the transfer of heavy metals from the soil to plants. These are: (i) the total amount of potentially available elements (quantity factor); (ii) the ionic ratios of elements in the soil solution (intensity factor) and (iii) the rate of element transferal from solid to liquid phases and the transfer to plant roots (Brümmer et al., 1986).

Treatments for flue-gas desulfurization wastewaters

Wetland treatment systems:

A very cost-effective way to treat wastewater is through a process called constructed wetlands (CW). Badejo et al., (2015) and Kouawa et al., (2015) have stated that this process offers a very promising alternative for treating industrial, domestic and agricultural wastewater. Kadlec and Wallace, (2009) plus Brix (1997) have explained that CWs can remove pathogenic microorganisms are persistent organic pollutants. Nutrients and trace elements from domestic and industrial wastewater by acting as a biofilter. Additionally, Kadlec and Wallace (2009) plus Brix, (1997) also found that CWs operate as engineered ecosystems that operate a controlled environment. Constructed wetlands use natural processes that are aimed at improving have water quality by using microbial assemblages and wetland soils and vegetation.

In two studies conducted by Maine et al., (2007) and Vymazal (2011) they found that CWs are complex matrix of distinct anaerobic and aerobic treatment zones. These CWs process wastewater in the same manner as do natural wetlands. Constructed wetlands are classified by either the water flow regime (horizontal or vertical flow, surface or sub-surface flow) or by the type of macrophylic, or aquatic plant, growth (rooted with either floating leaves, free-floating, emergent or submerged). According to the studies done by the U.S. EPA (2004) plus Arias and Li (2009), CWs have been broadly classified into sub-surface flow or vegetated submerged bed and surface flow or free-water surface categories. Ayaz and Akca (2000) and Badejo et al., (2015) have found that for developing countries CW is reportedly better suited in offering lower maintenance and construction costs for domestic wastewater treatment.

Researchers Wang et al., (2002); Chaney et al., (1997); Salt et al., (1998); Garbisu and Alkorta, (2001); Chen et al., (2008) have pointed out, for an extended period, that within the natural environment (wetlands, soils, etc.) there is a high capacity for pollution remediation or removal. Kadlec and Wallace (2009) have found that a wide range or variety of pollutants, including nutrients, heavy metals, pathogens suspended solids and organic compounds, have been removed using CWs. Additionally, CWs improve water quality by utilizing natural processes. These processes include associated microbial assemblages, wetland vegetation and soils.

In recent years, CWs have been received the most attention in the urban wastewater treatment, particularly in decentralized sanitation, for the reason that they are affordable, reliable, simple in design and operation, and offer environmentally sound approach (Wu et al., 2011).

These ecologically engineered systems are known to be effective at removing many pollutants such as organic compounds, suspended solids, pathogens, nutrients, and emergent pollutants (Valipour and Ahn., 2017). They are designed to get the advantage of the same processes occurring in natural wetlands however within a more controlled environment. Gaining a better understanding of the mechanisms associated with CWs has driven to a wide variety of designs and configurations to achieve a more efficient domestic sewage treatment, for example, single-staged modification (Chale, 2012; Kumari and Tripathi, 2014), multi-staged in series (Melian et al. 2010), and/or combination with other treatment technologies (Singh et al., 2009).

In constructed wetland treatment systems receiving inflows of FGD wastewater containing elements such as Hg, Se and As, these elements may concentrate in new vegetation. Both planted and naturally occurring macrophytes in constructed wetlands have been used for the remediation of a wide variety of contaminated wastewaters. These toxic wastewaters include industrial and municipal wastewater, acid drainage from mining, storm water and agricultural runoffs (Moshiri, 1993).

Salt tolerant plants

Due to increased salinity of cultivated land worldwide, there is a great need to increase salt tolerant crops (United Nations Food and Agricultural Organization (UN FAO, 2002). The problem is that fresh water supplies are becoming scarce. This phenomenon is because of increasing demand by a growing world population (United Nations, 2010). Furthermore, because of global warming, increasing saline and dry conditions in the future will reduce the available arable land. Also, sea level rise will particularly threaten all coastal lowlands or littoral areas. Most conventional crop species that are salt sensitive include rice, chickpeas, corn and most fruit crops. Munns and Tester (2008) reported several challenges that plants face in growing on saline soils. This includes water uptake that is hampered because of the soils' potential low water capacity.

The search for salt-tolerant crops to handle higher concentrations of soil salinity has been an important research quest over the last several decades. This is especially true in combatting soil salinity and water scarcity in semi-arid and arid regions. In fact, some salt-tolerant species, such as the halophytes, will increase the availability of productive land resources (Rozema and Flowers, 2008). These crops can also achieve highly economical or lucrative yields (Ventura and Sagi, 2013). Also, these salt-tolerant plants can improve the economy of some regions (Glenn et al., 1999). Previous studies have primarily concentrated on halophyte seeds that can preserve seed viability for elongated time periods. These studies also showed that during exposure to hypersaline conditions plants can start germination when salinity levels or stress is reduced (Ungar, 1982; Woodell, 1985; Keiffer and Ungar, 1995; Khan and Ungar, 1997; Gul and Weber, 1999).

There have been studies reporting this variation in recovery responses that could be because of the differences in temperatures that seeds are exposed to (Khan and Ungar, 1997). However, germination studies with *Arthrocnemum indicium, Haloxylon recurvum, Suaeda fruticosa, Zygophyllum simplex,* and *Triglochin maritima* clearly demonstrate that recovery responses are more significantly influenced by changes in temperature regimes.

Recovery of germination responses has been well-demonstrated in *salicornia europaea* (Keiffer and Ungar, 1995). Salicornia *europaea* is an annual halophyte species *(Chenopodiaceae),* which is broadly distributed in coastal and inland salt marshes (Riehl and Ungar, 1982). Salicornia is also an herb that is small and easy to grow in salt water. The plant has a very high percentage of unsaturated oil. There are different factors to be considered while developing salicornia. The plant will have a huge impact on the development of biofuel stock. However, salicornia cannot be planted with different types of crops because of the oil contained inside it will have a negative effect on the other plants (Singh et al., 2014).

Grattan (et al., 2008) reported that halophytes can be used for several purposes, namely: recycling of hypersaline drainage water for posterior use in agriculture, revegetation of salt-affected tidal flats, fodder crops (Bustan et al., 2005; Ruan et al., 2008; Khan et al., 2006; El Shaer, 2010), extraction of bioactive compounds, and food for human consumption (Khan et al., 2006; Ventura et al., 2011).

Growing Salicornia in brackish water

Many researchers argue that one of the most encouraging examples of halophyte cultivation is most likely with salicornia. This plant colonizes naturally temperate and subtropical regions. This annual plant can also be irrigated with brackish water or seawater since it shows great salt tolerance (Davy et al., 2001; Rozema and Schat, 2013). Rozema and Schat (2013) reported that salicornia species are commercialized as a singular vegetable for the US and European food markets. However, stimulation of this market requires efficient agronomic approaches for large/medium scale production. Salty taste and high nutritional value (e.g., minerals, antioxidants, and vitamins) are the main reasons for its demand (Lu et al., 2001; Ventura et al., 2011).

Although the recovery and use of non-productive, saline or brackish areas is a promising niche for agriculture expansion, the development of specific agronomic techniques for the growing of halophyte crops is required (Glenn et al., 1999; Oliveira et al., 2009). In general, there is a range of agronomic limitations on halophyte species. This includes low harvest index, woody growth of the forages, a small size of the seeds, lodging of plants at maturity, shattering of the seeds, and an existence of antinutritional compounds in the seeds and leaves (Flessa et al., 2013). Furthermore, conventional agronomic techniques should be adapted to each plant species, plus salinity range of the soils and irrigation waters, and soil type need to be considered to significantly increase crop yields.

Salicornia can sustain itself and stay alive in saltwater. There is a special task carried out throughout the functioning of the biofuel feedstock system. Concurrently, there is extensive research required to obtain the right amount of oil in the feedstock system. The main function of salicornia is to retain moisture even when the surrounding area is infused or saturated with high quantities of salt. When a nitrogen-based fertilizer is added, this tends to increase the plants' growth rate plus boost plant height (Singh et al., 2014).

An entire marine culture is developed so required levels of sustenance maintenance throughout the feedstock system is achieved across the network. There is a considerable level of activity which supports the use of salicornia for different domestic and industrial purposes. It emphasizes the development strategies and techniques that have an immense impact on the development of related research elements throughout the system (Flessa et al., 2013).

Salicornia is considered the right candidate for reclamation of salt flats, barren lands, and sea-shores. In short, the plants can be considered for seawater agriculture (Katschnig et al., 2013). Most wetland plants are afflicted by widespread damage from toxic substances including salicornia. These succulents are easily affected by metal and oil spill stressors for example. Yamamoto (et al., 2009) reported that *salicornia europaea* shows tolerance towards a high salinity of 3% NaCl. This plant is grown at the edges of wetlands, marshes, seashores, and mudflats. It is also grown on most alkaline flats (Smillie, 2015). Plus, the plant is distributed across four continents: North America, Asia, Africa and Europe. Salicornia also has some features such as spongy stems with diminutive scale-like leaves, inconspicuous flowers, and fruits. The green plant switches color from orange or pink to a reddish hue in autumn before dying in winter.

Salicornia has a huge role in providing animal feedstuff. The plant is also used as a biofuel. Since conventional crops cannot be grown near coastal areas due to exposure to salt in the air, salicornia can tolerate those conditions. Additionally, through proper and adequate use of these plants in the industrial sector, additional value is obtained by producing cellulose and its related elements. These plants tend to have overall high cellulose contents. However, their roots and stems are different from the tender tips and other parts of the plant in having different levels of cellulose. This content rises to 30% of the plant throughout the production of biodiesel in the industry (Cloete, 2013). The process aims to achieve the required parameters throughout the functioning of biofuels production. The tip of the tender stem contains a low percentage of cellulose or around 9.2%. There is a higher value of cellulose content required for the overall development of biofuel which is used in industrial applications (Singh et al., 2014).

Salicornia can also be used for removing salts from the soil. The salt is absorbed through the plant and eventually released into the atmosphere where it is dispersed by the prevailing winds. The volatilization rates of salicornia are high as compared to the other species of plants. It uses the carbon fixation (C_3) method for absorbing CO_2 from the atmosphere. There are higher levels of CO_2 absorption throughout the processing of the salicornia plant in the system. This prevails throughout the stem and roots of the plant. They are effective in absorbing gases and the plant produces slurry-free waste from these unwanted gases. These gases would cause great damage if they were released into the atmosphere (Samaras, 2011).

Annuals are common in seaboard and inland salt marshes either in mixed stands or monoculture. They inhabit extremely variable natural and biotic environments (Noble et al., 1992). Thus, the genus salicornia contains plant species that are succulents with reduced vegetative parts and jointed photosynthetic stems that end in fruiting spikes, the internodes of which are embedded with reduced flowers (Noble et al., 1992). There are two cymules (flower clusters) per internode in salicornia species. Each cymule bears three flowers each containing one seed (Ellison, 1987). Some species, including *salicornia patula* and *salicornia europaea,* exhibit seed dimorphism in which seeds originate from the central flower of each cymule that are larger and germinate more quickly than seeds from the two lateral ones (Berger 1985, Ungar 1979).

Studies have also been conducted on the suitability of the salicornia species. As a wastewater biofilter, (Shpigel et al., 2013) demonstrated that a constructed wetland planted with Salicornia *persica* were had effectively in the removal of Nitrogen (N), Potassium (K), and total suspended solids (TSS) from a 1,000m^3 commercial, intensive, even semi-recirculated aquaculture system growing 100 tons of gilt-head sea bream (1-500g in size). It was estimated that about 10,000m^2 of wetland planted with salicornia *persica* would be required to remove N and 14 TSS in wastewater in one year. This study also found that an average yield of 10,000m^2 of *salicornia persica* could be expected to produce about 28.8 tons of plant produce (2.88 kg m-^{2} y $^{-1}$). The upper part constitutes approximately 80% of the yield. Therefore, the investment yield would be about 23 tons of fresh produce (Shpigel et al., 2013). Although using CWs for effluent treatment demand a relatively extensive area, a cost-effective analysis conducted by (Cardoch et al., 2000) found that treatment of wetland costs approximately 75% less to the farmer than conventional on-site treatment. (Shpigel et al., 2013) stated that the use of a CW to treat aquaculture wastewater can be even more cost-effective if the wetland is sowed with a crop that has a potential or real market demand.

Studies of salicornia have focused mainly on *salicornia europaea* and other species common either to Europe or the US east coast. Other studies were conducted on detailed on the plant's distribution growth and morphology. By comparison, there are very few investigations of *salicornia bigelovii,* which inhabits Pacific coastal salt marshes from northern Baja California, Mexico, to Los Angeles County, California (Wiggins, 1980). *Salicornia bigelovii* plays an environmental role like *salicornia europaea*. Both species are typically the first colonizers of bare space in the high tidal zones of North American salt marshes (Ellison, 1987; Zedler et al., 1992). Such bare patches are characterized by extremely saline soils, the mechanism by whatever salicornia species tolerate extreme soil salinities is their intracellular ability to accumulate sodium (Na^+) in vacuoles (Ayala et al., 1994). The salt is partitioned away from salt-sensitive metabolic reactions in the cytoplasm, and the vacuoles provide the cell itself with a high osmolality enabling tolerance of osmotic stress (Ayala et al., 1994).

Germination challenges

As technology progresses, there are different techniques and methods developed to grow a plant from its seeds. It is through the process of the seed sprouting that will generate the plant. There is also the formation of seedlings from the angiosperm or gymnosperm in the seed. Germination also refers to the development of spiraling in the process of hyphae formation from the fungal spores. There are three main requirements for germination of seeds. These are water, appropriate temperature, and suitable soil. There is a vast range of academic and scientific literature published about germination of seedlings. However, it is still a mystery about exactly how this activity occurs. So far, the process cannot be entirely explained by any thesis as developed by any scientific researcher. There is a high level of Nature's involvement in seed germination. Also, Nature tends to play an imperative role in the development of a seedling (Nonogaki, et al., 2010).

It has been observed that germination is a process in which seeds need to recover from the drying phase. There is a high rate of metabolism that occurs during the seed's development into a plant. There is also an extensive level of protein synthesis involved in carrying out the process. This synthesis helps in estimating cell integrity. This synthesis is also needed for the required plant development to shape the seedlings. The process of seed development is very peculiar. It is the dormancy period that supports the process of germination throughout the formation of a plant from seedlings (Bradford and Nonogaki, 2007).

There are some problems related to germination. All these problems concern the development of seeds. There are different processes that have been witnessed between the several stages of development during its growth. The physiology of the seeds must be understood throughout the whole germination process. These problems which persist were developed on the basis of that germination process. There have also been many experimental studies carried out on the process. It was determined that the process of germination is a combination of various factors. These factors must work in synchronization to create a successful process that results in the development of a plant (Crocker a Barton, 1953).

These problems have been described below:

- Seed planting 1.5 inches is a major problem if they are planted too deep. Then the seeds cannot get moisture from the soil. The seeds also do not get the proper sunlight due to deep planting.

- The quality of the soil is also a significant problem, especially when there is a requirement of developing the seedlings into plants. When a heavy soil is used for planting seeds, the seedlings will not spring into plants.
- Temperature is one of the most influential elements in the germination of seeds that form seedlings. It is through the application of proper warmth and maintaining the correct temperature so the seeds will germinate.
- Lack of, or improper, moisture is one of the most common challenges faced in the germination process. The seeds and their surrounding area should be kept from drying out. For this purpose, plastic domes covering seed-holding metal or plastic trays are used to prevent dehydration. Soil elevation will increase drainage and provides other features that help retain moisture (Baskin and Baskin, 1998).
- The controlled use of herbicides tends to be one of the most critical challenges throughout the germination process. Using inefficient or an improper number of herbicides for the germination process carries a certain amount of related success factors. These factors support seed germination (Nonogaki et al., 2010).
- Soil salinity is also a very significant factor in obtaining proper plant growth from seedlings. Direct observation of large terrestrial areas of crops that were affected by soil salinity before being harvested clearly demonstrates a negative impact on growth. It is also seen that there is considerable growth of the plants through the germination process carried out in the agricultural sector without soil salinity (Baskin and Baskin, 1998). Some plants are highly salt-tolerant such as the annual *salicornia* species, but their response to salinity levels will vary (Philipupillai and Ungar, 1984). Ungar (1967) did a study that found that

salicornia *europaea* showed a 10% germination with a 5% solution of sodium chloride (NaCl).

Growing Salicornia in FGD water

Salicornia plants helps mainly in the gas removal process by producing CO_2. These properties of the plants are very much required for the FGD process to occur, which in turn helps in the removal of sulfur (sea water; Singh et al., 2014). There is an enormous amount of challenges and problems seen during the process of developing salt-tolerant plants like salicornia. In this process, soil salinity is the primary factor contributing to the reduction in the fertility of the soil. Producing salt-tolerant crops is a significant task. This effort is taken into consideration due to its industrial benefits. This whole process involves a high level of commercialization. There are different setup procedures related to the development of functionalities across the system. It also influences FGD, where the main elements related to the development of FGD for commercial use. There are different concentrations of slurry generated through power generation elements. These have a significant impact on the development of strategies for creating a more suitable plant (Yamaguchi and Blumwald, 2005). Wet scrubbing involves the usage of seawater where there is a consecutive absorbing carried out by a slurry of alkaline substances which act as a sorbent. There is a consecutive level of gas developed throughout the water structure. It can be stated that there is a continuous process of FGD carried out throughout its processes, which promotes the use of the sorbent slurries. The higher absorption rate of salicornia makes it suitable to be used as a sorbent along with the seawater in the FGD (Hudson and Rochelle, 1982). The distribution of *salicornia europaea* is highly correlated with saline habitats. Factors such as soil salinity, soil water potential, precipitation pattern, and level of the water table, influence the growth and distribution of *salicornia* *europaea*. Intraspecific and interspecific competition may also influence the behavior of this species under field conditions (Ungar et al., 1979). Shpigel et al. (2013) and Ungar et al., (1979) found that level salinity is of one of the main reasons in determining both distribution and the growth of *salicornia europaea* in salt-marsh environments. Also, the distribution of *salicornia europaea* is usually limited to the most saline portions of both coastal marshes and inland salt marshes.

Salicornia is the most tolerant plant used along with the sea water to absorb the SO_2 and remove sulfur from fuel used in the FGD treatment. There is also carbonate used for generating water and CO_2 from hydrogen ions. The presence of salicornia in sea water helps in obtaining CO_2 from the carbonate (Salo et al., 1996).

SO_2 (gas) + H_2O (liquid) + $\frac{1}{2}O_2$ (gas) $\rightarrow SO_4^{2-}$ (aq) + $2H^+$

$HCO_3^- + H^+ \rightarrow H_2O$ (liquid) + CO_2 (gas)

There are many types of research carried out about obtaining softened water from hardened water. This concern is during the water treatment process function which is adopted in the FGD. It has the potential to increase plant efficiency through the removal of hardened water with the help of a plant like salicornia. There is the formation of a bitumen-like substance which is derived from the steam. This has functionality across the development of different stages of bitumen throughout the surface water in obtaining CO_2 from the carbonate (Salo et al., 1996).

The scrubbing agent used has a high sulfur content. This arises from the procurement of softened water across the absorption of CO_2 from the carbonate. There is a high level of absorption capacity required for removing the CO_2 from the water. As a result, salicornia, which has high cellulose content, is very much appropriate for the purpose. Softened water can be recovered through the development of processes with the establishment of suitable strategies that are related to the functioning of the system in the process (Salo et al.,1996). The functions related to the absorption of CO_2 from the FGD process are carried out with the help of a scrubber. This scrubber has the capacity to provide the required amount of dryness plus add stability to the system by removing the hard water in the process. It also tends to provide an alkaline water stream throughout the functioning of the bitumen recovery process adopted in the FGD plant.

There is a high level of saline waste produced during the process of FGD. This is carried out with the help of sea water and plants like salicornia. There are higher levels of salts present in this water. It can be developed through the process of absorption that is carried out in the system. There is a high level of absorption required for the functioning of salt plants. Plus, its quality would be deteriorated. Hence, it is required to develop saline waste through disposal sites which are already present in the system’s development (Salo et al.,1996).

Wetland treatment system for trace element sequestration

Galkaduwa et al., (2015) reported that the constructed wetland treatment systems are effective in removing Se from flue-gas desulfurization wastewater, leading to the complete Se retention by the soil columns. As for Boron, retention was weak, appropriate with field observations. Changing redox conditions in the soil led to mobilization of the initially-retained Se (~ 4 to 5%). They used micro-XANES analyses that indicated the retention mechanism of Se from the FGD wastewater was via the transformation of Se into reduced/stable forms [Se(IV), organic Se, and Se (0)]. These studies present useful information to improve the performance, and longevity of a full-scale CWTS for FGD wastewaters.

Pilon-Smits et al., (1999) reported that phytoextraction of selenite in contaminated water from wetland species is five times greater than selenite in contaminated water. Factors such as salinity, pH, temperature, levels of other pollutants, and competitive strength affect the performance of the wetland plant species (Pilon-Smits et al., 1999). It is important to mention that high Se accumulation in plant tissues (shoot and root) is a threat to wildlife, and their disposal in an appropriate way is important (Pilon-Smits et al., 1999). Operating halophytes as a plant bio-filter making use of living material to capture and biologically degrade pollutants of marine aquaculture effluent is a low-cost opportunity to mitigate potential negative impacts on the environment (Buhmann et al., 2015).

A recent study by Díaz et al. (2013) found that a number of halophytic species (*salicornia bigelovii*, *distichlis spicata*, *spartina gracilis*, *atriplex lentiformis*, *allenrolfea occidentalis*, and *bassia hyssopifolia*), which were grown under irrigation with saline drainage water over a 4-6-year period in the San Joaquin Valley of California, grew very successfully and also can effectively reduce saline drainage effluent. Halophyte tissues contained high levels of salts that total ash content ranged between 6 and 52% and accumulated in Na^+, Cl^-, SO_4^{2-}, NO^{3-}, B and Se to levels close or above the maximum tolerable levels. The halophyte species that accumulated Se at the highest rate was *salicornia bigelovii* with an average concentration of 16.3 mg kg^{-1}, and B concentration ranged up to 1680 mg kg^{-1}.

Salicornia europaea has also been shown to have significant potential in the treatment of aquaculture effluent. Webb et al., (2012) described a wetland filter bed planted with marsh samphire to evaluate its ability to treat the wastewater from a commercially operated marine fish and shrimp farm. The results demonstrated the effectiveness of the marsh samphire wetland in removing N and P from the wastewater was 91-99% of influent dissolved inorganic N and 41-88% of influent dissolved inorganic P.

However, Zhang et al., (2015) and Türker et al., (2014) reported only rarely successful attempts have occurred in treating wastewater high in B, salts, and Se by using CW. This is due to both the high B and salt toxicities that the remediating plants experience. Bonilla et al., (2004) have found that complicated eco-physiological responses caused by the interactions of B and salt in plants occurred. It affected their ability to absorb various elements. However, constructed wetlands were found to offer the possibility for treating saline drainage effluent that is high in B and Se under challengeable conditions. This can be expected if the wetland plants possess superior growth rates, the ability to accumulate B, Cl, Na, and Se in their shoots and exhibit high B and salt tolerances. Kadlec and Wallace (2009) review the removal of B in wetland treatment; however, they point out that most wetland treatments are not designed to remove B because B is not generally the important contaminant. For the proposed Westar treatment wetland, it is anticipated that successful reduction in the concentration of other contaminants will result in sufficient reduction in the concentration of B, also Talley, 2012 conducted research at the pilot-scale CWTS at the JEC. This provided an opportunity to assess the retention behavior of FGD wastewater constituents under real field conditions. Galkaduwa et al. (2017) compared the removal efficiency of each constituent by the saturated soil column system and the pilot-scale CWTS. The removal efficiencies of the flushed columns were used to compare the performance efficacy of the pilot-scale CWTS as those were more realistic for the field situation. The effectiveness of removing Se from the FGD wastewater by the soil columns was 100% whereas that of by the pilot-scale CWTS was 80%, it was assumed that the variation of field conditions (e.g. occasional drying) might have influenced the Se removal by the pilot-scale CWTS.

The information on the removal of B in various types of CWs is limited. Also, the removal processes in CWs that are responsible for B removal, have not been clearly understood. The chemistry of B differs from that of other trace elements, and the overall B removal process in CWs is very complex, making the identification of specific removal pathways more difficult. Nevertheless, several experiments have been carried out to determine B removal path-ways in CW systems (Ye et al., 2003; Gross et al., 2007; Allende et al., 2012; Türker et al., 2013). So far, the studies have been indicated which the processes responsible for the removal of B from CWs are sorption and plant uptake. However, environmental factors such as temperature, pH, transpiration rate, solution composition and competing species, hydraulic retention time, filtration media and operational factors are also important factors in B removal process in CWs.

Constructed wetlands have also been shown to remove significant amounts of Se from waters contaminated with agricultural irrigation drainage water (Johnson et al., 2009 and Gao et al., 2003). The principal mechanisms of Se removal by wetlands are (1) the dissimilatory anaerobic reduction of Se oxyanions (selenate and selenite) to less soluble or insoluble forms (e.g., Se^{2-}, Se^0) (Masscheleyn and Patrick, (1993), and (2) biological volatilization of Se to the atmosphere through assimilatory reduction and methylation by plants and microbes (Dungan and Frankenberger, 1999; Terry et al., 2000), a process that is particularly desirable because it leads to a net loss of Se from the local ecosystem, thereby preventing its entry into the food chain. In designing constructed wetlands, there are a number of treatment options for altering the distribution of Se removal between the two pathways. One of the options is by plant species selection. Lin and Terry (2003) reported that the vegetated wetlands were able to significantly reduced Se from the inflow drainage water, and on an average of 69.2 percent of the total Se mass in the inflow was removed. Most of the Se was retained in sediment, and <5% of the Se was accumulated in plant tissues (Gao et al., 2003), upon the addition of organic amendments (Frankenberger and Karlson, 1994), artificially varying levels of aeration (Chazarenc et al., 2009), and manipulation of plant‒substrate arrangement, composition, and structure (Davies and Hart,1990).

Micronutrients

The primary source of nutrients in the soil is the weathering of parent material in the Earth's crust. Table (2) shows the presence of the key elements in the Earth's crust. Except for nitrogen (N), all the other essential elements for plant growth – potassium (K), calcium (Ca), magnesium (Mg), and iron (Fe) - are widespread in the crust. Manganese (Mn), Phosphorus (P), and sulfur (S) are less prevalent. Zinc (Zn), boron (B), molybdenum (Mo), nickel (Ni), copper (Cu), chloride (Cl) are relatively scarce and considered as micronutrients. Also, Fe is a micronutrient, as it is an essential mineral element required in small quantities for both plant and human development.

Table 2.1 Average levels of micronutrient elements in the Earth's crust, ranked according to level (Source: Rudnick and Gao, 2003). mg kg^{-1} = milligram per kilograms.

Nutrient	mg kg^{-1}
Potassium	23,200
Calcium	25,600
Magnesium	15,000
Iron	39,200
Manganese	775
Phosphorus	655
Sulfur	621
Zinc	7
Boron	17
Molybdenum	1.1
Nickel	47
Copper	28
Chloride	370

Micronutrient malnutrition - a global problem

Micronutrient malnutrition - also called “hidden hunger” - is a widespread global problem (Welch, 2002). In addition to the direct health effects, the existence of micronutrient malnutrition has profound implications for economic development and human productivity, especially in terms of the potentially huge public health care costs and the loss of human capital formation (World Health Organization, 2006).

Micronutrient malnutrition, i.e. low dietary intake of Zn, Fe, vitamin A and iodine (the “big four”) threatens more than 2 billion people, predominantly in developing countries (Stein, 2010). Previous studies have primarily concentrated on Zn and Fe deficiency. These two are the most diffuse nutritional disorders. They have been estimated to cause 2.4 and 1.9%, respectively, of the world's diseases or ailments (Rodgers and Randolph, 2000).

Micronutrient deficient soils are widespread throughout the world. Many millions of hectares of arable land are deficient in one or more micronutrient elements. This fact has been brought into sharp focus over the last decade because of the large proportion of people are deficient in micronutrients, mainly Fe and Zn. Biofortification (Olsen and Palmgren, 2014; Vaz Patto et al., 2015) via agronomic means is becoming widespread, especially in the case of Zn (Olsen and Palmgren, 2014; Tan et al., 2015). This situation is dependent on the interactions between various soil and plant factors.

Rengel (2002) and Marschner et al., (2011) reported that Plants suffer nutrient deficiency stress when the availability of soil nutrients, and/or the amount of nutrients taken up, is below which required for sustaining metabolic systems in a particular growth stage. Therefore, this may result from in low of nutrient level in the soil, low mobility of nutrients inside the soil, poor solubility of the given chemical form of the nutrient, or the soil-microbe-plant interactions.

Zinc is an essential micronutrient for plants and microorganisms. In calcareous soils, the solubility of ferric (hydr)oxides can only provide iron concentrations that are far below the needs of most plants. Calcareous soils are among the most micronutrient deficient soils in the world (Malakouti, 2008). The micronutrient deficiency problem in these soils is related to high soil pH, low organic matter content, low soil moisture, high soil and water alkalinity (Malakouti, 2008; Karami et al., 2009).

Zinc deficiency in soils

Deficiencies of Zn is observed in many parts of the world on a wide range of soil types. However, semi-arid areas with calcareous soils, tropical regions with highly weathered soils and sandy textured soils in several different climatic zones tend to be the most severely affected. According to Lindsay (1972), the soils most commonly associated with Zn deficiency problems frequently have one or more of these characteristics. Calcareous soils generally with > pH 7.4 have relatively little available Zn because the solubility of Zn decreases with increasing pH. Very often the total Zn content of calcareous soils is similar to those in soils of other types, or even higher, but the availability is low. Adsorption of Zn onto the $CaCO_3$ is also a contributory factor. An excess of phosphorus can interfere with the metabolic functions of Zn. High bicarbonate and magnesium in soil or irrigation water can also lead to high salt concentration in soils (Lindsay, 1972).

Soils may have low total Zn content (e.g., some leached acidic soils in tropics) or might have relatively high total Zn content, however, available plant fraction is low because of soil chemistry that favoring the formation of sparingly soluble Zn complexes (Rengel, 2002). Major factors affecting Zn uptake and reducing solubility and mobility, which be high soil pH, low organic matter content, low soil moisture, and high calcium carbonate. Soils with low plant-available Zn, incorrectly called Zn-deficient soils because only living organisms can be Zn-deficient, are common in tropical and temperate climates; also are widespread in regions with the Mediterranean type of climate (Sillanpää and Vlek, 1985). Rehman et al., (2012) reported low plant-available Zn in soils of various characteristics: high and low organic matter, high and low pH, calcareous, sandy, sodic, wetland or ill-drained, limed acid soils, etc. Zinc deficiency is common on neutral to the alkaline pH soils, including more than 10 g organic matter/kg as well on calcareous soils used for upland rice production which rice is grown.

Zinc is an essential element for all living organisms. In recent years, human Zn deficiency has aroused worldwide concern, especially in developing countries (Wessells and

Brown, 2012). Diets are predominantly from low-Zn cereal grains, such as wheat, rice, and maize, and daily Zn intake cannot satisfy the nutrition requirement of residents (Gibson, 2012). Agronomic (Prasad et al., 2014) and genetic biofortification (Bouis and Welch, 2010) are proposed to increase Zn concentration of cereal grains to address the nutritional disorder. Agronomic Zn biofortification measures such as a foliar spray of $ZnSO_4$ with or without soil Zn fertilization, have been proved to be effective in many developing countries, where soil is often deficient with available Zn (Joy et al., 2015; Zou et al., 2012). However, this practice has not been widely adopted by local farmers, due to relatively high economic and labor cost (Wang et al., 2016). Comparatively, genetic Zn biofortification measures aimed at breeding high Zn cultivars are easier to be accepted by farmers and consumers and evaluated as the most cost-effective method by disability adjusted life year (DALY) analysis (Joy et al., 2015; Ma et al., 2008). Hidden deficiencies of Zn are generally associated with growing Zn-inefficient varieties on soils of marginally low available Zn status. It is often found that Zn-efficient varieties can yield satisfactorily on the same soils (Graham and Rengel, 1993). The global distribution of cases of Zn deficiency in crops is shown in Fig. 2.1. Reproduced with permission from the International Zinc Association (IZA) and the International Fertilizer Industry Association (IFA).

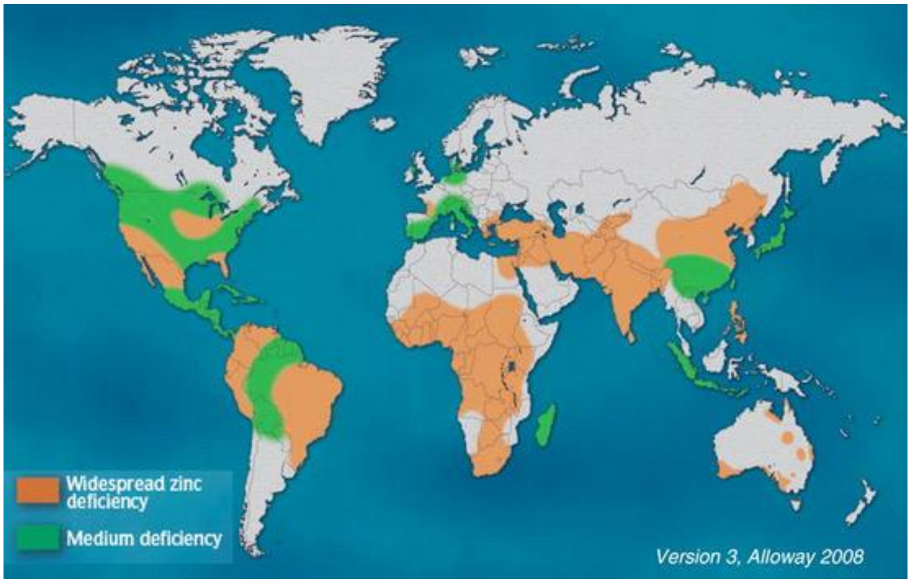

Figure 2.1 Global distribution of reported cases of zinc deficiency in crops adapted from Alloway (2008).

The plant-available forms of Zn in soils are ions (Zn^{2+}), soluble inorganic and organic complexes, and labile (easily-desorbed) Zn (Kiekens, 1995). Most of the soils with low plant-available Zn can be applied with Zn fertilizers to correct crops Zn deficiency. Several different Zn sources, including $ZnCO_3$, $Zn(NO_3)_2$, ZnO, $ZnSO_4$ and $ZnCl_2$, are currently being used as fertilizers. Concentrations of Zn in the soil solution are generally low (4–270 μg L^{-1}) compared with average total concentrations of around 64 mg Zn kg^{-1}. However, in very acid soils, soluble concentrations of 7-137 μg L^{-1} have been found, indicating that solubility is strongly but inversely linked to soil pH (Kabata-Pendias, 2001). The main soil factors controlling the amounts of plant-available forms of Zn in soils include: the ‘total’ Zn content, calcite ($CaCO_3$), pH, redox conditions, organic matter, concentrations of all ligands capable of forming organic or inorganic-Zn complexes, concentrations of other trace elements, microbial activity in the rhizosphere, concentrations of macronutrients (especially P), and the soil moisture status.. Zinc deficiency could occur on alkaline soils and sandy soils with poor organic matter content. High levels of P coupled with low levels of Zn in soil, may induce Zn deficiency (Soaud et al., 2011).

The Sources of Zn and Their Effectiveness

According to a study conducted by Boawn (1973), crop responses to the use of Zn fertilizer will be primarily attributed to the movement of Zn in soils. Under certain conditions, the soil's ions will vary with the Zn fertilizer source. Several studies conducted by Prasad (et al., 1976); Dhillon and Dhillon, and Hergert (et al., 1984) have reported that the application of non-chelated Zn fertilizers to calcareous soils is less effective than chelated forms of Zn under greenhouse conditions. Natural organic Zn compounds, such as Zn ammonium acidic (Zn-AA), are used to make Zn sources. However, there is only limited data available when comparing its effectiveness with synthetic Zn compounds. In a study conducted by Goos et al., (2000), it was found that Zn-citrate, Zn-fulvate, Zn-lignosulfonate, and Zn-humate lignosulfonate were less effective sources for maize than Zn-EDTA and Zn-DTPA. It was also determined in other studies conducted by Maftoun and Karimian (1989) and Thind et al., (1990) that in calcareous soils, the availability of Zn is largely governed by soil pH. Other important factors that will also govern the availability of Zn are the kind and number of anions in the soil solution, type of soil minerals, and Zn carriers. Metal-chelates that are used in soil application must be resistant to microbiological decomposition. These metal-chelates must also not be easily precipitated by colloids or ions in soils.

In a study conducted by Bolton et al., (1993) several soil samples were used to determine the biodegradation of synthetic chelates. The researchers also found that the relative order of chelate persistence was EDTA-DTPA-nitrilotriacetate. In another study conducted by Adriano (1986), it was reported that some cations, such as Zn^2, could enter the crystal lattice of the layer silicates. This is accomplished through isomorphous substitution or the solid-state diffusion then the cations can enter the crystal structure.

However, this process may be irreversible; it has been found that some applied metals may be irreversibly fixed by the application of clay. The chelating agents, including DTPA, EDTA and HEDTA, are some of the strongest synthetic chelating agents in existence. Norvell (1983) found that these agents will form much stronger chelates with Zn than naturally occurring organic ligands. In a study conducted by Dwyer and Miller, (1964), the long-chain natural organic compounds, such as Zn-AA, are intermediate in chelating strength. Consequently, according to (Hsu, 1986), amino acids can effectively supply Zn when it is applied to soil. Although it was noted that this occurs at a slightly higher rate of application than is required for chelates.

Kiekens (1995) and Li and Shuman (1997) have determined that the continuous application of large amounts of Zn chelates to soil has caused concern. This concern is regarding potential harm to the environment and the possible accumulation of trace elements, as Zn can be transported downward in soil and ground water quality may be possibly be negatively affected or deteriorate. Prasad and Sinha (1976) found that the uptake of Zn by maize is markedly influenced by its diffusion rate to the absorbing roots.

Adriano (1986), Modaihsh (1990), and Alvarez (et al., 1996) all have determined that chelating agents, such as DTPA, EDTA, and HEDTA, have been shown to largely contribute to Zn movement in soils by irrigation or under conditions of excessive rainfall, in general. Additionally, Mikkelsen and Brandon (1975), Mikkelsen and Kuo, (1977) and Kiekens, (1995) found that this movement may produce long-term Zn deficiency in soil. The movement may also complicate the study of the behavior of different Zn sources. In this manner, Goos (et al., 2000) has demonstrated that Zn-EDTA was not a superior source for maize more than Zn-humate-lignosulfonate and $ZnSO_4$ in calcareous soil in the long-term.

Additionally, chelation of Zn^{+2} by chelating agents will decline over time in calcareous soils (Lindsay, 1979). This is due to Zn^{+2} being slowly replaced on the ligand by Ca^{+2}. The Mehlich-3 and DTPA extractions are both methods usually employed to diagnose Zn bioavailability for plant uptake (Reed and Martens, 1996). Additionally, Zn soil behavior was evaluated by using sequential fractionation (Obrador et al., 2003). The Mehlich-3 and DTPA extractions in calcareous soil was correlated with the water-soluble. It also correlated to extractable DTPA and an exchangeable fraction.

The Zn distribution in various soil fractions provides a better understanding of Zn behavior in relation to the transformation of Zn added to the soil (Iyengar (et al., 1981). In a study conducted by Viets (1962), focus was on the following pools: adsorbed, chelated, or complexed metals bound to organic ligands; water-soluble the fraction present in the soil solution; clayey secondary minerals and insoluble metallic oxides; exchangeable ions bound to soil particles by electrical charges; and primary minerals. It is relatively easy to partition an element into different pools in soils conceptually. However, such fractionation is fraught with difficulties, at least regarding some extractants. This is because of the overlapping selectivity. The magnitude of this problem is known to increase with the stage in the sequential extraction procedure.

For example, Shuman (1985) found the acidification performed to assess the oxidation of organic matter can dissolve carbonate-bound Zn, and sodium hypochlorite (NaOCl) can dissolve Mn oxide (MnO_2). Although hydroxylamine hydrochloride seems to be an efficient extractant of Mn oxides in sediments, its efficiency with agricultural soils appears to be considerably lower.

Additionally, this is due to due to the no significant correlation between the Mn-oxide-bound Zn fraction and Zn uptake by plants. Pickering (1986) has been reviewed these topics. Despite these severe limitations, sequential extraction procedures have been commonly used to characterize Zn forms. According to McBride (1989), the speciation of metals indicates that organic acids are suitable chelating ligands. This speciation could explain the higher levels of metal complexation observed in soil solutions. Also included could be N-containing ligands, such as amino acids. The water-soluble plus exchangeable metal fraction characterizes are the most mobile. They are also (Li and Shuman, 1997) the most immediately bio-availability forms. These are the most labile metal forms in the soil environment. They also (Shuman, 1991) have greater leaching potential than the other forms.

Biofortification of Zn

Biofortification is a process in which plants are allowed to take up the mineral (Zn) from the soil and accumulate them in the grains to produce nutritionally rich grains that support a dietary requirement of the human. This approach has proved to be sustainable, relatively low cost, and highly efficient (Patel et al., 2004). Biofortification requires that agricultural research makes direct linkages with the human health and nutrition sectors. Plant breeding (e.g., genetic biofortification) and application of Zn fertilizers (e.g., agronomic biofortification), are two important agricultural tools that can improve the grain concentration of Zn (Cakmak et al., 2010). These processes facilitate the uptake of micronutrients by plants and further translocated to grains, which circumvents the impact of anti-nutritional factors (Phytic acid).. Fertilization is necessary for those trace elements whose accumulation in the edible parts of plants is limited by the low availability in soil. Approximately half of the world’s agricultural soils are deficient in

Zn for crop production, and these soils are generally associated with Zn-deficient humans (Cakmak, 2008).

It is this principle in Zn-deficient soil that the application of Zn fertilizers is effective in enhancing both crop yield and Zn concentration in the grain. Foliar application of $ZnSO_4$ to wheat crop at a late growth stage increased grain Zn concentration by 3-fold. In severely Zn-deficient soils, the addition of Zn fertilizer to soil increased yield, but not the concentration of Zn in rice grain, probably due to the effect of Zn immobilization in the soil (Wissuwa et al., 2008). Mineral Zn is supplied to plants either by soil application, foliar spray, or seed priming. The method of fertilizer application affects the overall yield and Zn concentration in grains of cereals. Soil type also influences Zn concentration in the root, shoot, and grains of cereals. Zinc sulfate is mostly used as soil Zn fertilizer (Rengel and Graham, 1995).

Zinc Minerals and Their Stability

The oxidation state of Zn in a natural environment like soils is Zn^{+2} exclusively. Metallic Zn formation is possible only in highly reduced environments; it is unlikely to occur in soil environments as demonstrated by the reaction given below:

$$Zn^{+2} + 2e^{-} \leftrightarrow Zn\,(c) \qquad \log K^{0} = -25.80$$

Considering Zn^{+2} to be 1 M, an electron activity (pe) of -12.90 is required to form Zn(c). As discussed earlier, the pe in soils ranges from -6 to -13 (Sposito, 1989). Therefore, it is unlikely that Zn concentration will reach 1 M in solution, and metallic Zn will form in soils.

Table 2.2 Equilibrium reactions of some Zn minerals at 25 °C in soils used for calculations of stability

No.	Equilibrium reaction	Log K°
1	Soil-Zinc+ 2H+ $\longleftrightarrow$ Zn+2	5.80
2	ZnO (zincite) + 2H+ $\longleftrightarrow$ Zn2+ + H_2O	11.16
3	$Zn(OH)_2)$ (amorphous) + 2H $\longleftrightarrow$ Zn+2 + $2H_2O$	12.48
4	$Zn2SiO_4$ (willemite) + 4H+ $\longleftrightarrow$ 2Zn+2 + $H4SiO_40$	13.15
5	$ZnCO_3$ (smithsonite) + 2H+ $\longleftrightarrow$ Zn+2 + CO_2(g)+H_2O	7.91
6	$ZnFe2O_4$(franklinite) + 8H+ $\longleftrightarrow$ Zn+2 + 2Fe+3+ $4H_2O$	5.80

Adapted from Lindsay (1979).

Since the specific minerals controlling the activity of Zn^{+2} in soils are not known, reference solubility is used that permits Zn^{2+} solubility relationships to be compared to those of known Zn minerals (Novell and Lindsay, 1969; 1972). Most of the Zn production is done from Zn sulfide minerals; sphalerite, and wurtzite as they have a higher percentage of Zn by weight compared to other minerals. Zinc sulfide minerals are common under reduced conditions; however, on exposure to oxygen-rich environment, ZnS is readily transformed into Zn silicate, oxide, carbonate, sulfate, and phosphate minerals.

The solubilities of different Zn minerals are plotted in figure 2.2. It shows that all of the $Zn(OH)_2$ minerals, ZnO (zincite), and $ZnCO_3$ (smithsonite) are too soluble to persist in soils. Willemite (Zn_2SiO_4) has intermediate solubility and is highly dependent on H_4SiO_4 in soils that are further controlled by quartz (SiO_2) (Lindsay, 1979). Franklinite can be the most insoluble of these minerals, depending upon the Fe(III) oxides controlling Fe^{3+} activity. Zinc minerals solubilities are highly influenced by pH, and with each unit of pH increase, the solubility of all Zn minerals is increased by 100-fold (Fig 2.2). In addition, the existence of Zn phosphate mineral (hopeite) is highly dependent upon the concentration of phosphate in soils (Lindsay, 1979).

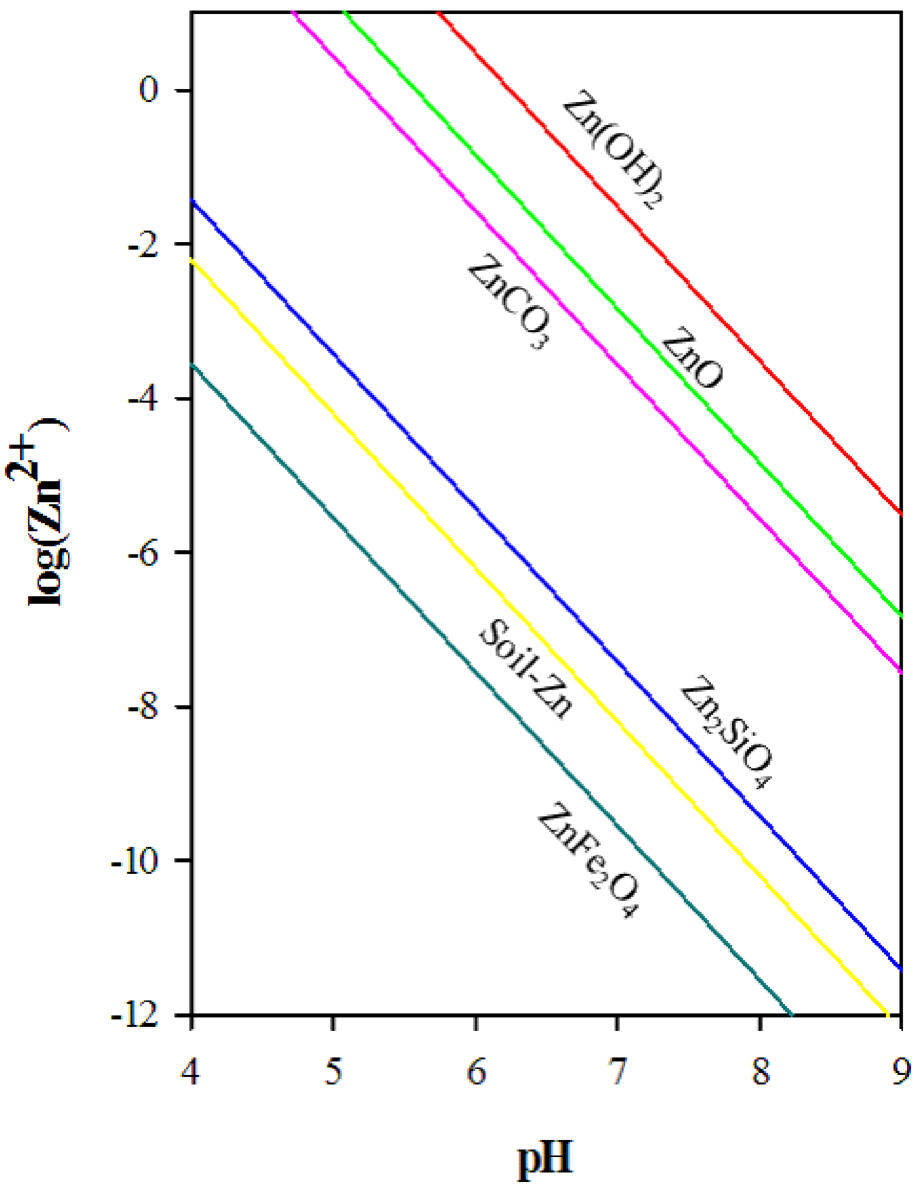

Figure 2.2 The solubility of several Zn minerals compared to soil-Zn.

Compost

In general, most of the calcareous soils showed poor hydro-physical and fertility characteristics. So, the addition of organic materials is of vital importance to improve physical and chemical characteristics as well as the fertility status of these soils (Habashy et al., 2008). Organic wastes include crop residues, animal manures, food processing wastes, also municipal biosolids, and wastes from some industries. These organic wastes are usually by-products of farming, industrial or municipal activities, also are regularly called “wastes” since they are not the primary product. Micronutrient deficiency is a widespread problem in plants grown on calcareous soils. Application of composted municipal waste can effectively alleviate micronutrient deficiencies in plants and especially cereals grown on calcareous soils. Majority of the agricultural arid and semi-arid soils are suffering from high lime, high pH; and low levels of organic materials. On the other hand, micronutrient deficiency. especially Zn-deficiency, is common on soils low in organic matter and of high pH (Hamidpour et al., 2012).

Compost obtained from the organic fraction of biosolid and from other selected waste biomasses can be used effectively as an organic fertilizer on agricultural soil. The high content of stabilized organic matter and the presence of nutrients of good quality compost are a guarantee of agronomic advantages; at the same time, the use of compost makes the reuse of organic wastes possible and has numerous advantages. An increase in soil organic matter and nutrient availability after compost application has been observed by many researchers (Hargreaves et al., 2008; Price et al., 2009).

The organic matter added by biosolid to arable land can improve some soil physical and chemical properties (Jacobs and McCreary, 2003), Ippolito et al., 2010) found increases in Cd, Cu, and Zn concentrations in soils amended with composted biosolid three years after application. Application of the municipal solid waste compost as biosolid to agricultural soils was previously found to improve micronutrient complex formation, which increases the availability of micronutrients to plants, stimulate the presence of beneficial soil organisms, and reduce the presence of plant pathogens. Moreover, it increases water holding capacity, soil buffering and cation exchange capacity, and it improves soil porosity. The primary benefit of biosolid compost is the high organic matter content and low bulk density (Soumare et al., 2003). Perucci, 1990; Crecchio et al., 2004; Garcia-Gil et al., 2004; Montemurro et al., 2006;). Walter et al., 2006 reported that repeated application of biosolid compost consistently increased soil organic matter content and soil C/N ratio to levels higher than those of unamended soil. Biosolid compost had a high water-holding capacity because of its organic matter content, which in turn improved the water-holding capacity of the soil (Hernando et al., 1989; Soumare et al., 2003). The addition of organic amendments to calcareous soils can effectively alleviate micronutrient deficiencies in plants grown on these soils (Hamidpour et al., 2012). Because of expensiveness and rarity of farm manure in arid regions, land application of biosolid (e.g., sewage sludge) may be considered as an alternative to animal manure for enhancing plant productivity (Achiba et al., 2010; Hamidpour et al., 2012).

Organic matter (manure, biosolid) plays a crucial role in governing the availability of soil Zn (Al-Chami et al., 2013). Biosolid materials not only desorption free Zn into soil solutions by decomposition but also alter the original solubility and mobilization of soil Zn by the formation of Zn organic complexes (Smith, 2009). Also, Organic amendments and biosolid were shown to change the distribution of Zn precipitated by calcium carbonate, which is supposed to be a signifciant factor in the loss of Zn availability in calcareous soil (Jalali and Khanlari, 2008). Also, mechanism adsorption and fixation of Zn takes place by the slow distribution of available

Zn into iron (Fe) and Mn oxides and clay minerals, and this is also closely related to the change amounting to Zn bound to organic matter (Pérez et al., 2012). The effect of organic matter on the availability of soil Zn depends on the maturity and ability of organic amendments. The availability of Zn is small, where mature organic materials are present as compost due to the formation of stable organic complexes with organic matter such as humic acid (Smith, 2009). In contrast, a rapidly degradable organic matter added to the soil could effectively dissolve initially insoluble Zn, which improves its solubility and availability in soil-plant systems due to of water-soluble or labile organic compounds rich in functional groups as amino, carboxyl, and phenolic which have strong chelating abilities (Fuente et al., 2011).

Humic substances

Humic substances, perhaps controversially described as refractory, are dark-colored, heterogeneous organic compounds produced as byproducts of microbial metabolism and are considered the most widely distributed organic materials on the planet (Stevenson,1994). So that, this assembly of organic "leftovers" in which contributes to vital properties to soils, including mobilization, sequestration, and oxidative or reductive transformation of organic xenobiotic molecules, including trace gases, and trace metal contaminants. Early efforts to describe this material resulted in the following fractionation scheme based on its solubility under acidic or alkaline conditions (Stevenson, 1994). Humin is the insoluble fraction of humic substances; humic acid (HA), is the fraction soluble under alkaline conditions but not acidic conditions (generally $pH < 2$); and fulvic acid (FA), is the fraction soluble under all pH conditions. Although chemical and physical variations do underlie those differences in solubility, the separation of humic substances into three portions is operational. It does not indicate, for instance, the existence of three distinct types of the organic molecule (Hayes et al., 1989).

So that, specific characteristics have been ascribed to HAs and FAs, which leads to deprotonation of acidic functional groups results in a negative charge, which is generally higher for FAs than that for corresponding HAs from the same sample. (Christl and Kretzschmar, 2001 and Zomeren et al., 2009) FAs have enhanced water solubility because of as a result, higher negative charge, and smaller size (Chin et al., 1997). In spite of the heterogeneous composition of HAs and FAs, (Jones and Bryan,1998), proton binding parameters for carboxylic and phenolic groups in HA and FA isolated from natural and waste materials are similar and fall within the range of typical values for HA and FA. (Milne et al., 2001). Thence, HAs, and FAs have been successfully used to model binding properties to predict the dynamics of pollutants in the environment (Christl and Kretzschmar, 2001 and Xiong et al., 2015).

Polymers

Polymers are formed of long molecular chains (monomers), which are held together by covalent bonds (Georgees et el., 2015). They may be natural or synthetic and are classified functionally as cationic, anionic, and nonionic polymers. Also, they provide physical bonding between the soil particles rather than forming a new mineral using chemical reaction (Tingle at el., 2007). Onyejekwe and Ghataora, 2015 mentioned that to get the full benefit from polymer stabilization, one must identify the type of soil for which the polymer can be used. Sometimes the polymer might not work for a specific type of soil. It is also necessary to determine the application rate for a particular type of soil. Furthermore, polymer treated soil must lose moisture to gain strength, so the additives must be applied in dry weather conditions. It is necessary to adopt a suitable construction technique that considers the stabilization mechanism of the polymer. There are various types of polymers, such as acrylic copolymer and polymers, liquid polymers of methyl acrylates and acrylates, copolymers of sodium acrylates and acrylamides, poly-acrylamide and copolymer of acrylamide, and hydro-colloid polymer, currently used for the purpose of soil stabilization (León and Matthews, 2003).

Several researchers (Georgees et el., 2015; Onyejekwe and Ghataora, 2015; Rauch et al., 2002; and Mousavi at el., 2014) have used various types of polymer to stabilize different types of soil. The problem associated with polymer stabilizer is that they are sold with a commercial name and most of the times the manufacturer does not disclose the chemical composition of the polymer. This makes it difficult to characterize the performance of the polymer.

Such as polymer, known as AVAIL, is designed to sequester antagonistic metals in the soil surrounding the fertilizer granule, also, reduce tie-up of phosphate and make phosphate more available to the plant from a label for AVAIL granular phosphate fertilizers (cation exchange capacity, CEC, is about 1000 $\text{cmol}_c\ \text{kg}^{-1}$ at pH 6.2). It is a long chain dicarboxylic acid (DCA) copolymer. The DCA, composed of maleic and itaconic acids, is reported to have a cation exchange capacity (CEC) of 18 meq g^{-1} of solid polymer. The proposed mode of action claims that this high CEC sequesters Ca, Mg, Al, Fe, Mn, and other multivalent cations, thus reducing their interaction with P in soil solution (Chien et al., 2014).

Plant type

Wheat is one of the three leading cereal crops worldwide and is the dominant crop used for human food. As the most widely planted crop on the planet, Wheat (Triticum aestivum) is one of the most important crops in the world (Leff et al., 2004). Cereals occupied about 52% of the world's cultivated lands (707 million ha), and one-third (232 million ha) belongs to wheat crop (Phillips and Norton, 2012). Wheat is considered one of the three majors of cereal crops worldwide and represents a main dietary source of calories, proteins, and micronutrients for the majority of the world's population, especially in the developing world (Shewry, 2009).

Wheat is responsible for up to 50 % of the daily calorie intake of the population living in rural regions and an important source for Zn for human beings living in the developing world (Cakmak, 2008). For example, wheat-based food products supply more than 20 % of dietary Zn in China (Ma et al., 2008). China, India, Kazakhstan, Mexico, Pakistan, and Turkey are some of the major wheat-producing countries and responsible for more than 30 % of the global wheat production (FAO database 2010). These countries are also among the regions where Zn deficiency is most frequently reported in soils, crops, and human population (Alloway, 2008; Hotz and Brown, 2004). Increasing grain Zn concentration in those countries representing about 45 % of the world's population would be, therefore, an important global achievement for better human nutrition. It is estimated that more than 40% of the wheat crop is cultivated on severely low Zn soils (Alloway, 2004; Malakouti, 2007), which produces poor grain yield with low Zn content, leading to Zn deficiency in human. In Iran, wheat is the predominant crop and staple food with approximate cultivation of more than 6 million ha per year (Emam, 2008). Almost all of this wheat is grown on the calcareous soils (Banaei et al., 2005).

Zinc uptake from soil

Zinc enters plants primarily via root absorption of Zn^{+2} from the soil solution, which is a dynamic and complex process. Also, the uptake depends on ion concentrations at the root surface, root absorption capacity, and plant request. Zinc reaches the plant root surface by three mechanisms mass flow, diffusion, and root interception mechanisms. The mass flow that considers to passive nutrient transport from the soil to the roots and is driven by transpiration. Meanwhile, the solution moving through the soil to the root contains a relatively large concentration of Zn, and so, the mass flow becomes the principal mechanism bringing Zn to the root surface (Sadeghzadeh, 2013).

The diffusion is playing an important role in the transport of Zn and other nutrients such as P, K, Fe, Cu and Mn to the root surface, when the Zn concentration is low, especially in soils with low plant-available Zn. The mass flow could only carry a small fraction of the nutrients required for the plants when the solubility of that nutrient is generally low. In contrast to mass flow, diffusion operates only in the immediate volume of soil surrounding a root (Sillanpää, 1990 and Sadeghzadeh, 2013).

The interception of nutrients by roots is a fundamental uptake mechanism for immobile soil nutrients such as Zn. Thus, root interception is also an important factor in determining plant availability of Zn, so that depends on root growth and root surface area. Weak root interception can limit Zn uptake if granules of $ZnSO_4$ are banded in the soil, especially at a low rate of $ZnSO_4$ application. Wheat grain zinc through fertilizer applications.

Crop improvement to develop micronutrient dense wheat is a longer-term breeding effort and requires extensive germplasm screening, crossing and developing early, intermediate, and final stage products, and intensive performance testing in target areas under different environmental conditions. In several target countries and target zones with a high prevalence of Zn and Fe deficiency, soils cultivated to wheat are prone to adverse chemical and physical conditions that reduce the chemical solubility and plant availability of Zn. Low soil moisture, high soil pH, and high $CaCO_3$ content and low amount of organic matter decrease solubility and availability of Zn in the soil severely. As a consequence, absorption of Zn at amounts adequate for better crop production and higher grain mineral concentrations are significantly depressed (Cakmak, 2008).

For example, increasing soil from pH 6 to 7 reduces the chemical solubility of Zn in soil by nearly 30-fold (Marschner, 1993). Under such adverse chemical soil conditions, the genetic potential of biofortified wheat can only be partially expressed. Hence, it is critical to maintaining an adequate amount of available Zn in the soil during plant growth because continued root uptake and transport into seeds during the seed-filling period is an important means of seed micronutrient accumulation (Waters and Grusak, 2008). Further, maintaining adequate Zn transport from vegetative tissues into the seeds during reproductive growth stages (Hasslett et al., 2001; Cakmak, 2008) can be an important strategy in reducing variations in grain mineral concentration caused by environmental fluctuations and in producing and further enhancing grain Zn concentration. When feasible in a given target country, Zn fertilizer approach seems to be an effective way to improve grain Zn concentration in wheat and a useful complementary approach to on-going breeding programs.

Soil or foliar application of Zn containing fertilizers greatly improves grain Zn concentrations in both durum and bread wheat (Cakmak, 2008). In field trials in Central Anatolia, a well-known highly Zn-deficient region of Turkey (Cakmak et al., 1996), applying $ZnSO_4$ to soil enhanced both grain yield (Yilmaz et al., 1997; Ekiz et al., 1998) and grain Zn concentration of durum wheat. An increase in grain Zn concentration by soil Zn application was almost twofold. In contrast, combined application of Zn through soil and foliage was more effective and resulted in a more than threefold increase in Zn concentration in durum wheat grain. Similar increases in grain concentrations of Zn in wheat following soil Zn application were also seen in Australia (Graham et al., 1992) and India (Shiway et al., 2008) under field conditions.

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Chapter 3 - Evaluating Soil- and Plant-based Treatment Options for Marginal Waters

Abstract

Plant systems have a significant capacity to remediate marginal waters through several phytoremediation processes including uptake (e.g., nutrients, trace elements), accumulation (e.g. salts), and assist with biotransformation of inorganic compounds (e.g., nutrients, trace elements). The halophyte, *salicornia europaea*, grows under extreme salt conditions and has been explored as a saline agriculture crop. Salicornia could be a suitable halophilic plant to capitalize on its salt-tolerance potential for treating marginal waters. The aim of this study was to determine ability of *salicornia europaea* to grow in a soil watered with flue gas desulfurization (FGD) wastewater, which is high in salts and potentially toxic trace elements such as selenium (Se); or brackish waters. A greenhouse experiment was conducted using five treatments: tap water100% (control), brackish water100%, FGD wastewater 100%, Hoagland solution and brackish water (50:50 v/v), Hoagland solution and FGD (50:50 v/v). For two months, 500 mL of these solutions were applied every two days to pots containing transplanted *salicornia europaea*. Shoots were harvested two times:1 and 2 months after initiation of treatments. Dry matter yield and uptake of constituents (including Se, nutrients, and salt) by *salicornia europaea* were measured. Dry matter yield was significantly higher in wastewater treatments than the control. *Salicornia europaea* tolerated high salinity and maintained relatively high growth rate at intermediate salinity. The results also demonstrated some enhancement in FGD wastewater N removal by *salicornia*. These results were further confirmed through a hydroponic study. *Salicornia europaea* has the ability to remove excess trace elements such as Se and B and salts in marginal waters, therefore, it has the potential for slowly reclaiming marginal waters. Enhanced biomass is

encouraging as it shows the potential of *salicornia* to provide valuable stock for biofuel and bio-based products from marginal waters.

Introduction

Today, the use of environmentally friendly technologies is considered a principal necessity in many countries, and employment of natural methods in wastewater treatment, including Constructed wetlands (CWs), has found its place in the planning process of various countries as well. Constructed wetlands are a natural, low-cost biological technology for wastewater treatment that emerges from the ecosystems of natural wetlands and are considered a potential replacement system and/or a complementary system within wastewater treatment (UN-HABITAT 2008). Plant systems have a significant capacity to remediate marginal waters through several phytoremediation processes including uptake (for example, nutrients, trace elements), accumulation (e.g. salts), and assist with biotransformation of inorganic compounds (e.g., nutrients, trace elements).

Coal-fired power plants are a type of power plant that makes use of the combustion of coal in order to generate electricity. Coal-fired power plants release many pollutants and also emit environmentally harmful gases that included sulfur (SO_x), oxides of nitrogen (NO_x), carbon dioxide (CO_2), and particulate matter, which are known to contribute to global warming and climate change (Munawer, 2018). Coal combustion wastes generated at thermoelectric, coal-burning power plants contain numerous trace elements such as arsenic (As), barium (Ba), boron (B), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), selenium (Se), and zinc (Zn) (Cherry and Guthrie, 1977; Walia and Mehra, 1998). Severe physiological effects on organisms or species may be caused by exposure to these trace elements when above tolerable concentrations (Chang, 1996). These power plants have now significantly decreased air emissions of sulfur dioxide (SO_2) by installing flue-gas desulfurization (FGD) scrubbers (US Department of Energy, 2000).

Constructed wetland treatment systems are an economically and environmentally feasible option to treat a variety of marginal water (wastewaters) such as municipal, agricultural, industrial wastewater, FGD wastewater, and inorganic and organic contaminated water. Galkaduwa et al., (2015; 2017) mimicked a pilot-scale constructed wetland treatment system that was being used to remove Se from FGD wastewater (WW) using a saturated soil column; their study indicated a complete Se retention by the soil columns. As for Boron (B), the study found that retention was weak, agreeing with field observations with the pilot-scale CWTS. Changing redox conditions in the soil led to release of some of the initially-retained Se, it was approximately 4 to 5%. They used micro-XANES analyses that indicated the retention mechanism of Se from the FGD wastewater was by the transformation of Se into reduced/stable forms [Se(IV), organic Se, and Se (0)]. A follow-up study conducted by Paredez et al. (2017) using 2X concentrated FGD WW compared to Galkaduwa studies, showed that Se sorption was inhibited by high salinity of the FGD wastewater. These studies present useful information to improve the performance, and longevity of a full-scale CWTS designed for FGD wastewaters and suggested that pre-treatment of FGD wastewaters to remove dissolved solids (trace elements, salts, and nutrients) would be desired.

Phytoremediation of Se is the use of plants and their associated microbes for environmental cleanup, through processes that include, phytoextraction, phytovolatilization, and rhizofiltration (LeDuc and Terry, 2005; Pilon- Smits, 2005; Robinson et al., 2009; Yasin et al., 2015). Water and soil Se-contamination that has resulted from coal production, and agricultural drainage has caused significant toxic impacts on aquatic wildlife, such as deformity of waterfowl in the Kester's on National Wildlife Refuge in central California. Phytoremediation is an alternative and sustainable remediation technology compared with traditional physical and chemical remediation approaches. The Se phytoextraction processes are based on bioaccumulation of Se that involves plant uptake, distribution, accumulation, and transformation of Se from the soil into the plant's matrix (Zhao and McGrath, 2009; Zhu et al., 2009; Centofanti and Bañuelos, 2015). Although the goals phytoremediation of Se are different, the processes can sometimes be closely connected to enhancing the efficiency of Se uptake and accumulation in plants (Vamerali et al., 2014). Therefore, it is important to better understand the rhizosphere physical, chemical, and biological processes that affect soil Se bioavailability, plant uptake distribution, and transformation of Se in the plant. Understanding and optimizing the critical processes will help to determine the success of phytoremediation of Se (Wang et al., 2014).

Halophytes are plants that can complete their life cycles in relatively high saline environments with NaCl concentrations of at least 200 mM (0.2 M), a condition similar to their natural environment (Flowers et al. 1986 and Flowers and Colmer (2008). The sequential reuse of saline agricultural drainage water has been proposed as part of an environmentally sound method for the reduction of saline drainage effluents, However, the effectiveness of halophytes in reducing drainage volume is dependent on their ability to tolerate extremely high levels of salinity and B over the long term, to maintain high rates of evapotranspiration, and to thrive in saline-sodic conditions with poor physical conditions (Benes et al., 2005). Ideally, it would be valuable not only to identify a halophyte with such characteristics, but one that has economic potential as well. However, the environmental and economic benefit of using such halophytes is related more to their contribution to the overall reduction in drainage water volume requiring disposal or treatment than to their potential as marketable crops.

The halophyte, *salicornia europaea*, grows under extreme salt conditions and it has been explored as a saline agriculture crop (Pasternak D.1990). *Salicornia europaea* plant is considered in general distributed in coastal and interior salt marsh, and it is an annual species of halophyte (Fernald, 1950; Chapman, 1974). Salicornia, also commonly and variably known as sea asparagus, and samphire is a halophyte, belonging to Amaranthaceae family by Patel (2016) and Singh et al. (2014). In fact, salicornia name has arrived from the Latin word meaning ‘salt.' *salicornia europaea* has a geographical distributing and also is known to inhabit interior and coastal salt marshes of North America, Europe, Asia and Africa (Fernald, 1950).

Saline agriculture which implies the application and irrigation of brackish and saline water for the cultivation of crops; the salinity of the irrigation water may vary from low up to that of seawater. Glasswort, *salicornia spp*. has become a favorable, attractive and tasteful saline vegetable crop year-round available in supermarkets. Such salicornia vegetable crop may be irrigated with brackish water and seawater and demonstrates a high-salt tolerance. Remarkably the relative growth rates of this halophytic crop even at low salinity is similar to that of glycophytic species and crops. Based on this trait productivity of seawater-irrigated salicornia could be similar to that of crops in freshwater grown conventional crops (Rozema and Schat, 2013).

Studies report that some species, such as *salicornia europaea* shows tolerance towards salinity as high as 3 % NaCl (Yamamoto et al. 2009). This plant is found at the edges of wetlands, marshes, seashores, and mudflats, actually on most alkaline flats (Smillie 2015). This plant has spongy stalks with diminutive scale-like leaves, inconspicuous flowers, and fruits. Also, the green plant turns orange, pink to reddish in autumn, before dying in winter. Salicornia considered to be the right candidate for reclamation of salt flats, barren lands, and sea-shores. In short, *salicornia* can be considered for seawater agriculture (Katschnig et al. 2013). Most wetland plants are afflicted by widespread damage, including salicornia that contain succulent is easily affected by metal and oil spill stressors. Webb et al. (2012) also used *salicornia europaea* as biofilters to remove nutrients from saline wastewater on marine aquaculture farms. Farzi et al. 2017 reported plants *salicornia europaea, salsola crassa*, and *bienertia cycloptera* would be considered good options for salt phytoremediation. Salinization of agricultural land is an increasing issue, due to their high tolerance to salinity, *salicornia europaea* could become models to study salt tolerance; they also represent promising saline crops. Due to all the above-mentioned reasons, salicornia will also be a suitable halophilic plant to capitalize on its salt tolerance potential for treating marginal waters or pre-treating FGD wastewaters prior to introducing to CWTS.

The present studies were designed to determine ability of s*alicornia europaea* to grow in FGD wastewater or brackish waters (BW); and to determine ability of *salicornia europaea* to remove excess trace elements, nutrients, and salt in highly saline wastewaters.

Materials and Methods

Part I (soil-based study)

Topsoil (0 to 10 cm) used for this study was obtained from Westar Energy's the Jeffrey Energy Center (JEC) in St. Marys, KS. The JEC is located in Emmett Township, Pottawatomie County, approximately 100 miles northwest of Kansas City, Kansas 39°17’10”N 96°07’01”W). The collected soil materials were individually mixed well for homogeneity. They were then gently ground using a ceramic mortar and pestle, sieved through 2 mm-screen, and air-dried. The soil material was analyzed for basic soil properties, such as texture, cation exchange capacity (CEC), pH, and organic matter (OM) content (Combs and Nathan, 1998) using established methodologies (see Table 3.1)**.** *Salicornia europaea* was used in this experiment because it is highly tolerant of salinity, it has favorable biomass properties, and it was available from plant distributors.

Table 3.1 Total element concentrations and soil parameters of topsoil and engineered soil

Total element concentrations and soil parameters	Value
Se, mg/kg	0.4
B, mg/kg	104.2
S, mg/kg	295.6
Mn, mg/kg	569.4
Fe, %	2.2
pH (1:5 soil:water suspension)	7.1
OM† %	5.1
CEC†, cmolc/kg	31.4
Texture (SIC)	7.7, 50.5, 41.8

†OM= organic matter; CEC=cation exchange capacity; SIC= Silty Clay

The greenhouse study was conducted under controlled environmental conditions at Kansas State University in Manhattan, Kansas. The study was conducted using *salicornia europaea* and five treatments, each replicated four times and arranged in a complete randomized block design: tap water 100% (control) (TW), brackish water (BW)100%, Flue Gas Desulfurization water (FGD)100%, Hoagland solution (MHS) and brackish water (BW) (50:50 v/v), Hoagland solution (MHS) and FGD water (50:50 v/v) (see Table 3.2). To ensure growth, pots were watered every two days (500 mL per pot) (see Table 3.3). A 15 hours (h) daylight and 9 h dark photoperiod regime was maintained with overhead sodium lights (150 Wm^{-2}). The temperature during the study was within the range of 17 to 23.9°C. The positions of treatment pots in the greenhouse were rotated weekly to secure that all pots received equal amounts of light.

Harvesting and Sample Preparation

Aboveground biomass (shoots) were harvested at 1 and 2 months after initiation of the treatments. Shoots were washed thoroughly with tap water and then deionized water, removing any adhering soil particles. Shoots were weighed, labelled, then placed in brown paper bags. Plant shoots were dried in a Fisher Scientific Isotemp oven (Marietta, OH) at 75 °C for 5 days or until reaching a constant weight before determining the dry matter yield, expressed as grams of dry matter (DM) per pot. Dried plant shoots were then ground in a stainless-steel Wiley mill (Swedesboro, NJ) and the powdered plant material stored in polyethylene vials until digested.

Chemical Analysis

Five-gram samples of ground plant material weighed into Teflon tubes were placed in microwave according to method EPA 3052A (CEM, Mathews, NC) accelerated reaction system, then the digestion unit was ramped up to 200°C and held for 15 minutes (1600 W @ 100%, 15-minute ramp time, 15-minute cooling time). Digested plant material was filtered with Whatman 42 filter papers into capped plastic scintillation vials under the fume hood. Samples were then stored at 4°C until analyzed for Na, P, Mo, Se, S and B on an inductively coupled plasma optical emission spectroscopy and inductively coupled plasma-mass spectrometer equipped (ICP-OES and ICP-MS). Standard plant reference materials (Pine Needles) from the National Institute of Science and Technology (NIST, Gaithersburg, MD) were 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 used for QA/QC where appropriate. Total N using a sulfuric peroxide digest (Matsunaga and Shiozaki, 1989).

The total elementals concentration in the soil was determined using a 30% hydrogen peroxide (H_2O_2) and aqua regia digestion procedure as described in Premarathna et al. (2010). First, air-dried samples were finely ground with an agate mortar and pestle. Then, 0.5 g of soil was added to a digestion tube and predigested with 0.5 mL of H_2O_2 for 10 min at room temperature followed by adding another 2.5 mL of H_2O_2. Pre-treated samples were allowed to react overnight at room temperature. Then, the tubes were heated on a digestion block at 90°C until the volume was reduced to ~1 mL. After the tubes were cool at room temperature, the soils were digested using 5 mL of aqua regia by ratio [1:3 (v/v) HNO_3/HCl] following the soil digestion procedure (75°C for 30 minutes, 100°C for 30 minutes, 110°C for 30 minutes, and 140°C until the acid volume decreased to ~1 mL). A 0.1% HNO_3 solution was then added until the total volume reached 25 mL. All samples were digested in duplicate. In each batch of digestion, three blanks and three samples of standard reference soil material (NIST 2711a-Montana soil) were included as a QA/QC control. Finally, the digested solution was filtered through a Whatman no. 42 filter paper. Prior to analysis on the ICP-MS-DRC, digested filtrates were further filtered through 0.2μm to protect the instrument by preventing finer solids from entering the system. The recovery of NIST 2711a was about 84%, and the spiked recoveries were in the range of 93 to 107%.

Analysis of Flue Gas Desulfurization Wastewater

Before analyzing the FGD wastewater and effluent samples on the ICP-MS-DRC, it was highly recommended to digest, filter, and acidify the effluent further. About 45 mL of aqueous effluent was digested with 3 mL of trace metal grade concentrated HNO_3 and 2 mL of HCl acids in a microwave digestion unit (MARSX press, CEM Corporation, Matthews, NC), following the EPA-3015A method (USEPA, 2007). This helped to minimize clogging of the ICP-MS nebulizer from a considerable amount of total dissolved solids in the FGD wastewater. Three blanks (acidified Milli-Q water only) and three replicates of NIST 1643e “Trace Elements in Water” SRM were analyzed as quality assurance/quality control (QA/QC) assessment. The recovery percentage was in the range of 82 to 116%. In addition, spiked recoveries were assessed by adding known amounts of a multi-element standard to randomly selected effluent samples. Recoveries for the spiked samples were found to be 84 to 117%.

Table 3.2 Water quality compositions among treatment solutions

Parameter	Units	FGD Wastewater	Tap Water	Brackish water
pH	pH units	6.8 ±0.018	8.72	-†
EC	mS/cm	11.2 ± 0.118	0.22	-
NH_4-N	mgL^{-1}	219.2±1.766	0.51	-
NO_3-N	mgL^{-1}	27.4± 0.47	0.29	-
NO_2-N	mgL^{-1}	6.57±0.029	-	-
Cl	mgL^{-1}	2038.39± 47.23	57.83	8140 mgL^{-1}
Ca	mgL^{-1}	1314.08± 24.2	13.75	-
Mg	mgL^{-1}	-	14.51	-
Na	mgL^{-1}	1395.20± 27.6	31.01	5270 mgL^{-1}
K	mgL^{-1}	243.93±9.81	7.92	-
S	mgL^{-1}	903.88±25.3	18.74	-
B	mgL^{-1}	13.55± 0.067	-	-
P	mgL^{-1}	2.48±0.47	-	-
Fe	mgL^{-1}	1650±118	-	-
Se	μgL^{-1}	148± 3.8	-	-
Zn	μgL^{-1}	10 ± 0.3	-	-
Mn	μgL^{-1}	1.9 ±0.3	-	-
Cu	μgL^{-1}	6.5 ± 0.2	-	-
Mo	μgL^{-1}	204±0.5	-	-

† Represents non-availability of the data

Table 3.3 Total of water used (L) and concentration of the constituent of interest (mg per L) through period study.

	Total volume of water used (L) *	Con. of a constituent of interest (mg L^{-1}) ‡	1 day††	unit	30 days††
NH_4-N	0.5	219.2	109.6	mg L^{-1}	3288
NO_3-N	0.5	27.4	13.7	mg L^{-1}	411
NO_2-N	0.5	6.57	3.3	mg L^{-1}	98.6
Cl	0.5	2038.4	1019.2	mg L^{-1}	30575.9
Ca	0.5	1314.08	657.04	mg L^{-1}	19711.2
Mg	0.5	-†	-	-	-
Na	0.5	1395.2	697.6	mg L^{-1}	20928
K	0.5	243.9	121.9	mg L^{-1}	3659
S	0.5	903.9	451.9	mg L^{-1}	13558.2
B	0.5	13.6	6.8	mg L^{-1}	203.3
Se	0.5	0.148	0.074	mg L^{-1}	2.22
Fe	0.5	1.65	0.83	mg L^{-1}	24.8
Zn	0.5	0.01	0.005	mg L^{-1}	0.15
Mn	0.5	0.0019	0.00095	mg L^{-1}	0.03
Cu	0.5	0.0065	0.0033	mg L^{-1}	0.10
Mo	0.5	0.204	0.102	mg L^{-1}	3.06
P	0.5	2.25	1.3	mg L^{-1}	33.7

† No data

* The total volume of water used in the greenhouse every two days

‡ Concentration of elements in wastewater

†† Total concentration of elements in wastewater that watered the plants every day and on the 30th day

Part 2 (hydroponic study)

An experiment was designed to determine if the composition of water used in a hydroponic system influenced the germination, survivability, and seedling height of *salicornia europaea* (Schmitz, E., 2018). This study was continued to determine the uptake of trace elements by S. europaea. In the study, two sources of industrial wastewater were used as well as Modified Hoagland’s Solution (MHS) and tap water. Industrial wastewater was obtained from two sources: FGD wastewater and Cooling tower blowdown water (CTBW) collected from cooling tower 1 located north of Seaton Hall on KSU Manhattan Campus.

Each experimental unit consisted of a 2 x 5-inch block of 1 x 1-inch Grodan rockwool cells placed inside of a clear plastic container (2.17 x 6.85 x 4.8 inches). The rockwool had a quarter inch circular depression in the center of the block for seed placement (Figure 3.1). One *salicornia europaea* seed was placed inside the center of each rockwool cell, for a total of 10 *salicornia europaea* seeds per experimental unit. To begin the experiment, 500 mL of treatment solution was added to each experimental unit. The experimental units were arranged in a randomized block design.

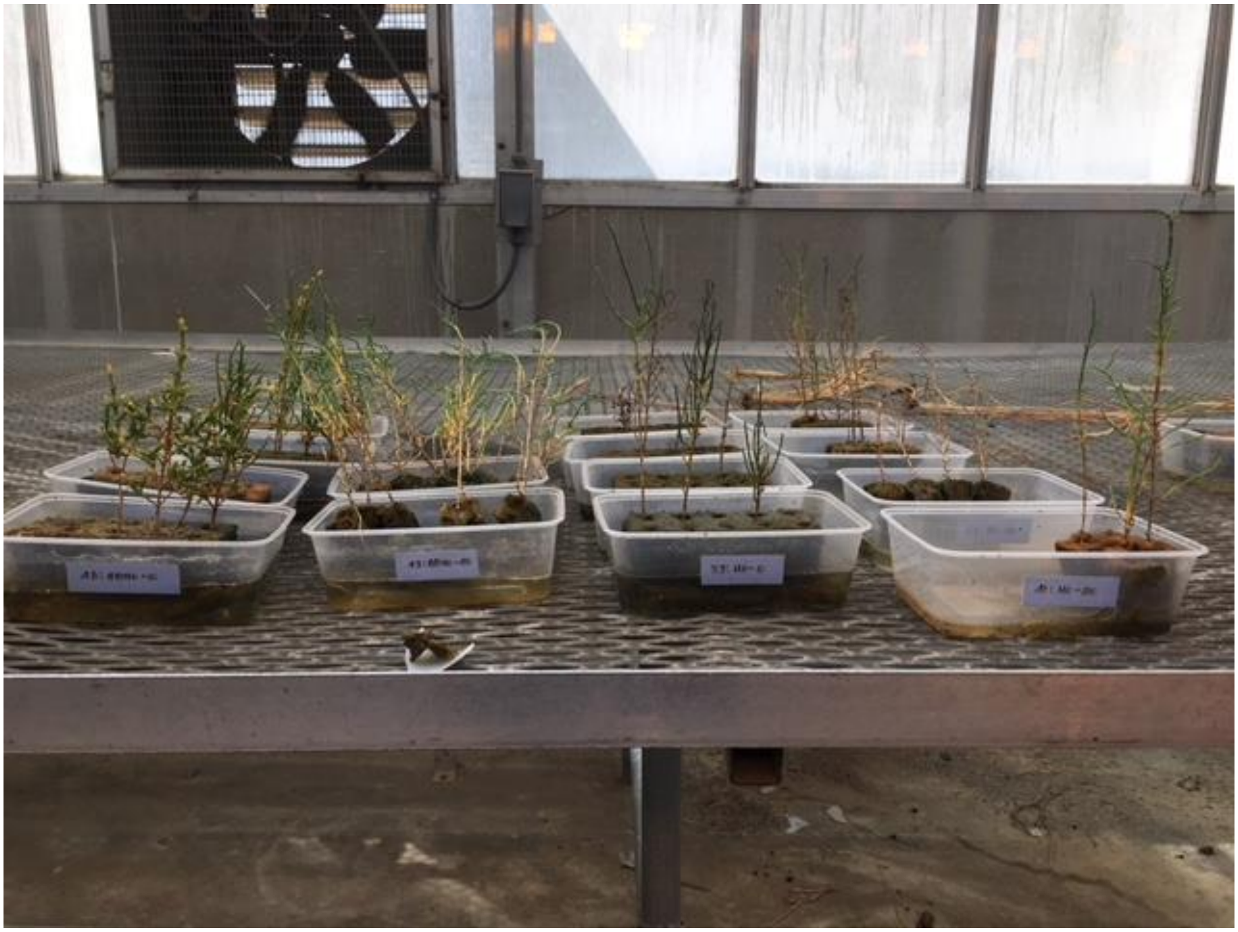

Figure 3.1 An example illustrating an experimental unit on day 65

The growth conditions of the growth chamber were set to a 16-hour day and 8-hour night photoperiod with a daily maximum temperature of 25°C kept for 8 hours and night minimum temperature of 20°C kept for 8 hours with a 4-hour transition period. After 21 days in the growth chamber, the experimental units were moved into the greenhouse, where the same measurements were taken with the exception of chlorophyll until day 65. After day 65, all experimental units were watered with DI water every 2-3 days and with MHS every two weeks. The shoots and the roots were harvested after day 65, then washed first with tap water and then DI water before drying five days at 75°C in a drying oven. Total dry biomass was determined as the percentage of dry weight of a known fresh sample. There was not enough plant material for harvest in some treatment units. Dried tissue samples were digested in nitric acid digestion (10 mL HNO_3) in a microwave accelerated reaction system, as mentioned above. Samples were then stored at 4°C until analyzed for Na, P, Mo, Se, S and B on an ICP-OES and ICP-MS. Total N using a sulfuric peroxide digest (Matsunaga and Shiozaki, 1989).

Statistical Analysis

Soils and plants data were analyzed using the PROC GLM Procedure in SAS (version 9.3, 2011; Cary, North Carolina). The experimental design was completely randomized block design for greenhouse which were then arranged in a randomized block design for hydroponic system. Data were analyzed using ANOVA for pairwise comparison of means before building a final model. The Tukey Pairwise Method was used as a comparison with all treatments at a 0.05 level of significance.

Result and discussion

Part I (soil-based study)

Biomass

Biomass was significantly affected by the treatments, and the magnitude of this effect changed over time (Figure 3.2). Also, all treatments showed significant deviation from the control. Biomass production in our experiment varied greatly between the treatments most likely because of differences in the amount of trace element, nutrients and/or other salts in each treatment. Plant biomass was mainly affected by all treatments: Hoagland 50% with Brackish water 50% had the highest overall final biomass, followed by Hoagland 50% and FGD 50%; Brackish water; FGD wastewater and Tap water, respectively. Concerning the treatment FGD wastewater and Brackish water, while 3-treatments were intermediate (Figure 3.2), untreated pots had less biomass than treatment pots did. The lowest biomass was observed in the FGD 100%; it was not clear if the decline in biomass was due to a salinity accumulation effect. The plant studied in this research tolerated a broad spectrum of salty water and wastewater. The results reveal that the capabilities of this salicornia halophyte showed hopeful results, as it could lower water salinity and concentrations of the other dominant minerals in the water and soil. Biomass of *salicornia europaea* plants was significantly higher with salt concentration; Ungar (1982) showed a high correlation between soil water potential and leaf water potential, indicating that *salicornia europaea* was able to adjust its water potential to more than –1 MPa, indicating that *salicornia europaea* can grow in more saline areas. Our results suggested *salicornia europaea* plant could grow in wastewater (FGD) as on the saline soil.

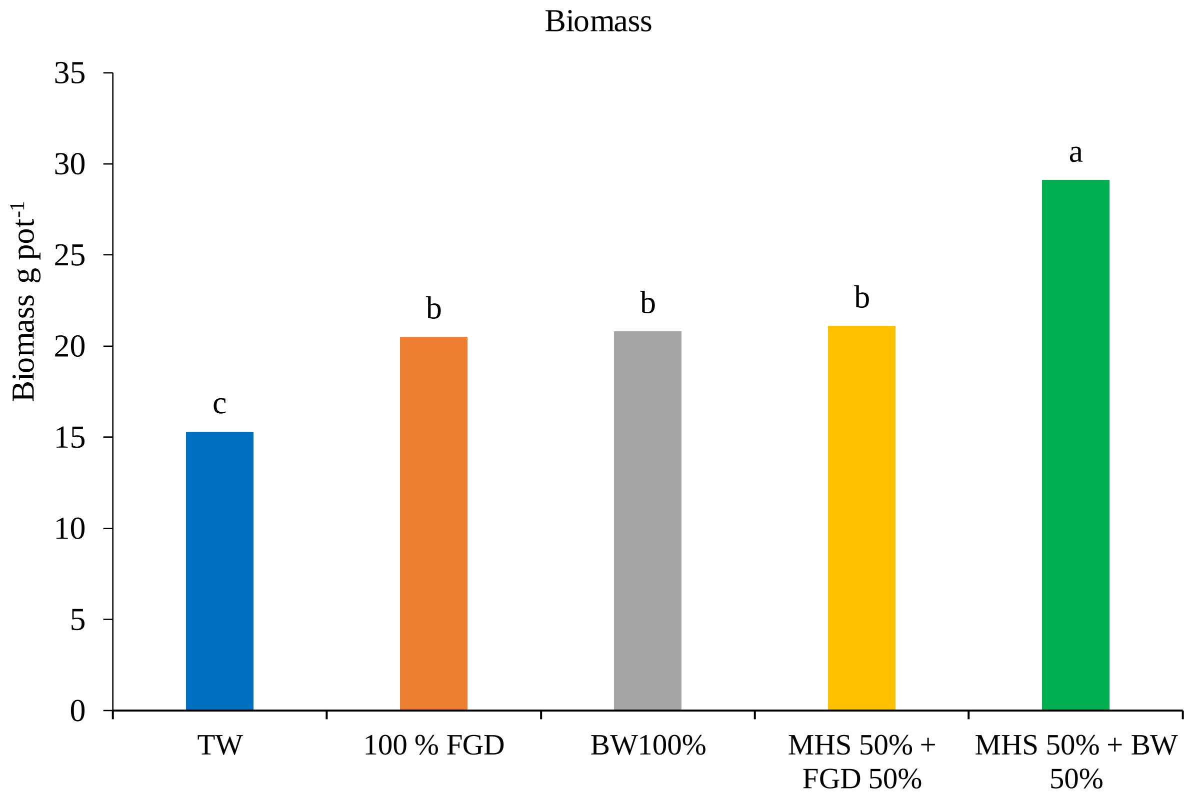

Figure 3.2 Total biomass (g pot^{-1}) after two cuttings of *Salicornia*. TW= Tap water; 100% FGD= Flue Gas Desulfurization wastewater; BW 100%= brackish water; MHS 50% and FGD 50%= Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v), Means with different letters are significantly different (LSD, α=0.05).

Selenium and Boron concentrations in *Salicornia europea*

Selenium and B concentrations of *salicornia europaea* increased progressively and significantly with increasing levels of Se and B in the soil from treatments (Figure 3.3). Webb et al. (2012) used *salicornia europaea* as a biofilter to remove nutrients from saline wastewater on marine aquaculture farms. *Salicornia spp*. has been suggested as a potential phytoextractor of salts and nutrients (Ozawa et al., 2010); Brown et al. (1999) used the halophytic species *Suaeda esteroa*, *salicornia bigelovii*, and *Atriplex barclayana* as biofilters to remove nutrients from the saline wastewater of marine shrimp farms. Figure 3.3 shows trace elements (Se and B) concentrations in *salicornia europaea* tissue during a study that were significantly higher in 100% FGD and 50% FGD treatments than in other treatments. Selenium concentration in salicornia shoots grown in 100%FGD and 50%FGD+ 50% Hoagland treated soils at 4 wk, and 8wk cuttings were 818%, 548%, 1332%, and 730%, respectively, higher than that of the shoots grown in the control pots, respectively. Hence, Se may accumulate to potentially toxic concentrations for ruminants even though the competitive effects of high-sulfate soil water usually reduce the uptake and accumulation of selenate, the dominant and plant available form of Se in the field environment (Grattan et al., 2008 and Tanji et al., 1988). Boron concentration in salicornia shoots grown in 100% FGD, and 50% FGD+ 50% Hoagland treated soils at 4 wk, and 8wk cuttings were 50.6 %, 60.2 %, 277 %, and 224 % higher than that of the shoots grown in the control pots, respectively.

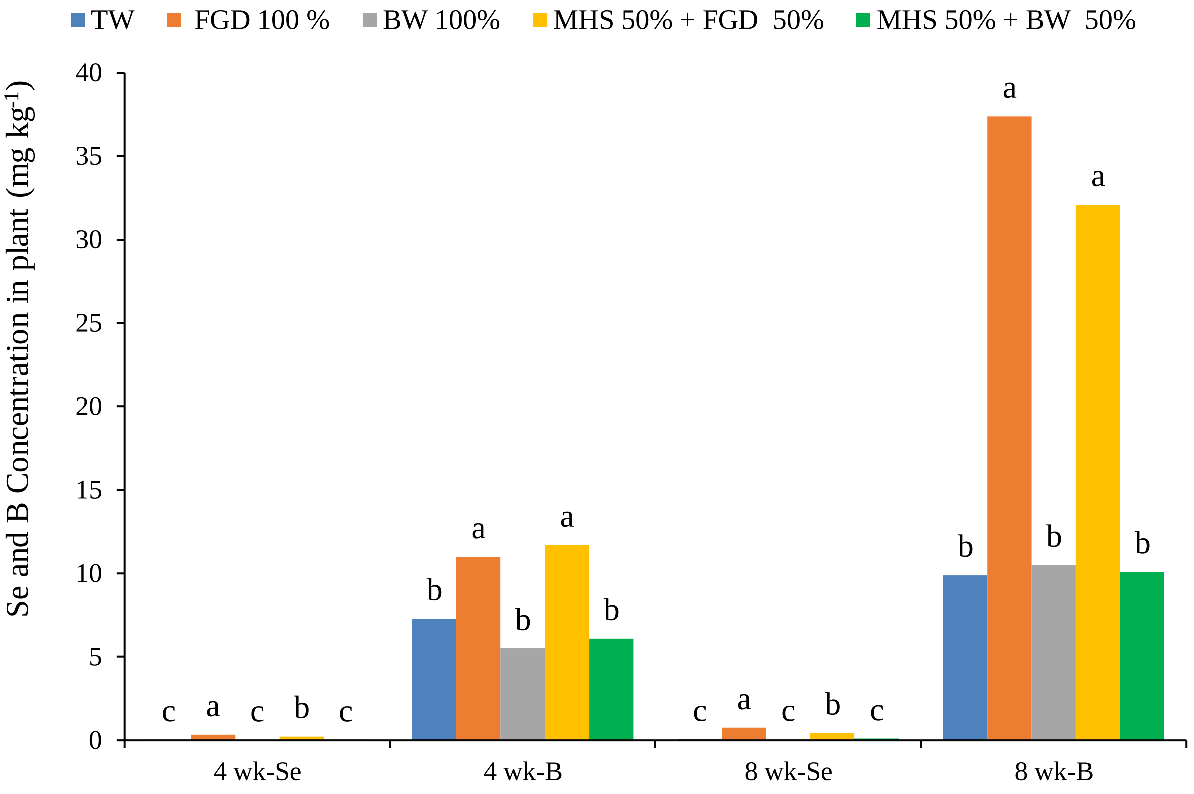

Figure 3.3 Selenium and B concentration in Salicornia mg kg^{-1} (dry weight basis) in two cuttings. TW= Tap water; 100% FGD= Flue Gas Desulfurization wastewater; BW 100%= brackish water; MHS 50% and FGD 50%= Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v), Means with different letters are significantly different (LSD, α=0.05).

Figure 3.4 shows total Se uptake by *salicornia europaea* tissue during the study that were significantly higher in 100 % FGD and 50% FGD treatments than the other treatments. According to these results, 4 weeks and 8 weeks Se uptake in salicornia shoots grown in 100% FGD and 50% FGD + 50% Hoagland treated soils were 833%, 500%, 1600% and 900%, higher than that of the shoots grown in the control pots, respectively. For instance, the *salicornia europaea* growing in the treated wastewater (FGD100% and FGD 50% saline wastewater) was effective at removing Se, they were 27.96% and 32.50%, respectively.

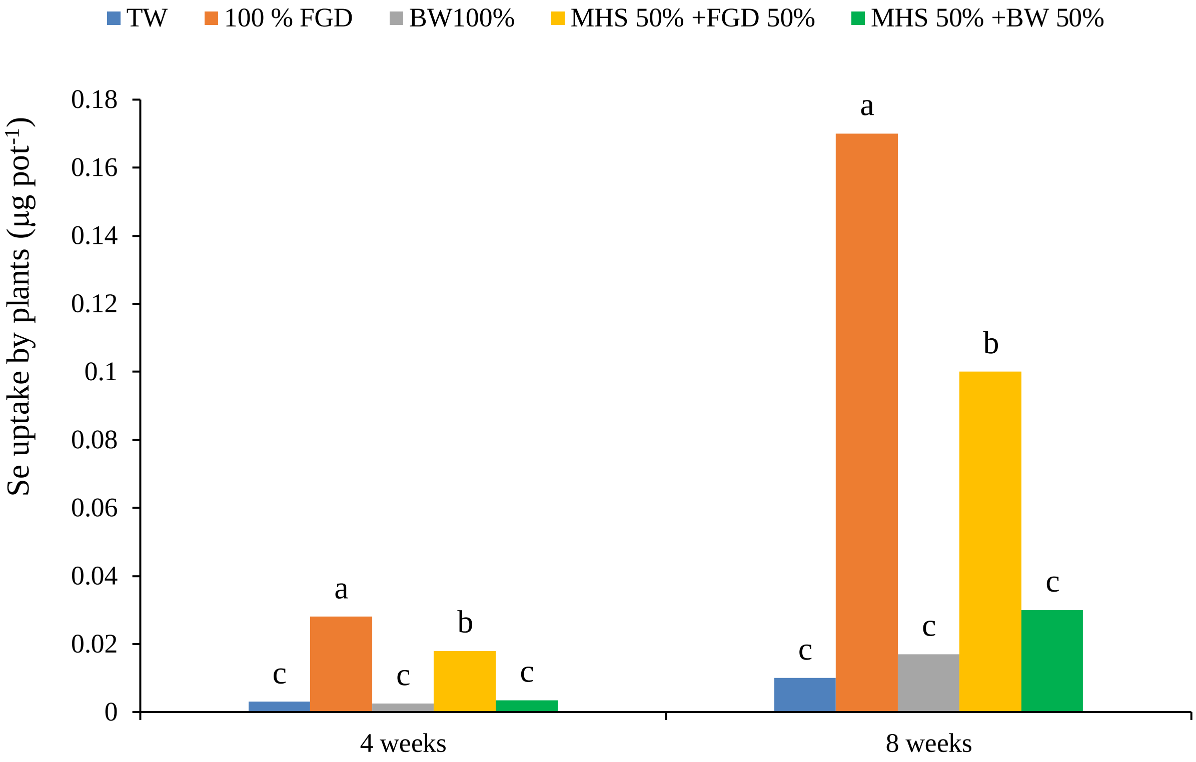

Figure 3.4 Total Se uptake by *Salicornia* (µg pot^{-1}). TW= Tap water; 100% FGD= Flue Gas Desulfurization wastewater; BW 100%= brackish water; MHS 50% and FGD 50%= modified Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v). Means with different letters are significantly different (LSD, α=0.05).

Boron uptake in *salicornia europaea* grown at 4 weeks and 8 weeks are presented in Figure 3.5. However, the greatest B uptake was observed for the FGD 100%. This was followed by 50% FGD. On the other hand, increasing B uptake at the 4- and 8-weeks harvest by salicornia grown in 100% FGD and 50% FGD+ 50% Hoagland treated soils were 55%, 51%, 453%, and 406% higher than that of the shoots grown in the control pots, respectively. However, the greatest B uptake was observed for the FGD 100% and FGD 50%, except other treatments did not uptake a significant amount, B in the FGD 100% and FGD 50% were retained in plant tissues 11% and 15.99%, respectively. The ability of *salicornia europaea* to passively accumulate total Se and B concentrations when there is a lack of new growth also appears to be evident, to a degree, with periods of growth. In phytoextraction, Se is absorbed by plant roots and translocated to the shoot, where it may be harvested and removed from the contaminated site.

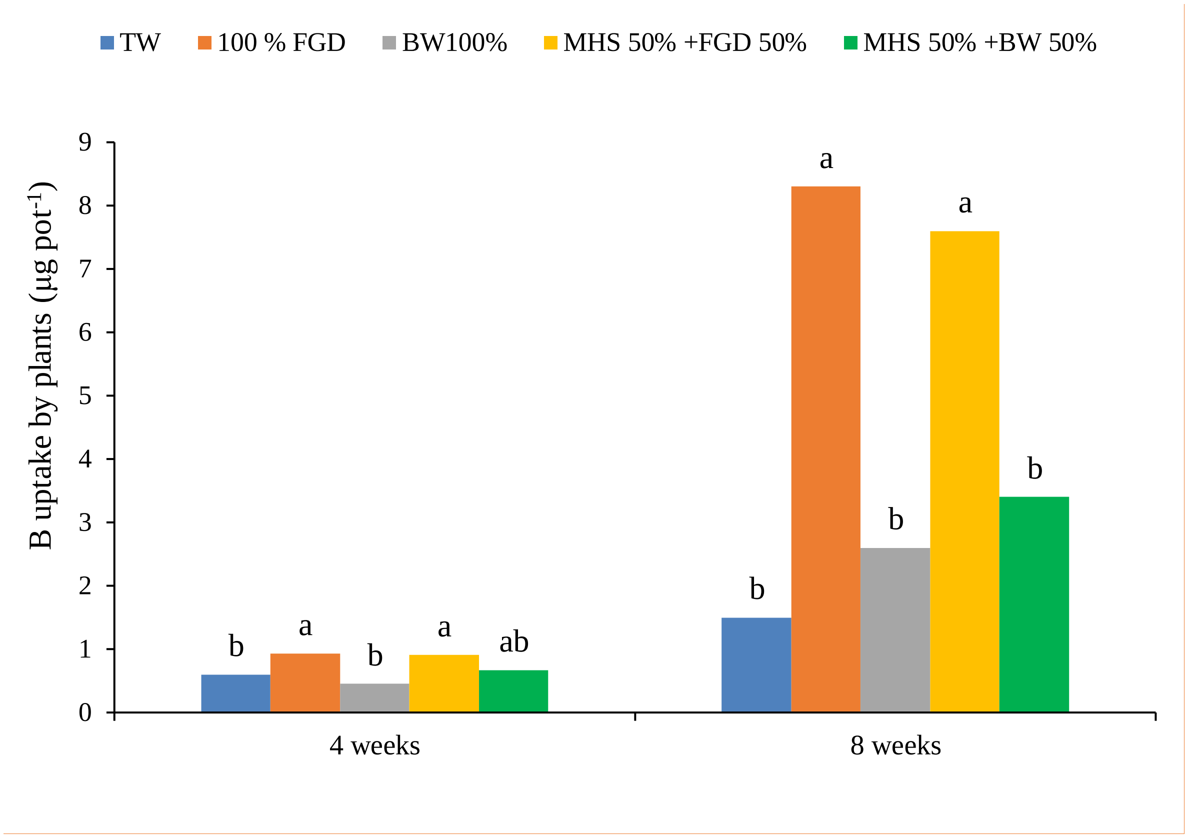

Figure 3.5 Total B uptake by Salicornia (µg pot^{-1}). TW= Tap water; 100% FGD= Flue Gas Desulfurization wastewater; BW 100%= brackish water; MHS 50% and FGD 50%= Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v). Means with different letters are significantly different (LSD, α=0.05).

Element concentration and uptake in *Salicornia europaea* shoots

Being a halophyte, *salicornia* has a remarkable ability to accumulate salt in its tissue with increasing soil salinity as a means of adjusting to the low osmotic potential in the soil water. According to these results over the time of the experiment, total N (as %) accumulation in plant tissue (estimated from N content, growth of plant shoot tissues and harvested biomass) was significantly higher under high salt conditions (Figure 3.6). Lin et al. (2005) observed removal rates of 17 mmol N m^{-2} d^{-1} and Konnerup et al. (2011) reported a removal rate of 27 mmol m^{-2} d^{-1}. The majority of nitrogen removal in wetlands is thought to result from the microbial processes of nitrification and denitrification and to a lesser degree, sedimentation, filtration, precipitation, and vitalization (Webb et al., 2012).

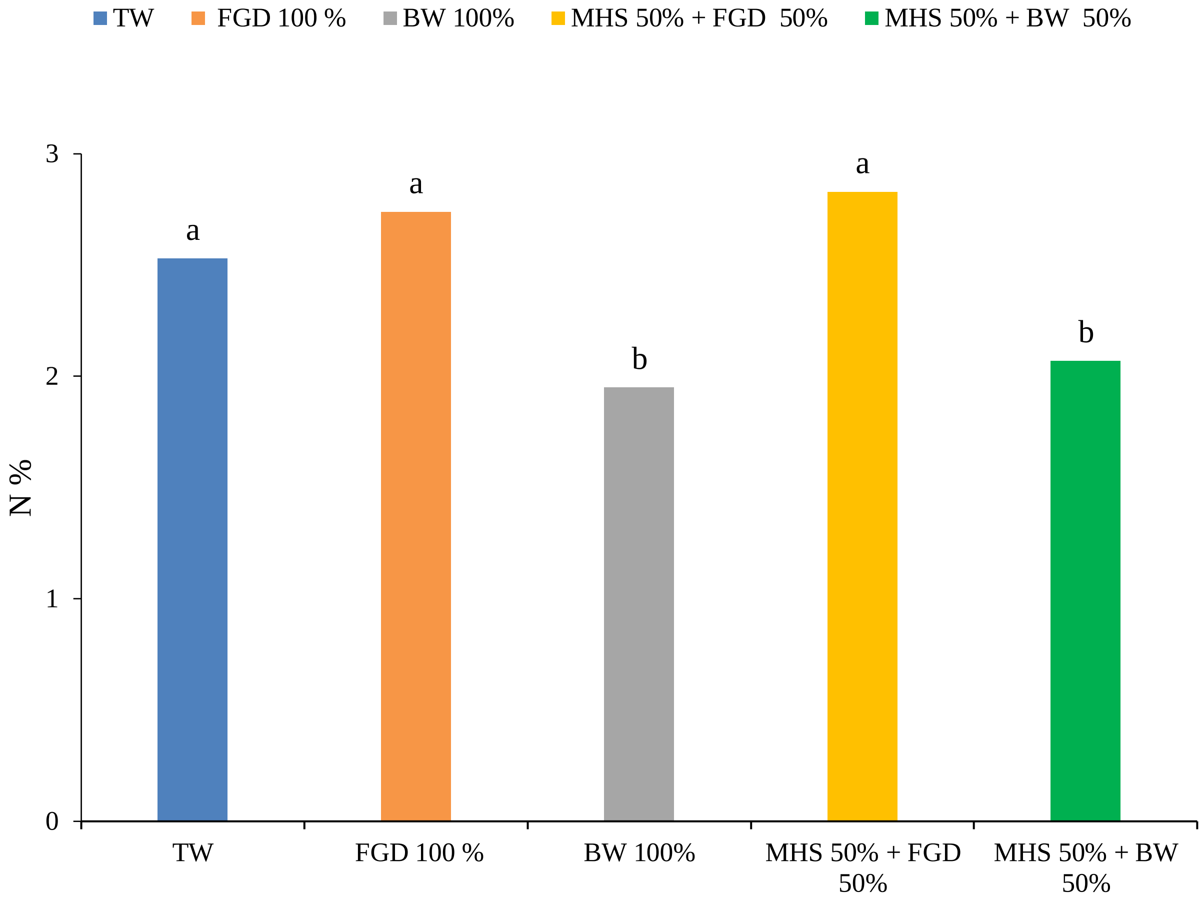

Figure 3.6 Total N % in *Salicornia*. TW= Tap water; 100% FGD= Flue Gas Desulfurization wastewater; BW 100%= brackish water; MHS 50% and FGD 50%= Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v), Means with different superscripts are significantly different (LSD, α=0.05).

Currently, the research available on the uptake capacity of *salicornia* spp. is sparse. In the present study, the highly effective removal of nitrogen by *salicornia europaea* may reflect an adaptation in plants that have evolved to compete for and sequester nitrogen in nitrogen-limited environments. Jefferies (1977) reported that nitrogen availability in salt marshes varies throughout the year as well as spatially in the environment. Phases of vegetative growth and increased growth in *salicornia europaea* shoots are preceded by peaks in available N. Webb et al. (2012) worked with *salicornia europaea* plants that were irrigated daily. This study observed an increase in above-ground growth when the N concentration was increased. Plants produced harvestable tips every eight weeks during the study period when grown in wastewater with high N concentration. Therefore, the results of the present study indicate a significant potential to increase N removal from wastewater. This causes the *salicornia europaea* increased growth and N uptake mechanism A relationship between variables such as N form, salinity, light, and temperature is established so that ideal growth conditions can be provided. Studies that have evaluated the performance of *salicornia europaea* in CWs reflect high N removal efficiency produces a regular supply of fresh succulent tips obtained by repeatedly harvesting (cropping) during the growing season (Webb et al. 2012).

In addition, the water concentrations of Na, S, P, and Mo were measured before and after the experiment. Table 3.4 shows that the plants, at four weeks and eight weeks, significantly reduced the concentrations of these elements: Na, S, and P. When compared to the untreated water, Na concentrations were reduced in the treated water by experimental plant species (Table 3.4). Also, slight reductions in Na concentration occurred in the control treatment which could be caused by soil absorption. However, there were significant differences in reductions of Na concentrations between plants in wastewater and the control treatment. In this study, *salicornia* showed promising capability to reduce water salinity and concentrations of the other dominant minerals in the wetlands. However, plant behavior during the growing period is an important variable to consider in regard to salt concentration. Shelef et al. (2012) reported that accumulation of salt occurred in greater concentrations in the leaves of the aerial organs compared to the stems. Moreover, as plants age and approach the flowering stage, the leaves decrease in size (Shelef et al. 2012), and the mechanism of salt tolerance changes from the accumulation of salt to inhibition of absorption. Over the same period, the wastewater had a significantly higher total P accumulation in plant tissue compared to the control (table 3.4). The HMS50%+BW50% treatment displayed significantly increased uptake of phosphorus than the other treatments. Lower P concentrations were seen in plants grown in FGD100%. Webb (2005) found that *salicornia* only had growth response to increasing P when treated with excess N supply, suggesting that P is not limiting to growth. In plant tissue, total sulfur concentrations were more than a magnitude higher than the control. Regardless of concentration, plants treated with FGD high in sulfate contained substantially more sulfur than plants in their corresponding MHS 50% and FGD 50% treatments. This outcome demonstrates that increasing salinity results in increased sulfate concentration when plants are treated with FGD. *Salicornia* is arguably one of the most salt-tolerant vascular plants (Ayala and O’Leary, 1995).

Table 3.4 P, S and Na concentration mg kg^{-1} and uptake in Salicornia µg kg^{-1} (dry weight basis) in two cuttings. TW= Tap water; 100% FGD= Flue Gas Desulfurization wastewater; BW 100%= brackish water; MHS 50% and FGD 50%= Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v). Means with different letters are significantly different (LSD, α=0.05).

Treatments	4 weeks						8 weeks						pH
	P		S		Na		P		S		Na		
	Conc.[†]	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	
			……………………………mg kg^{-1} and µg kg^{-1} ………………………………										
TW	890b	0.07c	4342bc	0.36bc	11549c	0.95c	823a	0.13c	8710b	1.37c	35370e	5.49c	8.2^{a}
FGD100%	789a	0.08a	6506a	0.55a	14789c	1.24bc	721bc	0.16bc	19514a	4.35a	63575c	14.1cb	7.5^{ab}
BW100%	1039a	0.08bc	3473bc	0.28c	20119b	1.64b	786bc	0.19b	5501c	1.35c	79351b	19.7b	7.7^{ab}
HMS50%+FGD50%	1023a	0.18c	5429ab	0.43ab	14068c	1.12c	792ab	0.19b	12331b	3.36ab	51242d	12.7c	7.1^{bc}
HMS50%+BW50%	967ab	0.11b	2970c	0.33bc	24592a	2.268a	703c	0.24a	7341bc	2.53bc	89782a	30.9a	6.4^{c}

[†] Conc.= concentrations of elements in plant tissue.

Using the results, total Mo concentration and uptake in plant tissue were graphed and analyzed (Figure 3.7 and 3.8). The high wastewater Mo concentrations caused high Mo concentrations in the plants that were 8 weeks grown. The uptake pattern of Mo in the plant that was grown in FGD 100% and 50% displayed a clear trend. Plant Mo uptake affected the standing biomass in almost all treatments; the highest final plant uptake at 4 weeks was shown by FGD wastewater 100%, followed by FGD 100% wastewater, Hoagland 50% with FGD 50%, Hoagland 50% with Brackish water 50% at 8 weeks. FGD 100% wastewater was the exception, showing the highest overall final plant uptake for both at 4 and 8 weeks (Figure 3.8).

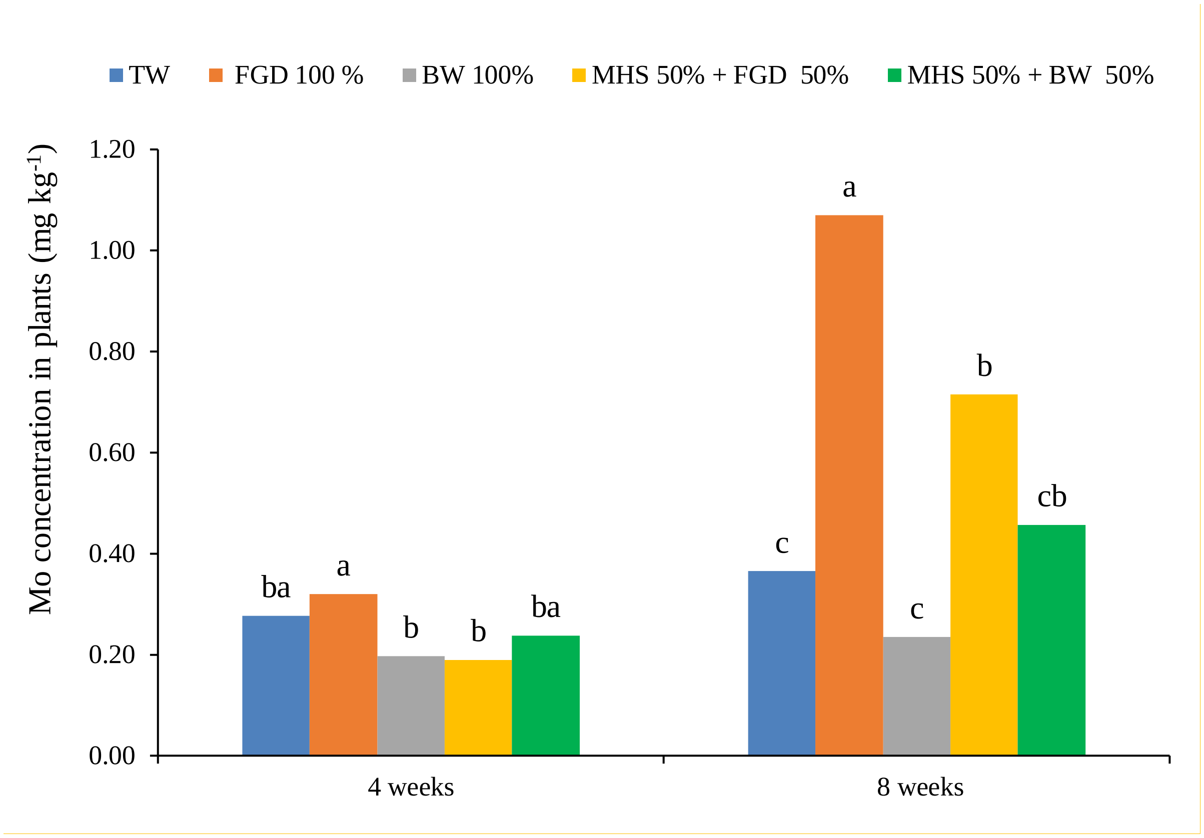

Figure 3.7 Mo concentration in *Salicornia* mg kg^{-1} (dry weight basis) in two cuttings. TW= tap water; 100% FGD= Flue Gas Desulfurization wastewater; BW 100%= brackish water; MHS 50% and FGD 50%= Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v). Means with different superscripts are significantly different (LSD, α=0.05).

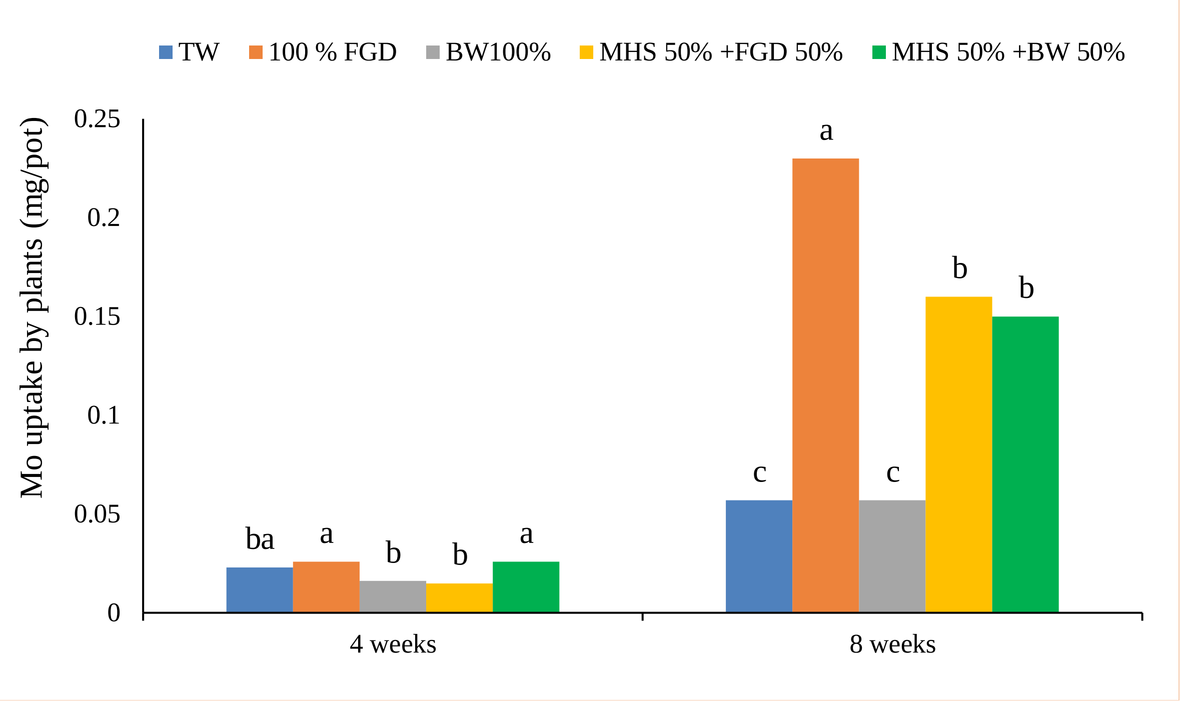

Figure 3.8 Mo uptake in *Salicornia* (µg kg^{-1}) dry weight basis in two cuttings. TW = tap water; 100% FGD = Flue Gas Desulfurization wastewater; BW 100% = brackish water; MHS 50% and FGD 50% = Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v). Means with different superscripts are significantly different (LSD, α=0.05).

Soil analysis

The concentration of Se in the influent wastewater that was delivered into the soil (in 8 weeks) was 2.22 mg L^{-1}. In the soil, Se concentrations found in soil at the FGD 100% and FGD 50% treatments varied from 0.75 to 0.79 mg kg^{-1} (Figure 3.9). Salicornia grown in the 100% FGD and FGD 50% took up a great portion of Se, ranging from 31.4% and 75.2% respectively. The soil accumulated a B concentration of 203.25 mg L^{-1} from the wastewater. B concentrations in soil treated with FGD 100% and FGD 50% wastewater range from 44 mg kg^{-1} to 48 mg kg^{-1} (Figure 3.10). The soil retained 40% and 80% of B for FGD 100% and FGD 50% respectively. Galkaduwa et al. (2017) reported that Se in the FGD wastewater was strongly (i.e., more or less irreversibly) retained by soil materials under reduced conditions, regardless of the soil type. Another study, Liang et al. (2017) mentioned when the Se input into soil is constant, a high concentration of total Se usually indicates that the soil has a strong ability to retain Se as well as low Se bioavailability. Galkaduwa et al. (2017) explored the transport characteristics, retention capacity, and transformation of selenium and other FGD constituents in the Constructed Wetland Treatment Systems (CWTS). This study reported complete retention of Se in the soil by effluent analysis, showing that Boron was partially retained in the soil.

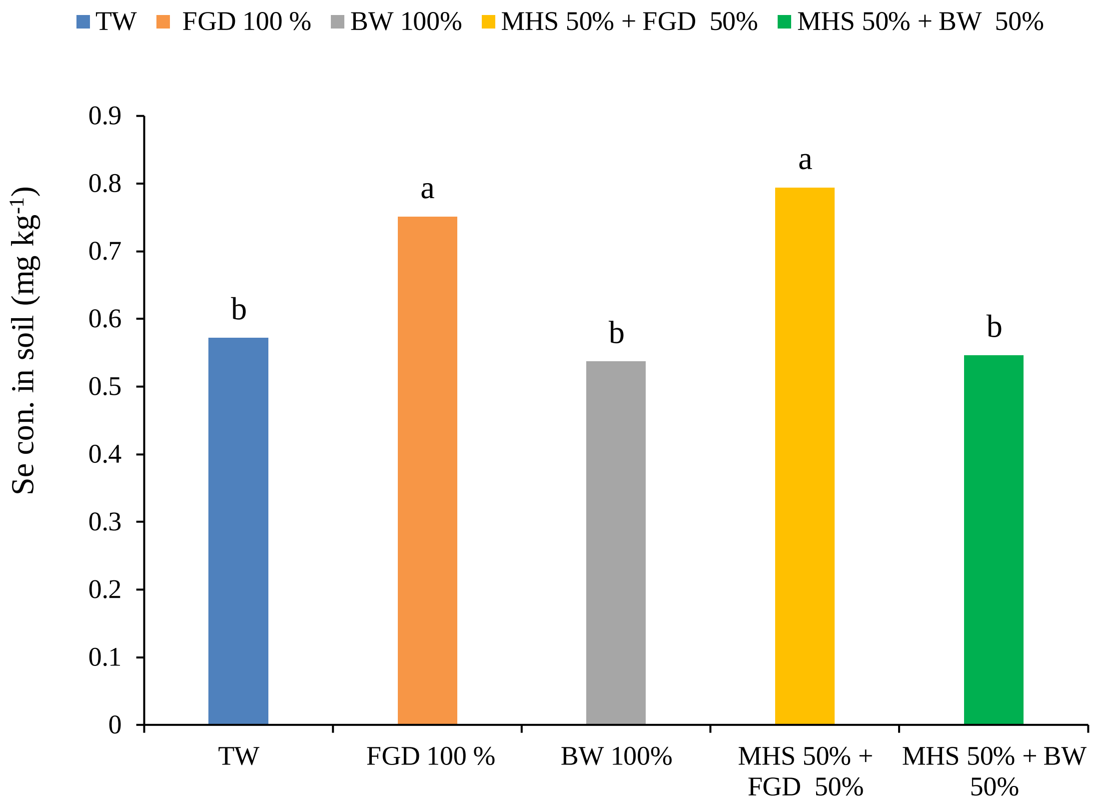

Figure 3.9 Se concentration mg kg^{-1} in soil after harvest. TW = tap water; 100% FGD = Flue Gas Desulfurization wastewater; BW 100% = brackish water; MHS 50% and FGD 50%= Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v), Means with different superscripts are significantly different (LSD, α=0.05).

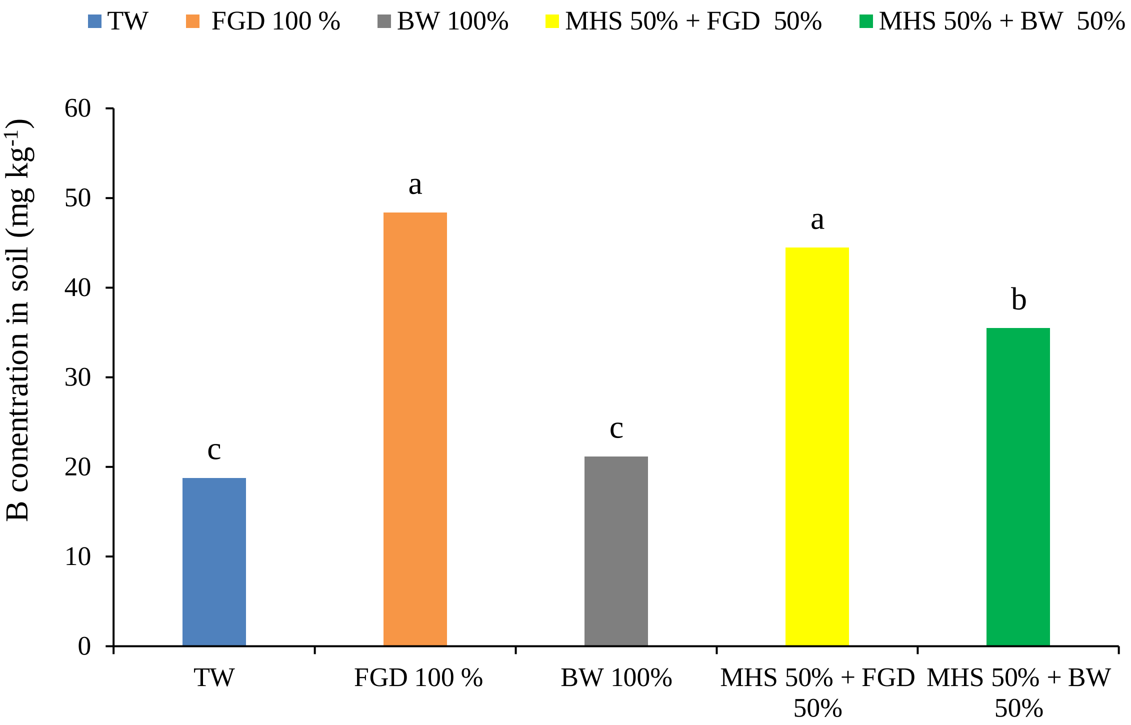

Figure 3.10B concentration mg kg^{-1} in soil after harvest. TW = tap water; 100% FGD = Flue Gas Desulfurization wastewater; BW 100% = brackish water; MHS 50% and FGD 50% = Hoagland solution and FGD (50:50 v/v); MHS 50% and BW 50% = Hoagland solution and brackish water (50:50 v/v). Means with different superscripts are significantly different (LSD, α=0.05).

Part 2 (Hydroponic study)

Biomass

A hydroponic system supplied with different concentrations of wastewater was used to evaluate units of *salicornia* which stimulate potential commercial civilization. Increasing the wastewater content also increased accumulated yield (roots and shoots). This was more apparent in BD and BD-20% FGD, especially when a plant was treated with BD 100% (Figure 3.11) and the yield accumulation was not affected by the salinity level of the irrigation solution. Dry biomass was not significantly affected by increasing the wastewater content in the growth medium. Although the ecotypes clearly differed in their dry matter, the highest dry shoots were observed in BD 100% and the lowest amount of biomass seen in MHS 100%. The highest dry roots were observed in the BD 100% treatment and the lowest amount of biomass in MHS 100%. There was a significant increase in salinity within the root fresh mass. In the root, the BD 100% treatment was significantly higher compared to treatment at BD-20% FGD, MHS-20% FGD, and MHS-0% FGD. According to Aghaleh et al. (2009), in species of *salicornia,* absolute dry mass values ranged from 2.3 mg plant^{-1} for *salicornia europaea* at 200 mM NaCl in the root medium for 45 d to 13.8 g plant^{-1} for *salicornia europaea* at 300 mM NaCl in the root medium for 45d. As might be expected, environmental factors also influence the shape and height of the growth response curve. Contributing factors include the availability of nitrogen and other minerals in the culture medium (e.g., Ca^{2+}) L as well as light intensity (Barbour 1970). Furthermore, differences in the experimental treatments influence the recorded growth (e.g., treatment period, starting age of plants) because differences in biomass between salinity treatments become more pronounced with time (Katschnig et al., 2013).

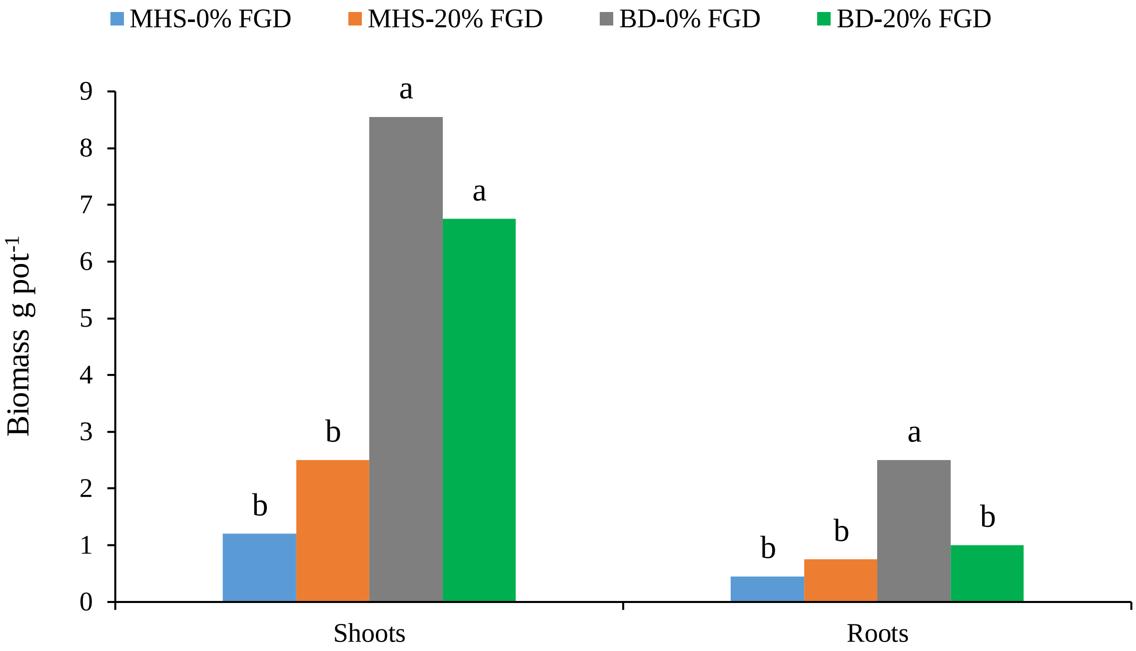

Figure 3.11 Total biomass of *Salicornia* (g pot^{-1}). MHS-0% FGD= Hoagland solution and Zero Flue Gas Desulfurization wastewater; MHS-20% FDG = Hoagland solution and 20% Flue Gas Desulfurization wastewater; BD-0%FGD = Blowdown water and Zero Flue Gas Desulfurization wastewater; BD-20% FGD = Blowdown water 20% Flue Gas Desulfurization wastewater. Means with different superscripts are significantly different (LSD, α=0.05).

Concentrations of different elements in *Salicornia europaea*

Salicornia europaea species was evaluated under the hydroponic system for its initial tolerance to highly saline irrigation water, which contained high concentrations of metals such as Se and B. The resulting data showed pronounced differences between Se and B accumulation and movement within the plant. Concentrations of Se and B were significantly higher in plant shoots grown on wastewater than compared to the control (Figure 3.12 and 3.13).

Shoot Se concentrations ranged from 30 µg kg^{-1} in control to 190 µg kg^{-1} in salt/ wastewater treatments. Selenium concentration was significantly higher in shoots when treated with HO-20%FGD than when grown under control conditions (Figure 3.12). With respect to B, it was apparent that all treatments used to grow an enhancement on the growth of the plant, but some treatments were more effective on B than others (see Figure 3.13). Boron concentrations in shoot ranged from 15.59 mg kg-1 in control to 33.84 mg kg-1 in salt/ wastewater treatments. In general, B concentration in shoots was significantly greater in wastewater treatment compared to control (Figure 3.13). However, the results reported in this study show that of *salicornia europaea* accumulated salts in its shoot tissue to a high level, indicating high Se accumulation potential; in particular, Se and B concentrations increased.

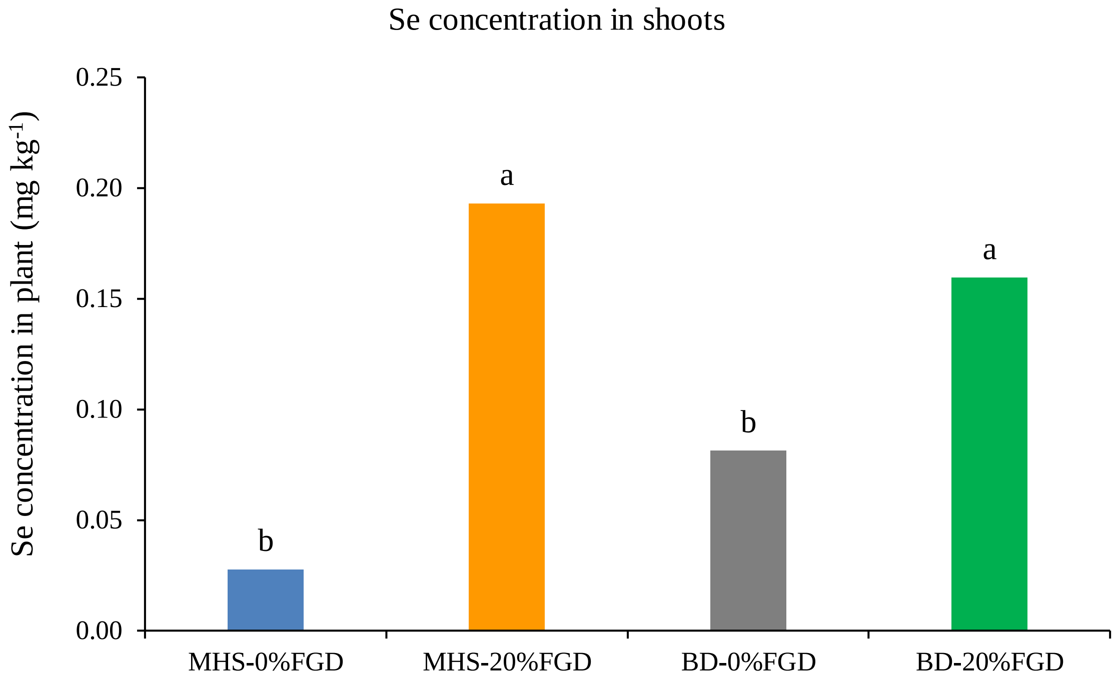

Figure 3.12 Total concentration Se in shoots of *Salicornia* mg kg^{-1}. MHS-0% FGD = Hoagland solution and Zero Flue Gas Desulfurization wastewater; MHS-20% FDG = Hoagland solution and 20% Flue Gas Desulfurization wastewater; BD-0%FGD = Blowdown water and Zero Flue Gas Desulfurization wastewater; BD-20% FGD = Blowdown water 20% Flue Gas Desulfurization wastewater. Means with different superscripts are significantly different (LSD, α=0.05).

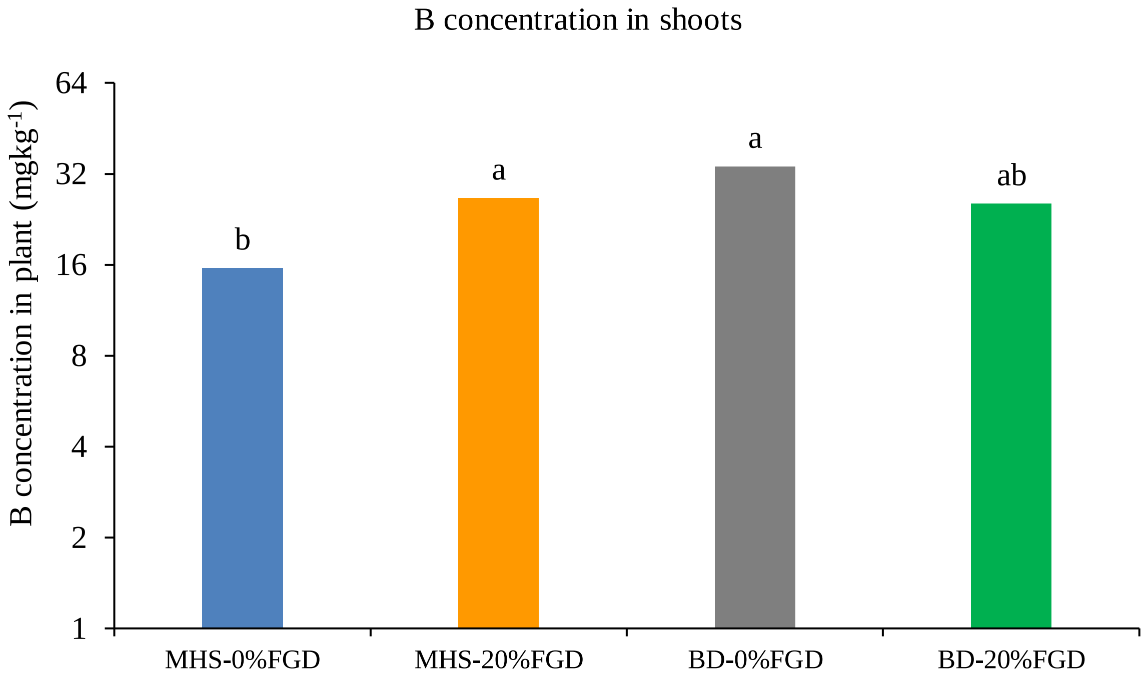

Figure 3.13 Total concentration B in shoots of Salicornia mg kg^{-1}. MHS-0% FGD = Hoagland solution and Zero Flue Gas Desulfurization wastewater; MHS-20% FDG = Hoagland solution and 20% Flue Gas Desulfurization wastewater; BD-0%FGD = Blowdown water and Zero Flue Gas Desulfurization wastewater; BD-20% FGD = Blowdown water 20% Flue Gas Desulfurization wastewater. Means with different superscripts are significantly different (LSD, α=0.05).

Selenium and Boron concentrations of roots are presented in Figure 3.14 and 3.15. Selenium concentrations in roots increased proportionately with increase in wastewater selenium concentration. Selenium concentration ranged from 320 µg kg^{-1} in control to 1800 µg kg^{-1} in salt/wastewater treatments as shown in Figure 3.14. Selenium concentration was significantly greater in roots when treated with 20% FGD than when grown under control conditions (Figure 3.14). However, the roots had higher Se concentrations than the shoots. Boron concentrations ranged from 21.28 mg kg^{-1} in salt/ wastewater treatments to 84.92 mg kg^{-1} in control (Figure 3.15). However, the concentration of B in roots reduced drastically with an increase in salt concentration. The roots of *salicornia europaea* accumulated much more heavy metals than the aboveground, which was in accordance with the previous work by Williams et al. (1994). In general, succulent-like halophytes species such as *salicornia*, *salsola*, and *suaeda*, salts are accumulated mainly in the vacuoles. For example, vacuoles occupy more than 70% of the leaf mesophyll cells in *suaeda maritima*, which has the ability to store salts at concentrations higher than 500 mM (Centofanti and Bañuelos, 2015 and Hajibagheri et al., 1984).

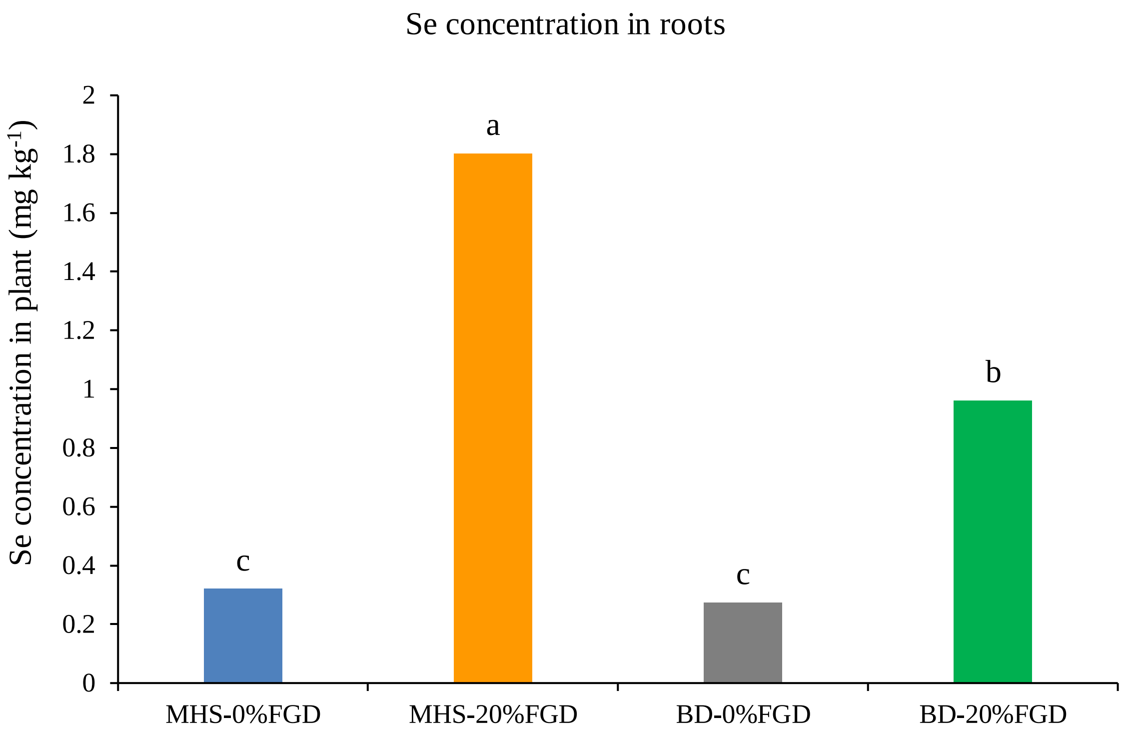

Figure 3.14 Total concentration Se in roots of *Salicornia* mg kg^{-1}. MHS-0% FGD = Hoagland solution and Zero Flue Gas Desulfurization wastewater; MHS-20% FDG = Hoagland solution and 20% Flue Gas Desulfurization wastewater; BD-0%FGD = Blowdown water and Zero Flue Gas Desulfurization wastewater; BD-20% FGD = Blowdown water 20% Flue Gas Desulfurization wastewater. Means with different superscripts are significantly different (LSD, α=0.05).

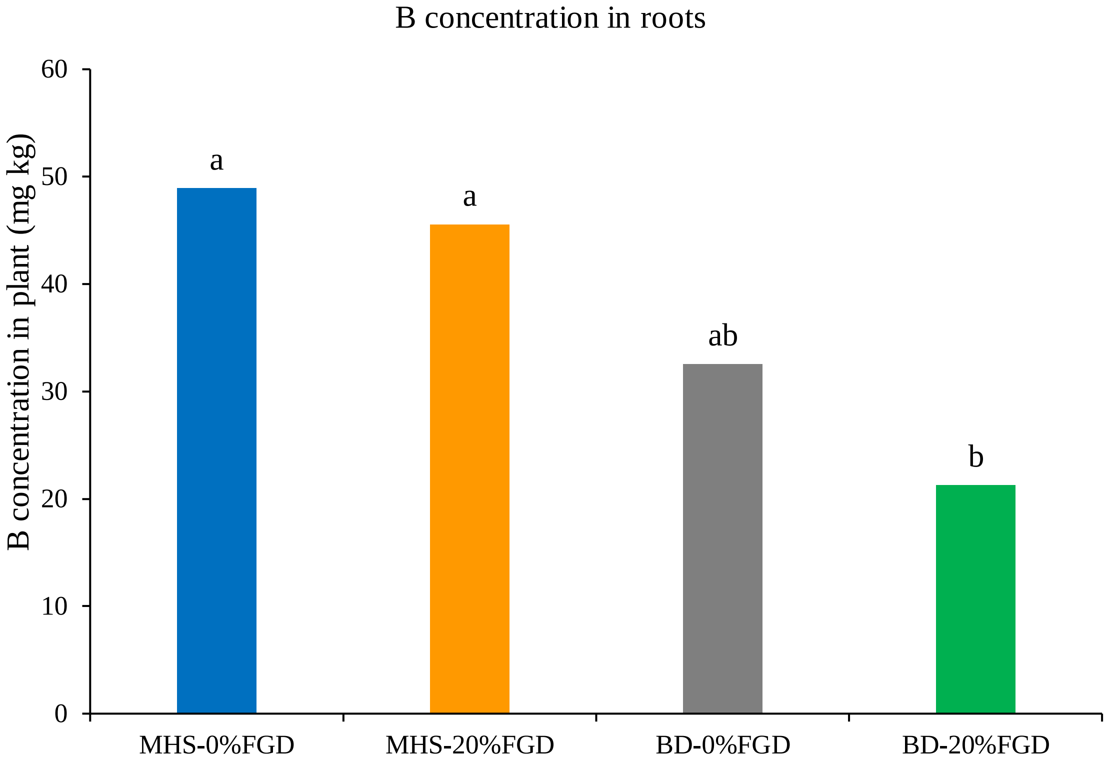

Figure 3.15 Total concentration B in roots of Salicornia mg kg^{-1}. MHS-0% FGD = Hoagland solution and Zero Flue Gas Desulfurization wastewater; MHS-20% FDG = Hoagland solution and 20% Flue Gas Desulfurization wastewater; BD-0%FGD = Blowdown water and Zero Flue Gas Desulfurization wastewater; BD-20% FGD = Blowdown water 20% Flue Gas Desulfurization wastewater. Means with different superscripts are significantly different (LSD, α=0.05).

The concentrations of Na, S, and P were significantly higher in plant shoots grown on BD-0% FGD than other treatment (Table 3.5) groups. Table 3.5 shows that the plants had considerable effects in reducing concentrations of these elements (Na, S, and P). Our results demonstrate that *salicornia europaea* accumulates and tolerates very high levels of Na in the shoots. This is one of the important characteristics for a plant to be considered as a viable alternative crop for saline soils. The limited mobility of the metals in the halophytes may be explained by the fact that metals are accumulated in the below ground levels rather than above ground parts of halophytes. This was also shown by the low translocation factor in all the investigated plant species. According to Ventura and Sagi (2013), sodium and chloride are the main ions accumulated when plants are irrigated with saline water. *Salicornia persica* shoots grown in seawater accumulated 1.6 g Na^+ and 2.9 g Cl^- $100g^{-1}$ of the edible portion which confers salty taste to the plant (Ventura et al., 2011). Related halophytic chenopods have increased shoot Na^+ on a dry mass basis with increasing external NaCl (Khan et al. 2000, 2005; Redondo-Go´mez et al. 2007). Plants treated with BD-0% FGD high in sulfate, irrespective of the concentration, had substantially more sulfur in their corresponding HO-20% FDG and BD-20% FGD treatments. Increasing salinity proportionately increased the sulfate concentration in plants treated with HO-0% FGD. Total P accumulation in plant tissue was significantly higher in BD-0%FGD wastewater than other treatments (table 3.5). Webb (2005) found that in *salicornia* a growth response to increasing P was only observed with excess N supply, suggesting that it is not a growth limiting factor.

Table 3.5P, S and Na concentration mg kg^{-1} in shoots of *Salicornia* (Hydronic system). HO-0% FGD= Hoagland solution and Zero Flue Gas Desulfurization wastewater; HO-20% FDG= Hoagland solution and 20% Flue Gas Desulfurization wastewater; BD-0%FGD= Blowdown water and Zero Flue Gas Desulfurization wastewater; BD-20% FGD= Blowdown water 20% Flue Gas Desulfurization wastewater. Means with different letters are significantly different (LSD, α=0.05).

Treatments	P concentration (mg kg^{-1})	S concentration (mg kg^{-1})	Na concentration (mg kg^{-1})
HO-0% FGD	2430^{c}	4184^{b}	10419^{c}
HO-20% FDG	971.7^{d}	3047.7^{b}	28210^{b}
BD-0%FGD	6726.5^{a}	6864.5^{a}	56825^{a}
BD-20% FGD	1660.7^{c}	2975^{b}	34770^{b}

Total Mo concentration in plant shoots are not different between either of the two treatments; control and wastewater as depicted in the Figure 3.16. Molybdenum concentrations ranged from approximately 4.08 mg kg^{-1} in control to 0.87 mg kg^{-1} in salt/ wastewater treatments. The concentration of Mo in *salicornia* showed a strong negative response to increasing selenium and sulfate in the growth medium, which was likely due to competitive inhibition for root transport. Plant uptake affected the standing biomass in all treatments; Molybdenum concentrations in HO-0% FGD had the highest overall final concentration; this was followed by BD-0% FGD. The plant treated by FGD treatment showed low Mo concentration because Mo levels significantly decreased in a plant with increasing sulfate and selenite (Harris et al., 2014). According to Harris et al. (2014), increasing Se supply had a strong inhibitory effect on leaf Mo levels.

Total Molybdenum concentration in plant roots was significantly lower in wastewater treatments (BD-0%FGD) compared to other treatments (Figure 3.17) over the course of the experiment. Approximately, Mo concentrations ranged from 1.47 to 4.45 mg kg^{-1} in wastewater treatments. Previous study has shown that the sulfate transporters play a vital role in the uptake of molybdate since, there exist a significant competition with S and Se at the transporter level (Schiavon et al., 2012). It is also possible that the interactions between Mo, S, and Se might occur at the level of various enzymes involved in sulfate assimilation (such as ATP sulfurylase), which may act on molybdate and selenate in addition to sulfate (Schiavon et al. 2012). The higher levels of Mo in the BD-0%FGD as compared to BD-20% FGD or HO-20%FGD in most conditions might well fit with the hypotheses that Mo finds its way into the root through sulfate transporters.

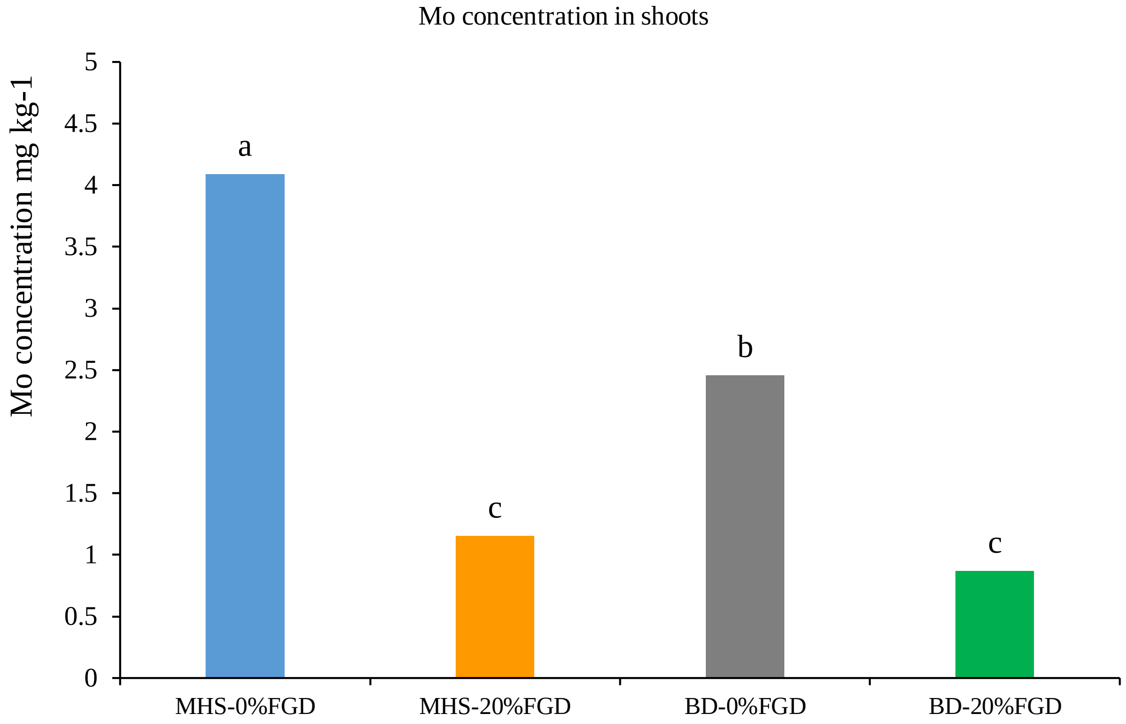

Figure 3.16 Total concentration Mo in Shoots of *Salicornia* mg kg^{-1}. MHS-0% FGD = Hoagland solution and Zero Flue Gas Desulfurization wastewater; MHS-20% FDG = Hoagland solution and 20% Flue Gas Desulfurization wastewater; BD-0%FGD = Blowdown water and Zero Flue Gas Desulfurization wastewater; BD-20% FGD = Blowdown water 20% Flue Gas Desulfurization wastewater. Means with different superscripts are significantly different (LSD, α=0.05).

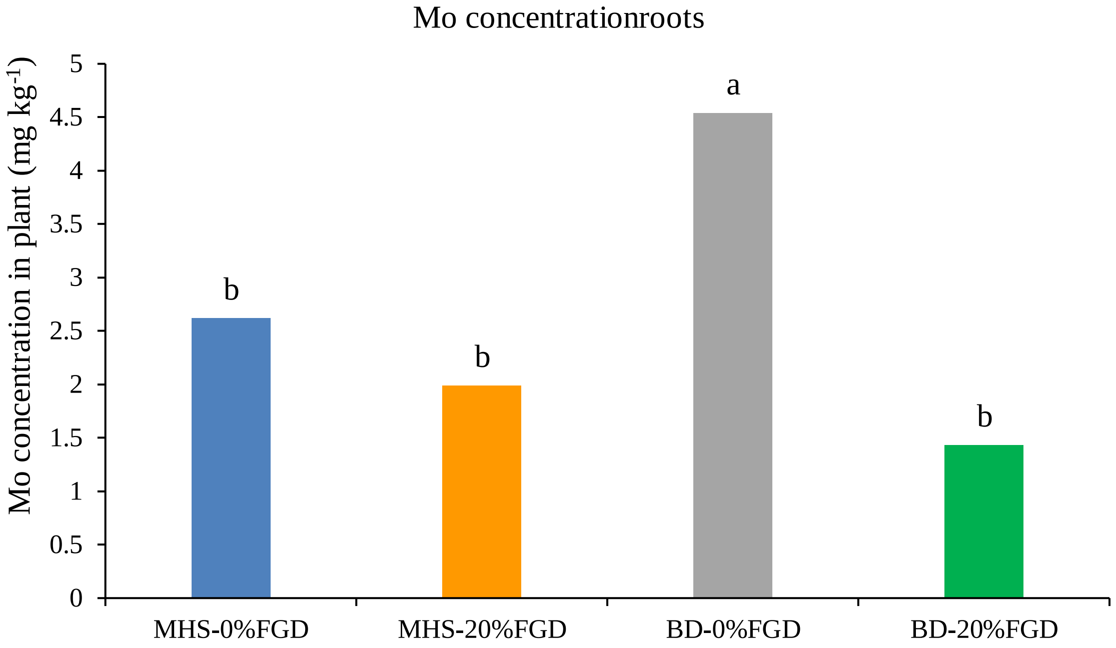

Figure 3.17 Total concentration Mo in roots of *Salicornia* mg kg^{-1}. MHS-0% FGD = Hoagland solution and Zero Flue Gas Desulfurization wastewater; MHS-20% FDG = Hoagland solution and 20% Flue Gas Desulfurization wastewater; BD-0%FGD = Blowdown water and Zero Flue Gas Desulfurization wastewater; BD-20% FGD = Blowdown water 20% Flue Gas Desulfurization wastewater. Means with different superscripts are significantly different (LSD, α=0.05).

Conclusion

The results demonstrate that *salicornia europaea* has the ability to remove excess trace elements such as Se, B, and salts in marginal waters, giving it potential for reclaiming marginal waters. *Salicornia* accumulates salts in shoot tissue which allows *salicornia* to thrive in hypersaline conditions. This was evident in the study; as the wastewater treatments were higher in Cl^-, sulfate, or B, and shoot biomass and shoot tissue concentrations increased. Although B concentration in shoot tissue was higher, there was no qualitative or quantitative indication of B toxicity observed. Selenium concentrations in shoot tissue were relatively low: 0.75 $\mu g\ kg^{-1}$ in 100% FGD and 0.44 $\mu g\ kg^{-1}$ dry wt. in 50% Hoagland + 50% FGD using to the low concentration of Se in the source waters. Enhanced biomass indicated that *salicornia* has the potential to provide valuable stock for biofuel and bio-based products from marginal waters and should continue to be further explored. The ability of *S. europaea* to tolerate high levels of Na in the shoots is a crucial characteristic for a viable alternative crop for the arid saline soils. The feasibility of cultivating *salicornia* by applying hydroponic system with 100% wastewater was a significant finding in the second study. The results showed the promising ability of *salicornia* to remove Se and B from wastewater indicating that *S. europaea* can be utilized for pretreating high saline wastewaters.

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Chapter 4 - Agronomic Biofortification of Wheat with Zinc through Co-Addition of Organic C with Zn Fertilizers

Abstract

Zinc (Zn) is an essential micronutrient for humans, plants, and animals. Zinc deficiency occurs in a wide range of soil types around the world. Soils that are frequently associated with Zn deficiency are calcareous soils, sandy soils, weathered tropical soils, saline soils, waterlogged soils, and heavy cracking clay soils. Agronomic biofortification is an effective way to increase micronutrient concentrations in grain crops. This study aimed to investigate the effectiveness of various inorganic and organic Zn sources with and without fertilizer co-additives on biofortification of wheat with Zn grown in a mildly calcareous soil. The study also focused on determining the distribution of stems/leaves, whole grain, and bran and flour, as well as bioavailability of Zn in different plant parts (bran and flour). A greenhouse study was set up as a factorial design experiment arranged in a randomized complete block with three replications consisting of 18 treatments. The treatments were no Zn, $ZnSO_4$; ZnO; $ZnSO_4$ incorporated-MAP; ZnO incorporated-MAP; and biosolids with and without two co-additives (AVAIL polymer and humic acid). All Zn sources with or without additive treatments increased the biomass production from 2 to 43% compared to the control pots. Zinc oxide resulted in the largest increase in whole grain Zn (2.45 µg Zn pot^{-1}), followed by ZnO + AVAIL (1.98 µg Zn pot^{-1}) and $ZnSO_4$ (1.91 µg Zn pot^{-1}). There was no difference in Zn uptake by ZnO and $ZnSO_4$; however, $ZnSO_4$ treatments showed the largest increase in soil Zn extractability. Although Zn treatments increased bioavailable Zn in wheat flour, it did not increase the bioavailability of Zn in wheat bran. Co-additives did not improve the soil Zn extractability, nor the Zn uptake by wheat. Results

also showed that DTPA extractable Zn in soil was superior to the soil Zn fractionation in predicting plant Zn.

Introduction

Micronutrient malnutrition affects over three billion people around the world. Malnutrition is the most prevalent and increasing in developing countries (Welch and Graham 2004; Graham 2008; White and Broadley, 2009). Zinc (Zn), iron (Fe) and iodine (I) are the mineral micronutrients that are most frequently deficient in humans. Zinc malnutrition ranks fifth in terms of the leading cause of disease in developing high-mortality countries (WHO, 2002).

Zinc, iron, and copper (Cu) are essential micronutrients for plants, humans and animals. There are estimates that indicate around three billion people worldwide are afflicted by Fe deficiency, and up to half of the population in developing countries are at risk of Zn deficiency (Cakmak, 2008; Cakmak et al.; 2004). Zinc deficiencies are known to be a common problem in populations that depend on cereals as a main essential food source and have little or no access to animal products (White and Broadley, 2011; Prasad et al., 1961). Wheat is a common food in many developing countries (Morgounov et al., 2007; Cakmak et al., 2004). Thus, the Zn concentrations of wheat grains are very important nutritional factors especially in developing countries.

Biofortification is an agricultural strategy with the goal of increasing the content of select micronutrients, including Zn, in essential food crops like rice, wheat, maize, pearl millet, and others (Hotz, 2009). Biofortification strategies include the application of Zn fertilizers and crop genotypes that acquire more Zn from the soil and accumulate it in their edible portions (White and Broadley, 2011). Adoption of biofortification approaches would result in Zn enhancement of the target tissue to a considerable extent. Nevertheless, there is a basic need to understand Zn absorption mechanisms in plants prior to the exploitation of such practical approaches. Zinc absorption is a complex physiological trait which is mainly governed by Zn transporters and metal chelators of the plant system (Gupta et al., 2016).

A major factor causing Zn deficiencies is the low bioavailability of Zn in cereals and legume-based diets (Hurrell, 2003). These plant based diets contain relatively high levels of antinutrient factors, such as phytic acid (PA), fiber, and tannins. These negative factors lead to a marked reduction in the bioavailability of essential nutrients (Raboy, 2001). Hence, it is important to improve the micronutrient quality of essential foods by increasing the levels of Zn and/or decreasing the content of the anti-nutritional chemicals.

Plant availability of added Zn and other micronutrients is reduced due to various soil reactions and plant factors. Soil reactions include the formation of Zn phosphate, high sorption of Zn in calcareous soils, soils with low-to-very-high organic matter (OM), and/or high soil pH. Plant factors include the low potential of cereal genotypes for Zn uptake and limited Zn transport within the plant due to interaction with phytate.

Phytic acid is a phosphate ester of inositol containing six phosphate groups; each group is capable of binding metal cations. It is a major storage element of P in cereal grains, and it is known to lessen the bioavailability of Zn and other micronutrients for monogastric animals and humans (Akhtar et al., 2019). Zinc is one of the elements most strongly complexed with phytate in plants and food (Kornegay, 2011). Phytic acid is found to decrease the bioavailability of Zn (Akhtar et al., 2019) and is considered as an anti-nutrient compound due to its negative impact on the availability of Zn (Hotz and Gibson, 2007). Phytic acid decreases Zn solubility in foodstuffs and limits its assimilation in the human body. Instead of the total amount of Zn in grain, its bioavailable portion is important for nutritional aspects, because total Zn content is not a fair indicator of its bioavailability in monogastric animals or human.

Increasing bioavailable Zn, by either increasing Zn in wheat grain or decreasing antinutrients in wheat grain, is generally recommended to solve the problem of human Zn deficiency (Hussain et al., 2011). In cereal grains, PA forms insoluble complexes with Zn, causing a reduced absorption inside the human body (Ciccolini et al., 2017). The PA concentration in grain, and consequently, the PA to Zn molar ratio, is widely used as an indicator of Zn bioavailability in human diets (Cakmak et al., 2010). It is also used as an indicator to assess bioavailable Zn in food stuff in general (Morris and Ellis, 1989). According to the World Health Organization (WHO) cut-offs, a PA: Zn (FAO, 2005) molar ratio of less than 15 is an indicator of high Zn bioavailability (Ciccolini et al., 2017).

Zinc deficiency occurs in a wide range of major soil types in many parts of the world that are frequently associated with Zn deficiency. These include calcareous soil (calcisols), sandy soil (arenosols), weathered tropical soil (ferralsols), saline soil, waterlogged soil (gleysols), and heavy cracking clay soil (Cakmak and Kutman, 2018). Graham and Welch (1996) reported that it is estimated that approximately 50% of soils utilized for cereal production in the world have a low standard of plant-available Zn.

Soil is the primary source of micronutrients for plants (Welch et al., 1991). Therefore, the transfer of micronutrients from soil to crop plants is important for both plant and human nutrition (Yang et al., 2007; Welch et al., 2002). The phytoavailability of soil Zn depends on soil properties such as total micronutrient concentrations, mineralogy, cation exchange capacity (CEC), pH, calcium carbonate ($CaCO_3$) content, organic matter content, soil moisture conditions, and available phosphorus (Alvarez et al., 2006; Martínez and Motto, 2000). This deficiency is traditionally corrected by application of soluble inorganic Zn salts such as $ZnSO_4$ to soils (Nayyar and Takkar., 1980). Arce et al. (1992) reported when Zn is added to calcareous soils in the form of soluble inorganic salts the deficiency can be corrected to a large extent (Arce et al., 1992).

The inorganic surfaces involved in Zn adsorption in soils are clays, metals oxides-hydroxides, metal carbonates, and metal phosphates (Bradl, 2004). Furthermore, the calcareous soil containing high levels of clay minerals increase the retention of Zn further in the soil. Since clays adsorbs Zn ions through both ion exchange and specific adsorption, Zn adsorption to clay minerals decreases Zn availability to plant (Farrah and Pickering, 1997). Zinc is a diffusion-limited nutrient in the soil, so more soluble sources of Zn tend to be more readily available because they allow increased movement of fertilizer away from the application point, increasing the fertilized volume of soil (McBeath and McLaughlin, 2014). Strong adsorption (i.e., fixation) of Zn takes place by the slow diffusion of Zn into Fe and Mn oxides and clay minerals. This is also closely associated with the change in the amount of Zn-bound to organic matter. The solubility of Zn and ratio of Zn^{2+} to organic-Zn ligand complexes increases at lower pH, especially in soils of low soluble organic matter. Zinc availability is reported to be associated with its transformation in soils and plant continuum through various mechanisms, such as mentioned before. Understanding the distribution of Zn among various fractions of soils will help to characterize the chemistry of Zn in soils and possibly its availability to plant uptake (Saxena, 2017).

We hypothesize that co-polymers and humic substances application will effectively increases the Zn availability in calcareous soils by increasing the amount of dissolved OM bound-Zn in solution, thereby reducing the accumulation of Zn in less readily available fractions such as Zn associated with calcium carbonate, Fe or Mn oxides and clay minerals; and ZnO and biosolids have the potential to better synchronize Zn solubility and plant Zn uptake.

The objectives of this research were to: (1) study the effectiveness of various inorganic and organic Zn sources with and without co-additives on biofortification of wheat with Zn; and (2) to determine distribution of stems/leaves, whole grain, bran, and flour, and bioavailability of Zn in bran and flour in different plant parts.

Materials and Methods

Soil collection

A calcareous soil from Garden City, KS was used for this study. The surface soil (0 to 10 cm depth) was collected, air-dried and sieved to < 4 mm by passing through stain-less steel mesh. Basic soil properties, including soil texture, pH, OC content, and CEC, were determined (Table 4.1). 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); CEC was determined by displacement method (Soil Survey Staff, 2011); carbonates were determined by Allison and Moodie, (1965); and OC content of soil was measured by loss on ignition method (Combs and Nathan, 1998). The concentration P in the Olson extract was measured using Murphy and Riley (Murphy and Riley, 1962). Available N (NO_3^--N and NH_4^+-N) was determined by using 1*M* KCl extraction (Keeney and Nelson, 1987). Concentrations of N in filtered extracts were determined calorimetrically. Available cations (K, Na, Mg, S, Cu, Mn, and Ca) were determined by the ammonium acetate extraction (Warncke and Brown, 1998). The maximum water holding capacity (MWHC) determination was made using the protocol from

Jenkinson and Powlson (1976). The soil was pre-moistened with 10% of the total distilled water needed for 80% MWHC.

Table 4.1 Initial soil characterization of chemical and physical properties.

Parameter	Value
pH (1:10 soil:water)	8.8
EC mS/cm	1.3
CEC[†], cmol$_c$/kg	18.3
TOC [‡] %	1.1
$CaCO_3$	5.6
Total Zn, mg kg^{-1}	52.1
Available Zn mg kg^{-1}	1.3
Total P, mg kg^{-1}	634
Available P (Olson) P, mg kg^{-1}	61.5
Available Ca, mg kg^{-1}	4461.9
Available Mg, mg kg^{-1}	418.1
Available K, mg kg^{-1}	767.9
Fe, ppm	5.1
Available Mn, mg kg^{-1}	8.6
Available S, mg kg^{-1}	4.8
Total Cu, mg kg^{-1}	12.8
Available Cu, mg kg^{-1}	1.1
Available NH_4-N, mg kg^{-1}	5.7
Available NO3-N, mg kg-1	34.9
Total- N %	0.2
Sand, Silt, and Clay §	24.9, 52.4, 22.7

[†]Cation exchange capacity; [‡]Total organic carbon; [§] soil texture

Greenhouse study setup

The experiment was carried out from January to April 2018, 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 plastic pots (23 cm diameter, 19 cm height). The following factorial combination of treatments were used: (1) control (CF); (2) control AVAIL (AV); (3) Control humic acid (HA); (4) $ZnSO_4$; (5) $ZnSO_4$+ AV; (6) $ZnSO_4$+ HA; (7) ZnO; (8) ZnO + AV; (9) ZnO+HA; (10) biosolids; (11) biosolids + AV; and (12) biosolids + humic acid; (13) $ZnSO_4$-incorporated-MAP; (14) $ZnSO_4$-incorporated-MAP+ AV; (15) $ZnSO_4$-incorporated-MAP + HA; (16) ZnO-incorporated-MAP; (17) ZnO-incorporated-MAP+ AV; (18) ZnO-incorporated-MAP + HA. There were 18 treatment combinations, all pots including the controls received N, and P. We mixed powder and granular fertilizers ($ZnSO_4$; ZnO; $ZnSO_4$-incorporated-MAP; ZnO-incorporated-MAP) with soil (0-3 cm), the liquid fertilizers ($ZnSO_4$; ZnO) and granular fertilizers ($ZnSO_4$-incorporated-MAP; ZnO-incorporated-MAP) mixed with co-additives (AVAIL and Humic acid) were spot-placed in four corners via a pipet syringe. Nitrogen was balanced using NH_4NO_3 (0.83 g N/8 kg of soil), P was balanced using MAP (1.61 g P/8 kg of soil) powder by mixing in each pot, and S was also balanced using $(NH_4)_2SO_4$ (1.56g S/8 kg of soil). The experimental design was factorial design arranged in a randomized complete block design with three replications.

Wheat (*Triticum aestivum L.*, *Zenda,* DVP number: 201700282) seeds were vernalized at 4°C (Seeds were planted into germination trays containing Sunshine® professional peat-lite growing mix in a greenhouse for one week and resulting seedlings placed in a vernalization chamber for a period of eight weeks at 4°C) and 100% RH for a minimum of six weeks. On the next day, 57 plastic pots were filled with 8 kg soil, fertilizer was added as described before.

Water (1674 mL tap water) was added to each pot to bring the moisture content to 80% of MWHC of the soil. Equal amounts of water were added throughout the experiment period to keep the soil moist. On January 9th, the plants were transplanted (five plants/pot). After eight days of transplanting, plants were thinned down to two plants per each pot (8 kg soil/pot). During the experiment, the greenhouse temperature was maintained within the range of 15.5°C to 18.3°C during the first month, then increased from 18.3 to 21.1°C for the last two months. To ensure good growth, pots were watered with tap water every two days. A 12 h daylight and 12 h darkness photoperiod regime was maintained with overhead sodium lights (150 Wm^{-2}) for two weeks, followed by 14 h daylight and 10 h darkness for the next two weeks. This was followed by 16 h daylight and 8 h darkness until harvesting. The pots were rotated weekly to ensure that all pots received equal amounts of light.

Harvesting, sampling, analysis of wheat samples

Plants were harvested after their maturity at 94 days after planting by cutting above the surface (above ground). The grain was collected separately. Above-ground biomass was cleaned by washing them in water to remove adhered materials and were placed in paper bags. Fresh weight of the biomass was recorded. Plant samples were dried at 68° C about five days, until they reached a constant weight and dry weights of the biomass were recorded. Roots were carefully separated by washing them in deionized water (DI) to remove adhered soil materials. Fresh and dry weights of the roots (after drying as described above) were determined. The plant samples were ground using a stainless steel Wiley mill (Swedesboro, NJ) and the powdered plant material was stored in polyethylene vials until digested. All grain samples were placed in their respective labeled weighing bags and stored in a cold room at 4°C prior to analysis.

Chemical analysis

Ground sub-samples (0.5 g) of roots, aboveground biomass, and whole grain were 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 @ 100%, 15-minute ramp time, 15-minute cooling time). Standard plant reference materials (Pine Needles) from the National Institute of Science and Technology (NIST, Gaithersburg, MD) were 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 used for QA/QC where appropriate. Sample digests were filtered with Whatman 42 filter paper into plastic scintillation vials under the fume hood, vials were capped and stored at 4° C until analysis. Samples were analyzed for Zn, P, Fe, and S using an inductively coupled plasma-mass spectrometry (ICP-MS) or inductively coupled plasma-optical emission spectroscopy (ICP-OES) base on their concentrations. Zinc uptake was calculated as follows: Zn uptake (g/pot) = (Zn conc. of plant tissue * dry weight of biomass) + (Zn conc. in grain * dry weight of grain).

The anion-exchange method was used to determine the phytate content (Ciccolini et al., 2017). Plant samples were accurately weighed at either 0.05-0.06 g (50 and 60 mg), then transferred into 25 mL conical flasks or glass tubes. The samples were extracted using 10 mL of 0.2 N HCl solution. Flasks were capped and continuously shaken vigorously for 2 h. A 0.5 mL of this extract is mixed with 1 mL of ammonium ferric sulfate solution ($NH_4Fe(SO_4)_2$) (dissolved 0.2 g in 0.2 N HCl) in a capped glass tube, and constant heat was provided by placing the tube in a boiling water bath for 30 min. After cooling in ice cold water for 15 min, the samples were allowed to adjust to room temperature. The samples were then centrifuged (at 3000 g for 30 min), 1 mL of the supernatant was transferred into a new test tube. Thereafter, a 1.5 mL of 2,2'-Bipyridine solution ($C_{10}H_8N_2$) (1 g dissolved in 100 mL of water with 1% v/v thioglycolic acid) was added to the content mixture. A measurement of the absorbance was taken via a Beckman-Coulter DU-800 spectrophotometer (Brea, CA) set at 520 nm. A calibration curve was prepared using a standard phytate solution made with sodium phytate, which was in turn expressed as mg g^{-1} dw. Molar concentrations both of phytate and Zn were used to calculate phytate/Zn ratios and used to estimate the potential bioavailability of Zn in flour and bran. All samples were then prepared and analyzed in triplicate.

Soil samples were taken from each pot at harvest, air-dried and sieved (2 mm). The total elemental concentration in the soil was determined using aqua regia digestion procedure. A 0.5 g of soil was added to a digestion tube, then 5 mL of aqua regia [1:3 (v/v) HNO_3/HCl] was added. Pretreated samples were allowed to react overnight at room temperature. The tubes were then heated slowly increasing the temperature from 75°C for 30 minutes, 100°C for 30 minutes, 110°C for 30 minutes, 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 #42 Whatman filter paper. All samples were digested in duplicate. In each batch of digestion, two blanks and two samples of standard reference soil material (NIST 2711a-Montana soil) were included as a QA/QC control. All digestates analyzed on the inductively coupled plasma- optical emission spectrometry (ICP-OES, Varian 720-ES, Santa Clara, CA).

Five-step sequential extraction procedure of Tessier et al. (1979) was also performed on soil samples (Table 4.2). Two grams of soil were placed in centrifuge tubes. In between each step the tubes were centrifuged for 10 minutes, the supernatant was decanted, and the tubes were weighed to allow for correction for metals remaining in the entrained solution prior to the addition of the next reagent. Step 1: the soil was shaken with Milli-Q water 3 days (water soluble); step 2: soil residue was then extracted with 25 mL of 1 *M* $MgCl_2$ for 1 h (exchangeable); step 3: 25 mL of 1 *M* $NaC_2H_3O_2$ (pH 5.0) for 5 h (carbonate-bound); step 4: Fe-Mn^{-1} oxide bound fraction is determined by adding 40 mL of 0.04 *M* $NH_2OH(HCl)$ in 25% (v/v) $HC_2H_3O_2$ solution for 6 h at 96° C; step 5: the organic-bound fraction was determined via adding 6 mL of 0.02 *M* HNO_3 and 10 mL of 30% H_2O_2 and heating at 85° C for 2 h. An additional 6 mL of H_2O_2 then is added followed by heating for an additional 3 h. After cooling, 10 mL of 3.2 *M* $NH_4C_2H_3O_2$ in 20% (v/v) HNO_3 and 10 mL of deionized water are added, shaken for 30 minutes. Step 6; the remaining soil residue is finally treated with 20 mL of 4 *M* HNO_3 for 2 h at 80°C to determine the residual fraction.

Table 4.2 Summary for the sequential extraction procedure of Zn.

Step	**Zn phases**	**Extraction**
1	Water soluble	25 mL Milli-Q water, shaken for 3 days[†], centrifuged[‡], filtered[§]
2	Exchangeable	25 mL of 1 *M* $MgCl_2$, shaken for 1 h, centrifuged, filtered
3	Carbonate-bound	25 mL of 1 *M* $NaC_2H_3O_2$ (pH 5.0), shaken for 5 h, centrifuged, filtered
4	Fe-Mn^{-1} oxide bound	40 mL of 0.04 M $NH_2OH(HCl)$ in 25% (v/v) HC2H3O2 solution, shaken for 6 h at 96° C centrifuged, filtered
5	Organic-bound	6 g of 0.02 *M* HNO_3 and 10 mL of 30% H_2O_2 and heating at 85° C for 2 h. An additional 6 mL of H_2O_2 is added followed by heating for an additional 3 h. After cooling, 10 mL of 3.2 *M* $NH_4C_2H_3O_2$ in 20% (v/v) HNO_3 and 10 mL of deionized water are added, shaken for 30 min, centrifuged, filtered
6	Residual	20 mL of 4 *M* HNO_3, heating for 2 h at 80° C, shaken for 30 min, centrifuged, filtered

[†]One reciprocal shaker at 130 rounds per minute; [‡]centrifuged for 10 min at 2500 g; [§]filtered through Whatman No. 42.

Statistical Analysis

Plant and soil data were statistically analyzed using the Proc MIXED Procedure in SAS (SAS 9.4, 2017). The experimental design was a randomized complete factorial design. Data were analyzed using ANOVA with Zn fertilizer treatment as the main treatment and dish sections as subplot treatments. The Tukey Pairwise Method was used for a comparison of all treatments at a 0.05 level of significance.

Results and Discussion

Biomass of stem and grain

Dry matter production at harvest as a function of the Zn source is shown in Figure 4.1. The interactions between Zn treatments and co-additives for each treatment were not significant (*p*-value=0.75, Table 4.3). Therefore, data are presented averaged over co-additives or averaged over Zn treatments. Total biomass dry weight at maturity (stem) significantly increased by Zn application. Compared to the control treatment (CF), all Zn treatments significantly increased the biomass except biosolids treatment. Zinc treatments had different effects on biomass yield of the wheat. For example, the increase in biomass (stem) production was significantly different among the fertilizer sources; all Zn sources increased biomass production 2 to 39% over the control treatments. However, there was no difference between the control and the co-additives AV and HA (Figure 4.1b).

The application of Zn significantly increased the whole-grain yield of wheat compared to control except for biosolids treatment (Figure 4.2a). The interactions between Zn treatments and co-additives were not significant (*p*-value=0.69, Table 4.3). A noteworthy point is an insignificant difference between grain yield in MAP-ZnO and MAP-$ZnSO_4$ treatments (Figure

4.2a). Results revealed a significant increase in grain yield when inorganic Zn was applied. Grain production was significantly impaired in control Zn level, an increase in grain yield indicated the need for Zn fertilization in these soils. However, there was a significant difference between the control and the co-additive AV, although the control and HA or the AV and HA treatments were not different (Figure 4.2b). Grain yield can be depressed to a relatively greater extent by Zn deficiency than the total dry matter production due to the impaired pollen fertility in the Zn-deficient soil (Marschner, 1995). The use of chemical Zn fertilizer increased the grain yield significantly. These results suggested that inorganic sources of Zn addition were beneficial and able to enhance the plant growth and whole-grain yield.

The rapid increase in the world population requires parallel increases in food production. Wheat is one of the most important foods to help increase food production. To preserve the environment and the existing natural resources, further increases in global wheat production must be along with proper management of fertilization. Integrated use of chemical fertilizers and organic wastes is beneficial in improving crop yield, soil pH, organic carbon, and available P, N, and K in soil (Rautaray et al., 2003). The results of the present experiment indicated that wheat grain yield and yield components increased significantly with the application of inorganic sources of Zn (Figure 4.1a and 4.2a). The application of Zn significantly increased grain yield in all cultivars. In another greenhouse experiment, Singh et al., (1979) studied the effect of different levels of Zn supplied through Zn amended poultry manure and $ZnSO_4$ on corn. It was found that both sources significantly increased the biomass and uptake of Zn. However, in this study, we applied biosolids at a very low rate, based on the total concentration of Zn in biosolids, and that is most likely reason for not observing yield benefit of the biosolids treatments. Cakmak et al., (1996) compared the genotypes of bread wheat and durum wheat and reported that under Zn deficiency condition, shoot dry matter production was decreased in all genotypes, but more distinctly in durum wheat genotypes.

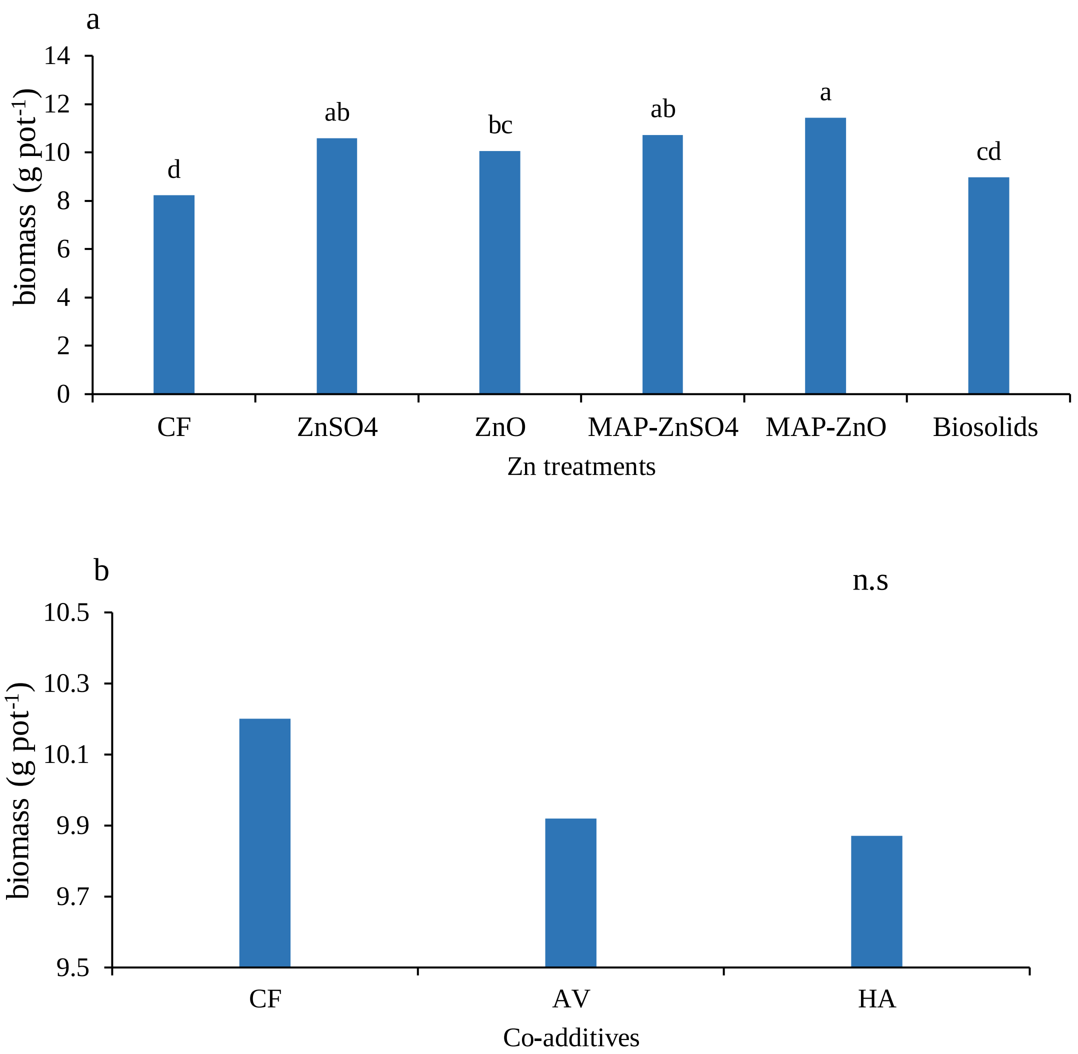

Figure 4.1 Effect of different Zn treatments on biomass (stem) of wheat. CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; HA= humic acid. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance. *NS=no significant difference in treatments**.**

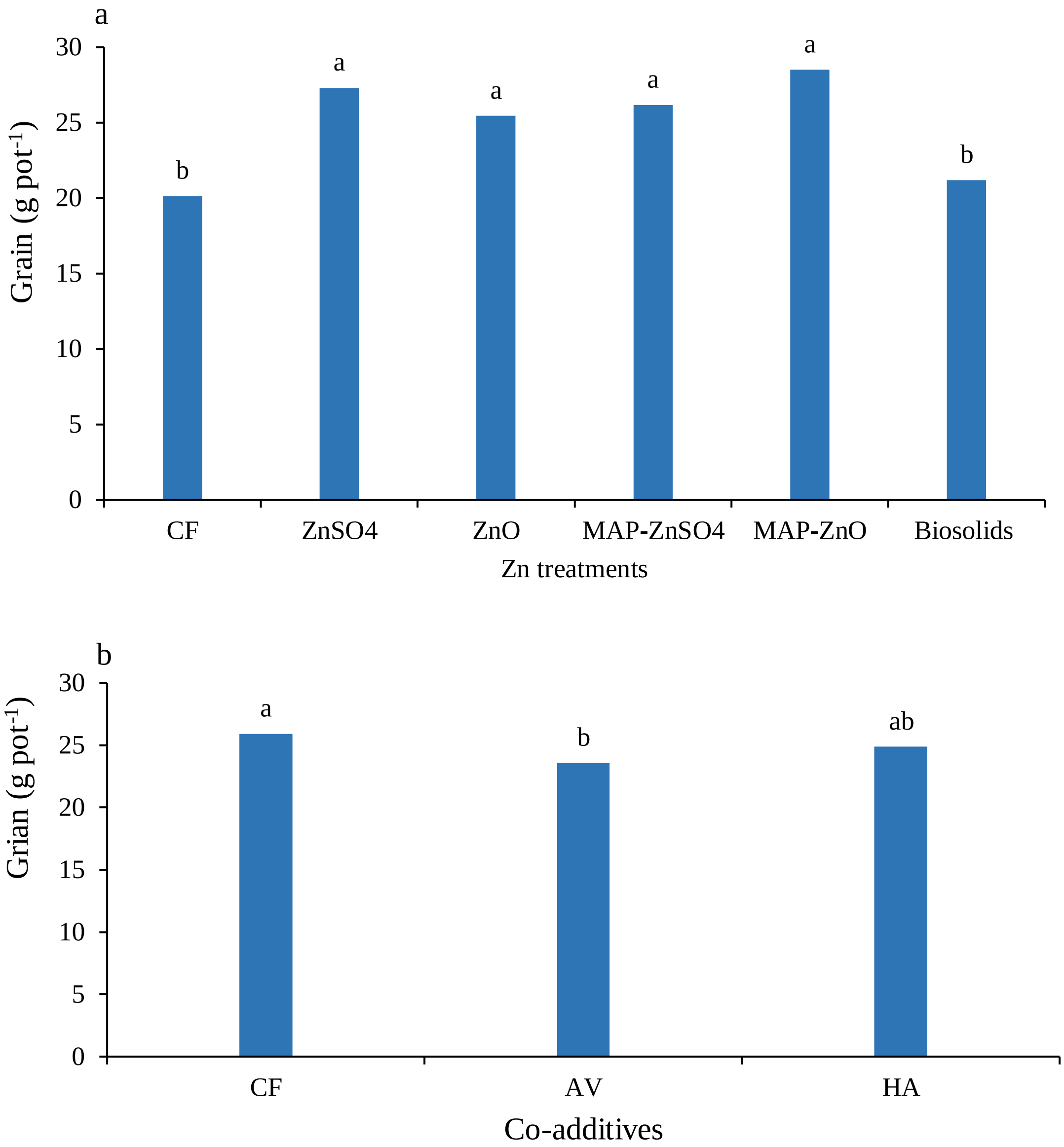

Figure **4.2** Effect of Zn treatments on grain yield of wheat. CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO-incorporated–MAP; Bio= biosolids; AV= AVAIL; HA= humic acid. Means within or between Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance.

Zinc Concentration and uptake in stem

The effect of the fertilizer treatments on Zn concentration and Zn uptake in the stem is shown in (Figures 4.3 and 4.4). The interactions between Zn treatments and co-additives were significant for both the parameters. The lowest Zn concentrations (5.9, 6.3, 6.5, 6.7, 6.7, 6.8, 6.8 and 6.9 mg kg^{-1}) were observed in the AV, MAP-ZnO+AV, $ZnSO_4$+HA, MAP-$ZnSO_4$, HA, MAP-$ZnSO_4$+HA, MAP-ZnO+HA, and FC, respectively (Figure 4.3). In contrast, the highest stem Zn concentrations were observed in those that were treated with ZnO+HA, followed by the ZnO+AV, $ZnSO_4$, ZnO and bio+HA treatments and the concentrations were increased by 230%, 115%, 110%, 104%, and 59% respectively compared to the controls (CF, AV, and HA).

The application of ZnO with or without co-additives (ZnO, ZnO+HA, ZnO+AV) increased the stem Zn concentration to a greater extent than the application of $ZnSO_4$, with average values being 14.3 mg kg^{-1} for ZnO, 15.1 mg kg^{-1} for ZnO + AV, and 23.1 mg kg^{-1} for ZnO+HA treatments. Gómez et al., 2017 reported that in a calcareous soil containing 2.4 % of free $CaCO_3$, the different Zn chemical species, ZnO and $ZnSO_4$, did not differ in their availability in the soil or accumulation in the leaves. This could be because, in the soil, there are other factors besides the solubility that have a significant impact on the plant availability of Zn. Soils provide a sink through sorption processes for the Zn^{2+} ions released, driving the dissolution of the ZnO towards completion. These mechanisms are particularly crucial in calcareous soils that can increase the sorption of Zn onto soil components, including carbonates (McBeath and McLaughlin, 2014). Moreover, in the calcareous soil, the concentration of Zn in the available fraction at equilibrium is very low. Therefore, the rate of Zn release from the oxides has a lesser influence in calcareous than in acidic soil. In hyperaccumulators, close contact between roots and trace elements is thought to be a major factor influencing the efficiency of metal uptake (Whiting et al., 2001). For any plant, it is important to facilitate close contact between wheat roots and $ZnSO_4$ fertilizer to increase the effectiveness of $ZnSO_4$ fertilizer in potentially Zn-deficient soil. Kar et al. (2007) conducted a greenhouse experiment on maize with various levels and sources of Zn. They reported that among all treatment combinations, and the maximum height was 56.5 cm achieved when 5 mg Zn was applied kg soil as Zn-HA.

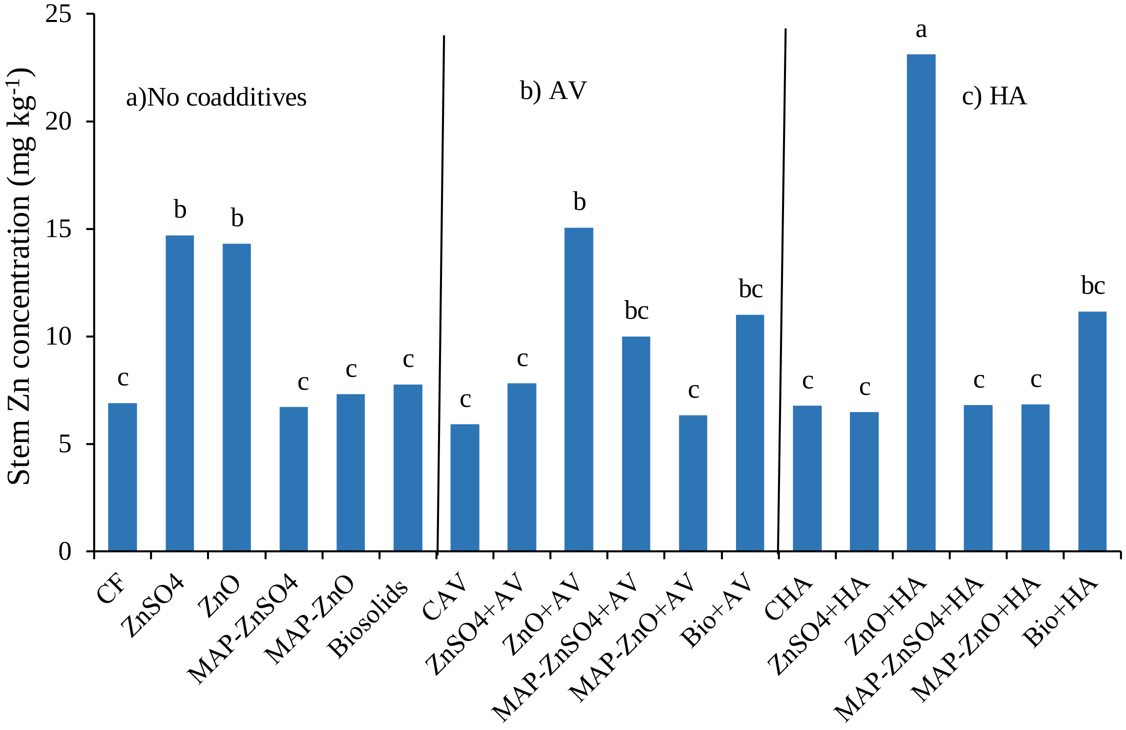

Figure 4.3 Zn concentration in aboveground biomass mg kg^{-1} (without grain), CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$= $ZnSO_4$ incorporated–MAP; MAP-ZnO= ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO+ VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey’s honest significance.

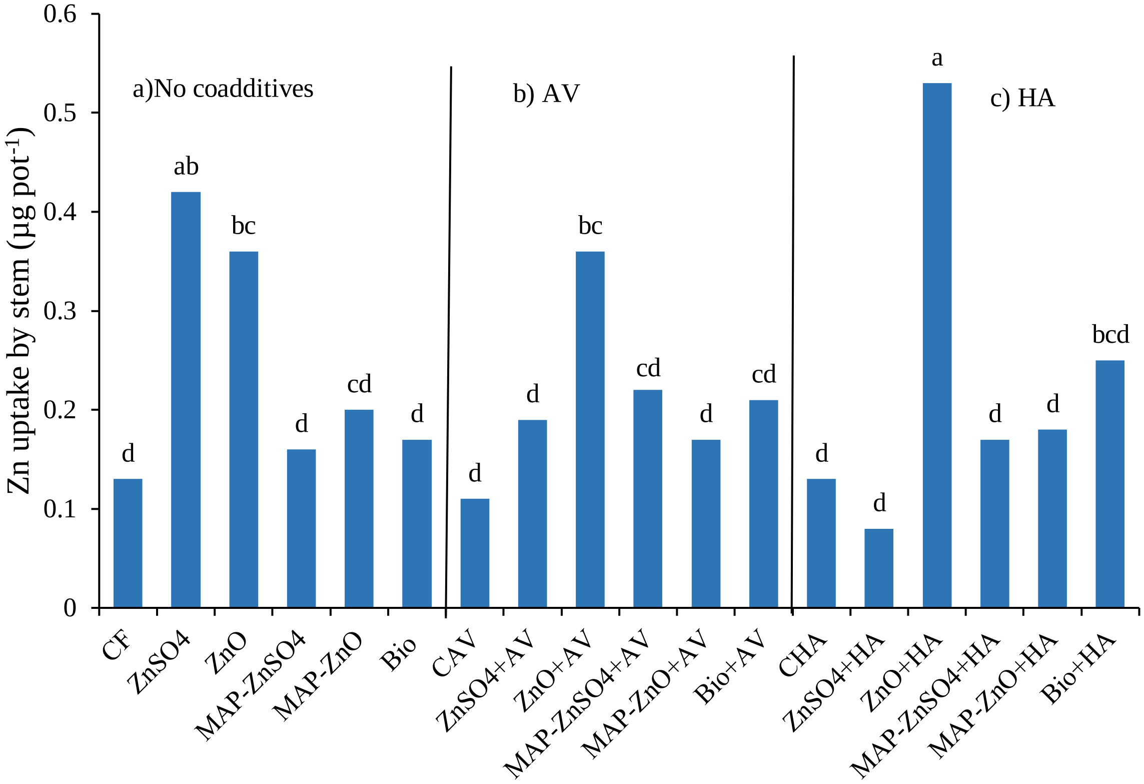

Figure 4.4 aboveground Zn uptake (µgpot^{-1}) (biomass), CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$= $ZnSO_4$ incorporated–MAP; MAP-ZnO= ZnO-incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO+ VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance.

Zinc concentration and accumulation in root

At the end of the harvest, root Zn concentrations in the ZnO treatments were higher than in all organic and inorganic Zn treatments (Table 4.5). The lowest Zn concentration was observed in MAP-$ZnSO_4$. All treatments contained increased levels of Zn, ranged from 3% to 132% compared to the control. Haslett et al. (2001) reported that root Zn concentrations in the Zn-EDTA and ZnO treatments were higher than in the $ZnSO_4$. However, it should be noted that root Zn concentrations for all treatments and all three harvests were in the range reported by the previous researchers for wheat.

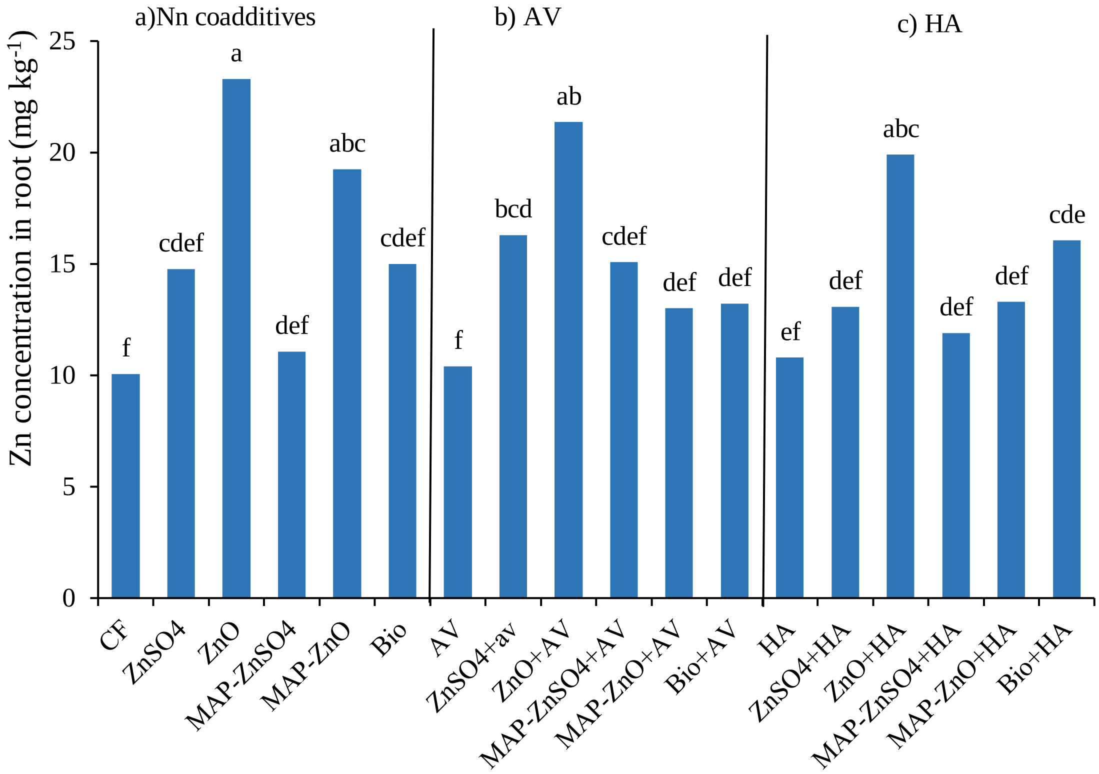

Figure 4.5 Concentration of Zn in roots (mg kg^{-1}). CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$= $ZnSO_4$ incorporated–MAP; MAP-ZnO= ZnO-incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO+ VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey's honest significance.

The concentration of Zn in whole grain, bran and flour

The effect of fertilizer treatments on whole grain, flour, and bran Zn are shown in Figures 4.6 through 4.11. The interactions between Zn treatments and co-additives for each treatment were not significant (*p*-value=0.055, Table 4.3). In comparison to the control treatment, Zn concentration in whole grain was 58 times larger for ZnO, 30 times larger of $ZnSO_4$, 0.14 times larger for of MAP-ZnO, although the effect of the treatment was significant only with ZnO, and $ZnSO_4$ (Figure 4.6a). Previous studies stated that generally, the Zn concentrations in grain within wheat cultivated regions range from 20-35 mg kg^{-1}, with an average value of about 28–30 mg kg^{-1} (Graham et al.,2007 and Singh et al., 2018). The application of ZnO treatments increased the whole grain Zn concentration to a greater extent than the application of $ZnSO_4$ treatments. The average values were 33.6 $mgkg^{-1}$ for ZnO, and 27.5 mg kg^{-1} for $ZnSO_4$ (Figure 4.6a). This could be because of the fast fixation of Zn added as $ZnSO_4$ into a calcareous soil (Martín-Ortiz et al., 2008). The reason for this fixation is that the presence of active $CaCO_3$ induced sorption of soluble $ZnSO_4$ and the precipitation of Zn as hydroxide or carbonate. It could also be due to the formation of insoluble calcium zincate (Rico et al., 1996). This lends the greater efficacy of ZnO with low solubility. The homogenous mixing of ZnO throughout the soil, provides soil minerals and organic matter to buffer pH increase due to ZnO dissolution (which reduces Zn solubility) and providing a sink (through sorption) for Zn^{2+} ions released through the dissolution process, thereby driving dissolution of ZnO towards completion. For these reasons, a greater portion of the applied Zn may have also gotten fixed in calcareous soils within a very short period. A greenhouse study performed by Zhao et al., (2016) showed that the $ZnSO_4$ ($ZnSO_4.7H_2O$; 22.6% Zn) mixed with soil and banded Zn-EDTA treatments significantly increased grain Zn concentration. The concentration of Zn in wheat grain needs to be at least 40-60 mg kg^{-1} to meet the demands of human beings (Cakmak, 2008; Ortiz-Monasterio et al., 2007). Soil Zn fertilization can effectively improve grain Zn bioavailability, the application of 10 kg Zn ha increased grain Zn concentration from 10 to 20 mg kg^{-1} (Cakmak et al., 2010; Pfeiffer et al., 2007). The difference between the minimum level of Zn required to prevent Zn deficiency symptoms and the level of Zn required to obtain high grain Zn concentration can be substantial (Liu et al., 2017a). Liu et al., (2017b) stated that to reach the Zn biofortification “target value” of 45 mg kg^{-1} Zn in grain, the concentration of available Zn in soil should be ≤4.09 mg kg^{-1}. Also, there was a significant difference between co-additives AV and HA (Figure 4.6b). Results revealed a significant increase in Zn concentration in grain yield when AV was applied compared to HA treatment, although AV was not any different from the control (FC).

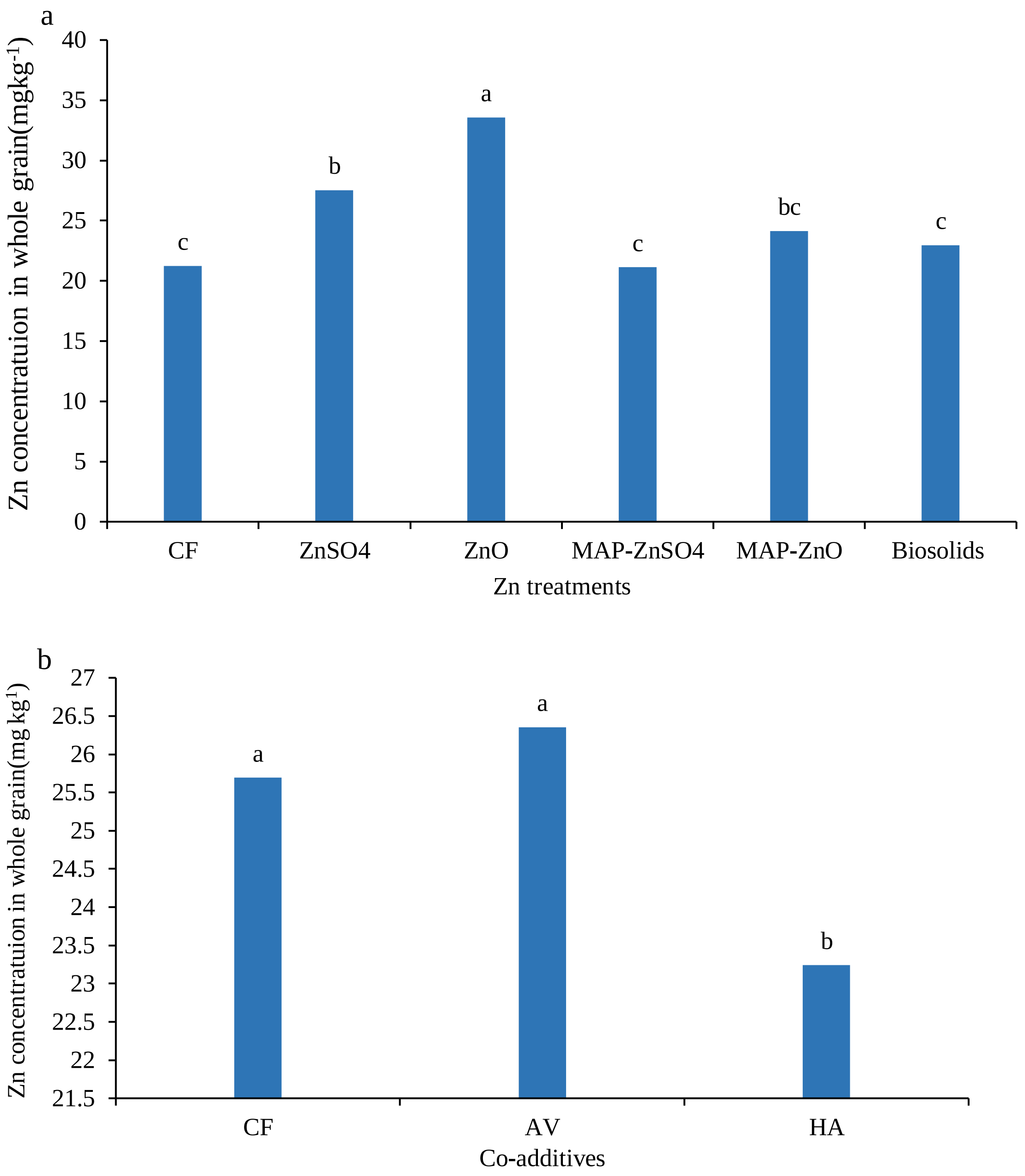

Figure 4.6 Zn concentration in whole grain (mg kg^{-1}), CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO-incorporated–MAP; Bio= biosolids; AV= AVAIL; HA= humic acid. Means within a Zn fertilizer treatment containing the same letter are not significantly different at $P = 0.05$ using Tukey’s honest significance.

To compare the biofortification efficiency of fertilizers, total Zn in whole grain was calculated per pot (Figure 4.7a). The interactions between Zn treatments and Co-additives for each treatment were not significant (*p*-value =0.52, Table 4.3). Zinc oxide produced the greatest Zn concentration in whole grain (2.3 µg Zn per pot). This followed by MAP-ZnO, $ZnSO_4$ that reached 1.83 and 1.79 µg Zn per pot, respectively (Figure 4.7a). Nevertheless, there was no significant deference between AV and HA (Figure 4.7b).

The interactions between Zn treatments and co-additives for the concentration of Zn in the white flour were significant. Figure 4.8 showed the concentration of Zn in the white flour. It shows that $ZnSO_4$, ZnO, ZnO+ AV, and ZnO+HA treatments produced significantly higher flour Zn concentrations compared to control. The Zn concentration in the flour increased as the Zn concentration in the grain increased. These results indicate that higher Zn concentrations in flour and grain can be synergistically achieved by Zn fertilization **(**Tang et al., 2008). The Zn concentrations in $ZnSO_4$ and ZnO treatments were 44.7 % and 35.8 %, respectively, higher than in control. Figure 4.9 shows the uptake of Zn in the white flour. Treatments ZnO, $ZnSO_4$, ZnO+ AV, and ZnO+HA had higher amount of Zn in flour compared to control. The fertilizer strategies used for agronomic biofortification of wheat with Zn can enhance the Zn concentrations both at the whole grain level and the endosperm level and this is crucial for target populations that consume large quantities of flour (Cakmak et al., 2010a; Kutman et al., 2011).

The Zn concentrations in bran were smaller than the concentrations in white flour. The interactions between Zn treatments and co-additives were not significant (*p*-value =0.26, Table 4.3). Figure 4.11 showed uptake in bran was small but still showed significant differences with the various of Zn treatments. Results showed that all treatments had significantly higher Zn concentrations in bran compared to control except MAP-$ZnSO_4$, and MAP-ZnO (Figure 4.10a).

It was also observed that bran Zn concentration differences were generally consistent across the treatments. The current results were consistent with a previous finding that Zn occurs largely in the aleurone layer, which is in the lateral and dorsal parts of the grain (Harland and Morris, 1995). The large difference in Zn concentration between white flour and bran might be inherent to these fractions relative to the total quantity of Zn in the wheat grain, indicating that the Zn application increased the Zn concentration in all fractions of wheat grain. There was no significant difference effect of co-additives (Figure 4.10b).

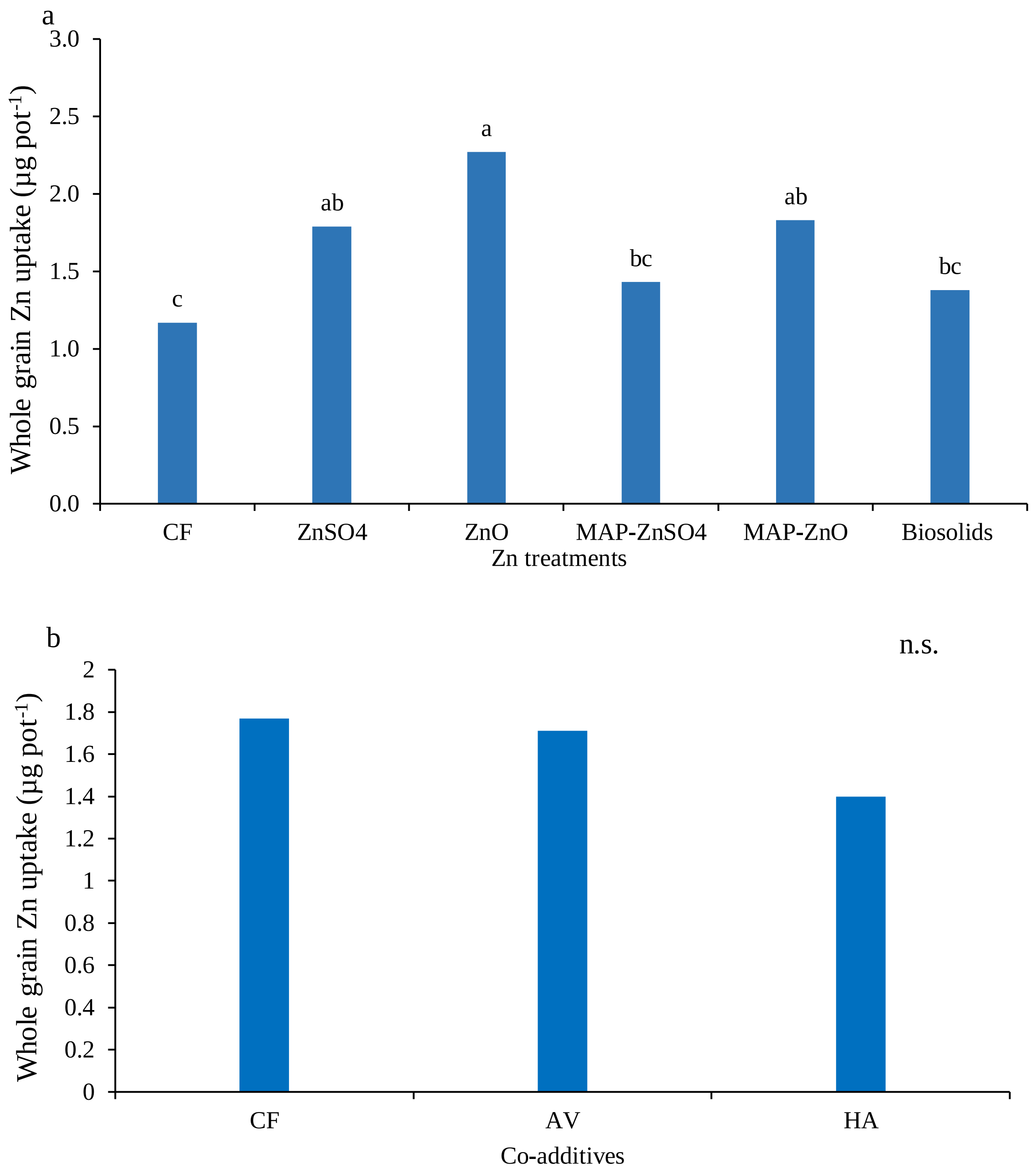

Figure 4.7 Whole grain Zn content (µg pot^{-1}), CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; HA= humic acid. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance. *NS=no significant difference in treatments.

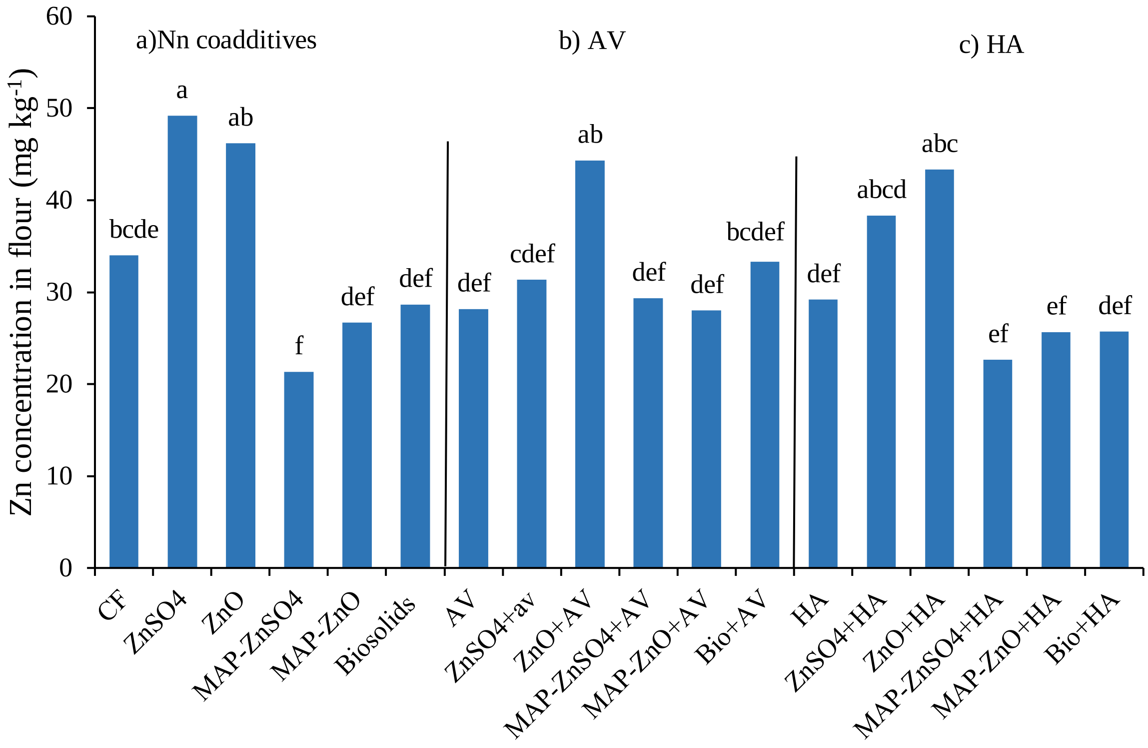

Figure 4.8 Zinc concentration in flour (mg kg^{-1}), CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance.

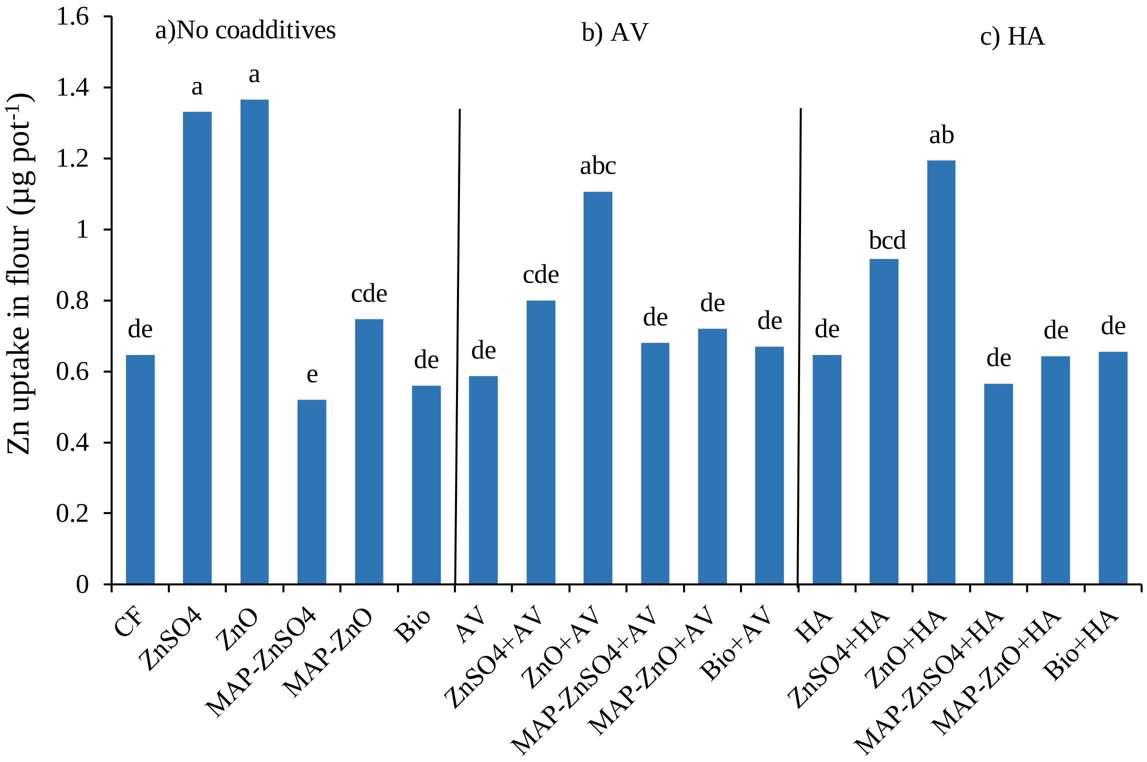

Figure 4.9 Total Zn content in flour (µg pot^{-1}), CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance. *NS=no significant difference in treatments.

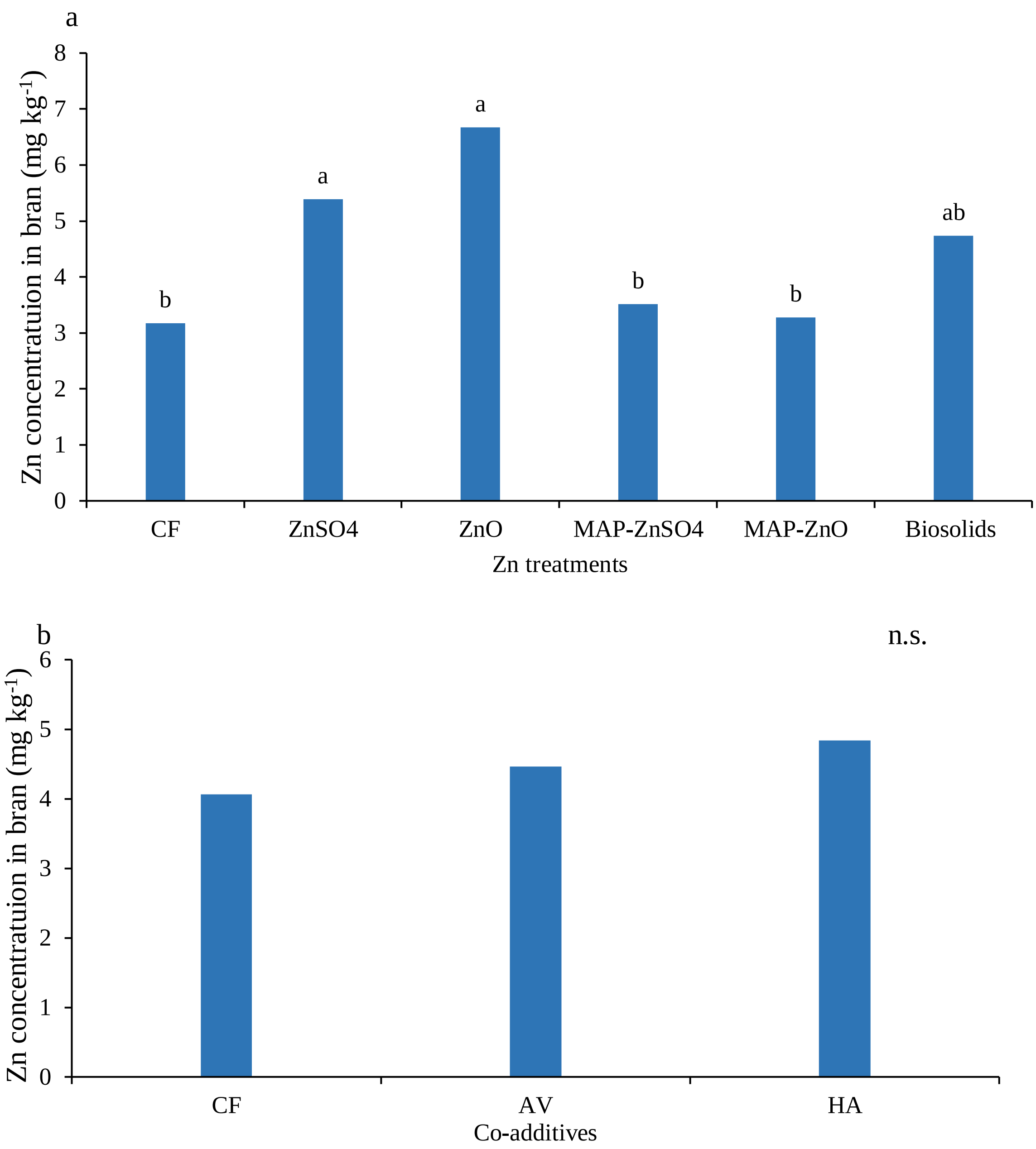

Figure 4.10 Zinc concentration in bran (mg kg^{-1}), CF= control with fertilizer; ZnSO$_4$= Zn sulfate; ZnO= Zn oxides; MAP-ZnSO$_4$= ZnSO$_4$ incorporated–MAP; MAP-ZnO= ZnO-incorporated–MAP; Bio= biosolids; AV= AVAIL; HA= humic acid. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance. *NS=no significant difference in treatments.

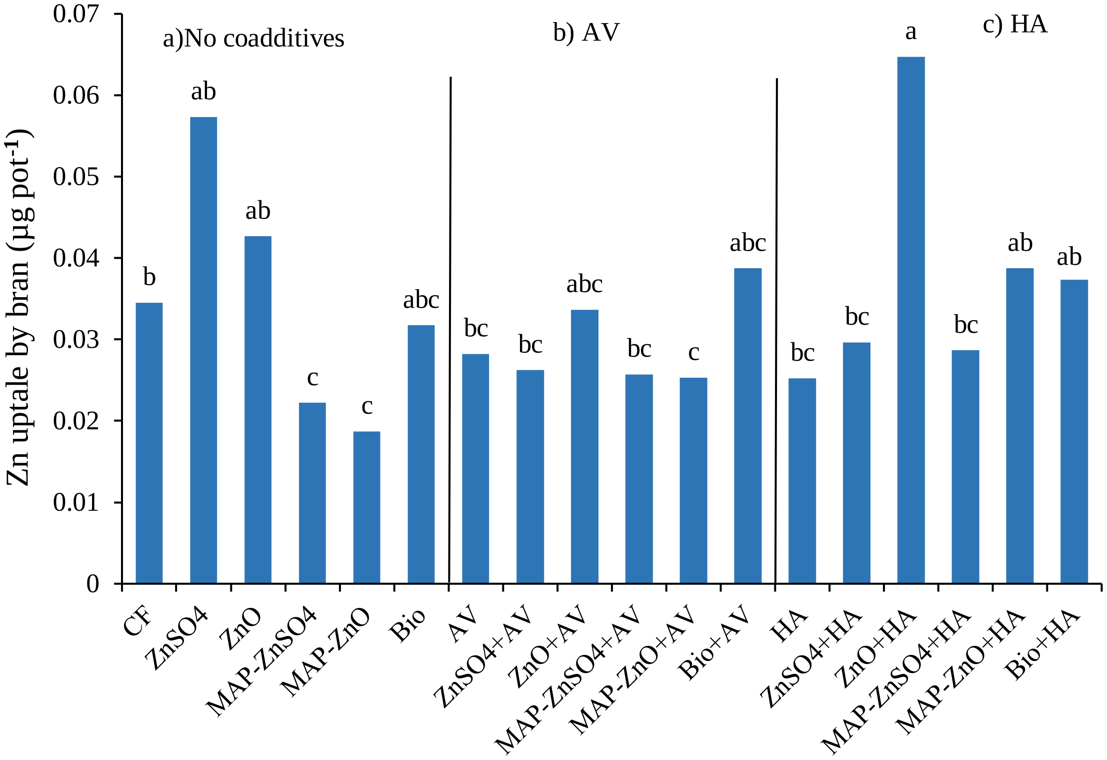

Figure 4.11 Zinc content in bran (µg pot^{-1}), CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance.

Table 4.3 The p-values for interaction treatments of Zn application effect on dry weight, concentration and uptake levels for within root, stem, whole grain, white flour and bran fraction along with total aboveground uptake levels in the plant. trt=Zn treatments; (F2) factor Co-additives (AV= AVAIL; HA= humic acid).

Effect	stem	Wholegrain	stem	wholegrain	flour	bran	Root	stem	whole	flour	bran	Total aboveground
	Biomass (dry weight)		………….. Zn concentrations…………….					………………………..….. Uptake ……...…………………				
trt	<.0001	<.0001	<.0001	<.0001	<.0001	0.0066	<.0001	0.0001	<.0001	<.0001	0.0033	<.0001
F2	0.4958	0.0300	0.4324	0.0075	0.2288	0.5634	0.0604	0.2503	0.9055	0.0379	0.1225	0.0320
trt*F2	0.7577	0.6999	**<.0001**	0.0550	**0.0127**	0.2668	**0.0005**	**<.0001**	0.5271	**0.0048**	**0.0103**	0.1205

Bioavailability of Zn in wheat white flour and wheat bran

Phytate (PA), which is the primary storage form of P in plants, can form chelates with divalent and trivalent metallic ions such as Zn, Fe, Mg, and Ca. Metal-phytate form poorly soluble compounds that are not readily absorbed in the gastrointestinal tract, thus decreasing their bioavailability (Gupta et al., 2016). The effect of PA on Zn absorption depends on the relative concentration of PA and Zn. The PA: Zn ratio is considered the best indicator of Zn bioavailability (Zhao et al., 2016).

Another way to increase Zn bioavailability in wheat flour is to decrease wheat flour phytate concentration. In our study, phytate concentration ranged from 334 to 366 mg100g^{-1}. Wheat flour phytate concentration significantly decreased with Zn application except with Bio+HA and MAP-ZnO+AV treatments. The decrease in the concentration of phytate in wheat flour might be related to an effect of Zn application on the uptake, translocation, and metabolism of P (Cakmak and Marschner 1986). Erdal et al. (2002) reported a decrease in grain phytate concentration with the application of Zn to Zn deficient calcareous soils. The decrease in grain phytate concentration could be related to Zn-induced changes in P uptake from the soil and translocation within the plant body (Huang et al., 2000).

Determining the bioavailability of micronutrients in plant food for human consumption is difficult but important. The PA: Zn molar ratio is widely used as an indicator to evaluate the bioavailable of Zn in plant food (Morris and Ellis, 1989). Usually, bioavailable Zn can be improved by keeping this ratio in a narrow range (15–20) and can be achieved by reducing phytate or enhancing Zn concentration (White and Broadley, 2011). Typically in ratios higher than 15 Zn absorption is considerably decreased. If affected by the decrease of PA to Zn molar ratio, Zn bioavailability cannot be less than 15. In this study, the PA: Zn ratio was decreased or influenced by Zn treatments to a much lower value (Figure 4.12a). The interactions between Zn treatments and Co-additives for each treatment were not significant (*p*=0.088). The reduction of PA:Zn ratio can be due to a simultaneous decrease in PA content and an increase of Zn in grain. The phytate-to-Zn molar ratio ranged from 7.1 to 12.2, indicating an overall desirable Zn bioavailability (based on [PA: Zn] ratio). Additionally, the PA: Zn ratio with the addition of Zn ($ZnSO_4$ and ZnO) to the soil was relatively lower than with the addition of granular Zn treatments (MAP-$ZnSO_4$ and MAP-ZnO). However, the increase of Zn in flour by Zn application may be remarkable because of the much lower phytate concentration and the existence of highly bioavailable ferritin in the endosperm (Zhang et al., 2010). Phytate is also affected by hydrolysis. This explains why bread has lower phytate content when compared to wheat flour. However, Co-additives (AV and HA) had no significant difference compared to control (*p*-value =0.1051). Abdoli et al., (2016) reported that Zn application methods decreased phytate contents in grain and decreased the PA: Zn molar ratio when compared with control. There was a minimum PA: Zn molar ratio of 8.8 and 10 in wheat grains (Abdoli et al., 2016). Human food is categorized as a low, medium, and high in Zn bioavailability for human consumption when it has a PA: Zn ratio of >15, 5–15 and <5 (Brown et al., 2001). Wheat grains generally have a PA: Zn ratio of > 25, whereas a PA: Zn ratio of < 15 is generally required for better human nutrition (Weaver and Kannan, 2002). According to Hussain et al., (2013) it was found that Zn applications decreased the PA: Zn ratio to values as low as 11 to 13, ensuring increased Zn bioavailability in humans. In addition to grain Zn concentration, grain Zn bioavailability is also crucial for human health. Phytic acid, already present in whole grains in relatively large quantities, can form insoluble complexes with Zn in the human intestine, thus reducing Zn absorption (Graham et al., 2007).

In wheat bran, the PA concentration ranged from 527 to 614 mg100g^{-1}. The phytate-to-Zn molar ratio ranged from 51 to 210, indicating that Zn bioavailability based on PA: Zn ratio was not enhanced in wheat bran to the desired levels. The PA: Zn molar ratios of the wheat bran (Figure 4.13) suggest that bran Zn bioavailability to humans is likely to be very low in all treatments. Bilgicli and Ibanoglu (2007) found that wheat bran PA ranging from 3116 to 5839 mg100g^{-1} dry weight.

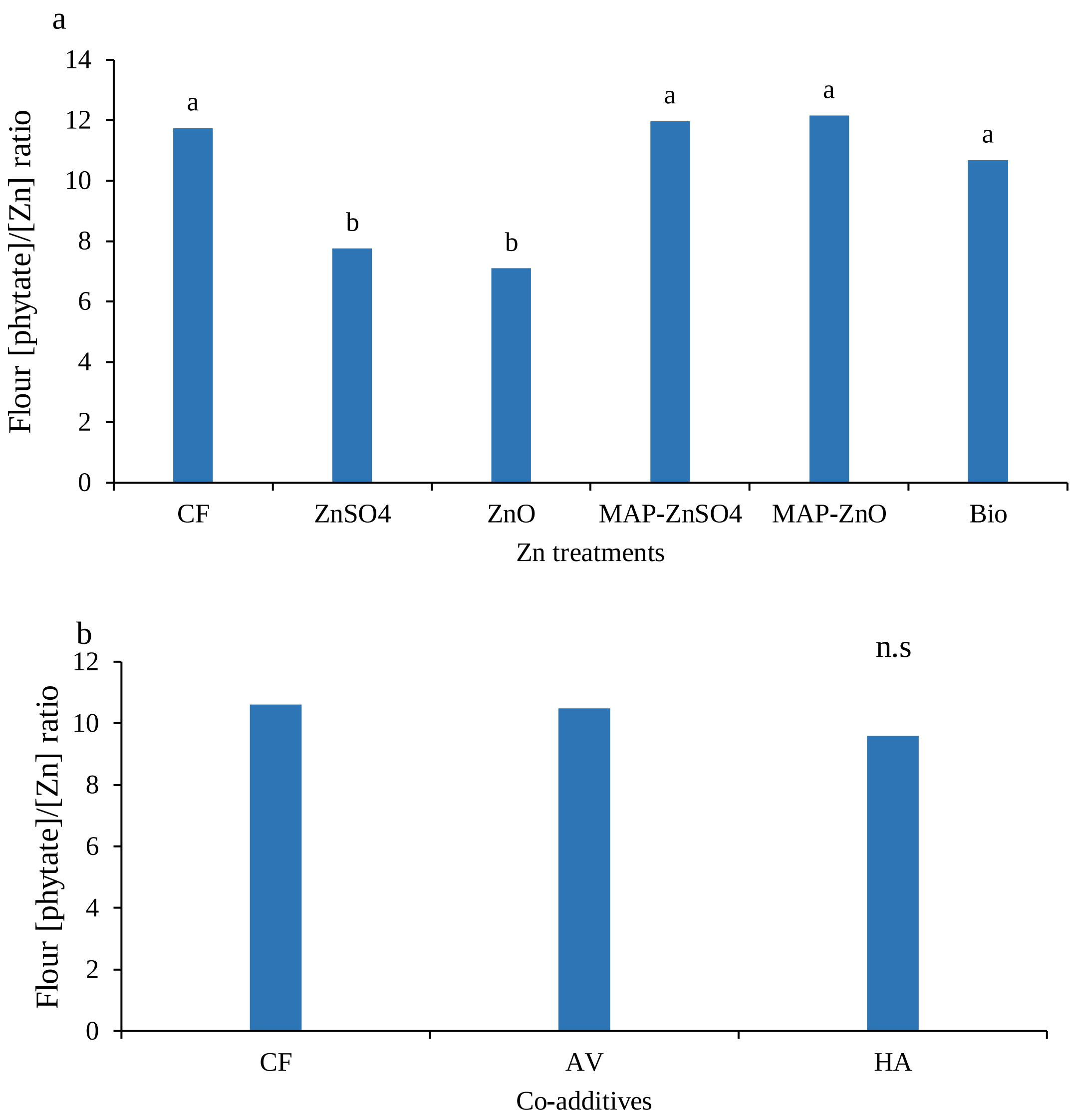

Figure 4.12 The [phytate]:[Zn] ratios in flour (mg100g^{-1}), CF= control with fertilizer; ZnSO$_4$= Zn sulfate; ZnO= Zn oxides; MAP-ZnSO$_4$ = ZnSO$_4$ incorporated–MAP; MAP-ZnO = ZnO-incorporated–MAP; Bio= biosolids; AV= AVAIL; HA= humic acid. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance. *NS=no significant difference in treatments.

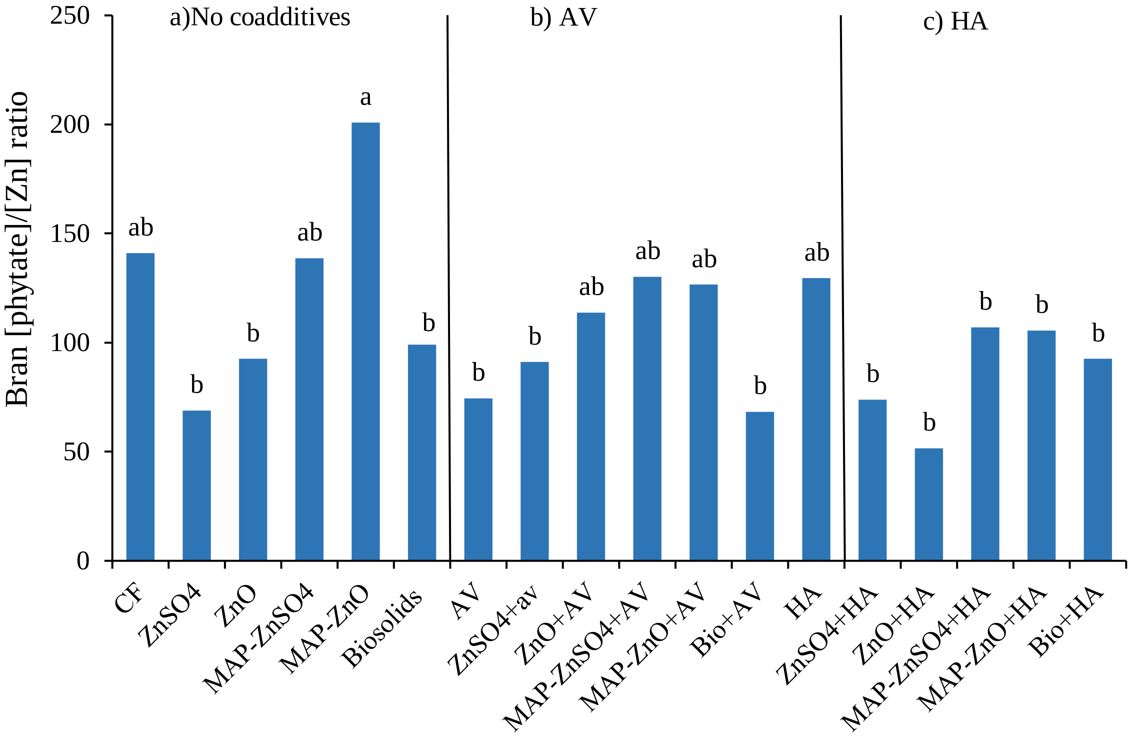

Figure 4.13 The [phytate]:[Zn] ratios in bran (mg100g^{-1}), CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO-incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance.

Table 4.4 The p-values for interaction treatments of Zn application effect on Zn bioavailability in white flour and bran. trt=Zn treatments; F2 factors; Co-additives (AV= AVAIL; HA= humic acid).

Effect	White flour	bran
	PA: Zn ratio	
trt	<.0001	0.0002
F2	0.1051	0.0103
trt*F2	0.0880	0.0143

Phosphorus, iron, and sulfur concentrations in wheat

Zinc treatments generally increased the grain concentrations of P, Fe, and S compared with control (Table 4.5). The concentration of P in biomass (stems) ranged from 346.9 to 1892.8 mg kg^{-1}. The largest P concentration was found in biomass treated with MAP-$ZnSO_4$+AV. The lowest P concentration was found in biomass grown in MAP-$ZnSO_4$+HA, but it was not statistically significantly different from the control. Phosphorus exerts P-Zn antagonism in plants (Singh et al., 1986). Phosphorus uptake in the plant stems ranged from 8.8 to 51 µg pot^{-1} (Table 4.6). The largest P concentration was found in biomass treated with MAP-$ZnSO_4$+AV, while the lowest P uptake was found in biomass grown in Bio+HA. According to Rengel and Graham (1996), soil Zn deficiency enhances plant P uptake and reduces Zn availability. Zinc application decreased grain phytic acid concentrations. This may be attributed to the inhibitory effect of Zn on root uptake and the accumulation of P in plant shoots (Erdal et al., 2002). According to Chattha et al. (2017), it is well-reported that Zn deficiency increases the potential of plants for P uptake.

The concentration of P in whole grain ranged from 2,437 to 4,839 mg kg^{-1} (Table 4.5). The highest P concentration was found in whole grain treated with MAP-ZnO+AV while the lowest P concentration was measured in whole grain grown in MAP-$ZnSO_4$+HA. According to Ertl and Goessler (2018), P ranges from 2,900 to 5,400 mg kg^{-1} and is far more present in grain and whole flour samples. This is in agreement with the results obtained in this study. In the literature, concentrations between 1,400 and 5,400 mg kg^{-1} in various grain samples were reported (Suchowilska et al., 2012; Araujo et al., 2007; and Ekholm et al., 2007). Total P uptake (content) in the whole grain ranged from 134 to 313 µg pot^{-1} (Table 4.6). The highest P uptake level was found in whole grain treated with MAP-ZnO+AV. The lowest P uptake level was measured in whole grain grown in Bio+AV.

The concentration of P in with flour arranged from 3,454 to 4,882 mg kg^{-1} (Table 4.5). The highest P concentration level was found in white flour treated with Bio+AV. The lowest P concentration level was measured in white flour in $ZnSO_4$+HA. According to Ertl and Goessler (2018), P ranges between 760 and 2,800 mg kg^{-1} in white flour and our values were greater than those reported by Ertl and Goessler (2018) study. Phosphorus uptake in the white flour ranged from 67 to 136.2 µg pot^{-1} (Table 4.6). The largest P uptake was found in white flour treated with MAP-ZnO. The lowest P uptake level was measured in white flour grown in Bio. However, the interactions between Zn treatments and Co-additives for each treatment were not significant (*p*-value =0.081, Table 4.6).

An increase in available P may also be ascribed to the decomposition of organic matter accompanied by the release of abundant CO_2 gas (Dash, 2018)**.** In calcareous soil, CO_2 gas plays a vital role in phosphate availability by lowering soil pH (Gaffar et al., 1992). Tandon (2005) reported that phosphate fixing capacity in soil was reduced due to the formation of protective cover on sesquioxide by organic matter. Higher grain yield also increases the phosphorus utilization by wheat grains (Singh et al., 2014).

Iron concentration ranged from 53.4 to 124.2 mg kg^{-1} in biomass (stems) (Table 4.5). The highest Fe concentration level was found in biomass treated with MAP-$ZnSO_4$. The lowest Fe concentration level was measured in biomass treated with ZnO+AV although it was not statistically significantly different from the control. Iron uptake in the plant biomass (stems) ranged from 0.8 to 4.2 µg pot^{-1} (Table 4.6). The highest Fe uptake level was found in biomass treated with MAP-$ZnSO_4$. The lowest Fe uptake level was measured in biomass treated with

$ZnSO_4$+HA. According to Kumar (2013), Zn applied the treatment, Zn concentrations in wheat shoots ranged from 12.3 to 26.9 mg kg^{-1}.

Iron concentration ranged from 32.6 to 79.8 mg kg^{-1} in whole grain (Table 4.5). According to Ertl and Goessler (2018)**,** mean Fe values ranged with an overall range between 3.1 and 42 mg kg^{-1} for grains. The highest Fe concentration level was found in whole grain treated with Bio while the lowest Fe concentration was measured in whole grain treated with Bio+AV, although it was not significantly lower than the control. Iron uptake in whole grain ranged from 1.5 to 4.9 µg pot^{-1} (Table 4.6). The highest Fe uptake level was found in whole grain treated with MAP-$ZnSO_4$+HA. The lowest Fe uptake level was measured in whole grain treated with Bio+AV. Again, the difference between the control and Bio+AV treatments was not statistically significant. According to Guttieri et al., (2015), consumption of whole grain food products has increased in popularity. Minerals are largely accumulated in the germ and bran of the wheat kernel. Fractions that are removed during conventional milling of grain into flour but are retained in whole grain products. Changes in whole grain mineral concentration have limited impact on milled flour mineral concentration (Peterson et al., 1986).

Iron concentration ranged from 19.6 to 27.2 mg kg^{-1} in white flour (Table 4.5). According to Ertl and Goessler (2018)**,** mean Fe values ranged with an overall range between 7.6 and 65 mg kg^{-1} for grains. This is in agreement with the results obtained in this study. White flour Fe concentration differences were statistically non-significant among the treatments. The literature is mainly available for whole wheat and wheat flour samples, and reported concentrations ranged between 13 and 103 mg kg^{-1} (Bermudez et al., 2011; Vrček and Vinković, 2012; Akinyele and Shokunbi, 2015; and Morgounov et al., 2007)**.** Iron uptake in white flour ranged from 0.4 to 0.7 µg pot^{-1}; neither treatments were statistically significantly different from the control (Table 4.6).

Iron concentration ranged from 46.8 to 148.9 mg kg^{-1} in the bran (Table 4.5). The highest Fe concentration level was found in bran treated with ZnO+AV. The lowest Fe concentration level was measured in bran treated with MAP-ZnO, but the differences were mostly non-significant. Iron uptake in bran ranged from 0.44 to 0.9 µg pot^{-1} (Table 4.6), and the differences were non-significant. Iron, Cu, and Mn concentration decrease with increasing Zn application, as reported by Adiloglu (2006).

Sulfur concentration ranged from 2,281 to 3,512 mg kg^{-1} in biomass (stems) (Table 4.5). The interactions between Zn treatments and Co-additives for each treatment were not significant (*p*-value=0.0940, Table 4.6). The highest S concentration was found in biomass treated with $ZnSO_4$. The lowest S concentration was measured in biomass treated with HA. Sulfur uptake ranged from 45 to 91 µg pot^{-1} in biomass (stems) (Table 4.6). The largest S uptake level was found in biomass treated with MAP-ZnO+HA. The lowest S uptake level was measured in biomass treated with AV and it was not statistically significantly different from the control.

Sulfur concentration ranged from 13,323 to 18,988 mg kg^{-1} in whole grain (Table 4.5). The interactions between Zn treatments and Co-additives for each treatment were not significant (*p*-value =0.9278, Table 4.6). The highest S concentration level was found in whole grain treated with MAP-$ZnSO_4$+AV. The lowest Fe concentration level was measured in whole grain treated with Bio. According to Ertl and Goessler (2018), S concentration range between 780 and 2,100 mg kg^{-1} for grain. Sulfur uptake ranged from 647 to 1,255 µg pot^{-1} in whole grain (Table 4.6). The largest S uptake level was found in whole grain treated with MAP-ZnO. The lowest S uptake level was measured in whole grain treated with Bio+AV, and again, it was not statistically significantly different from the control.

Sulfur concentration ranged from 13,763 to 18,110 mg kg^{-1} in white flour (Table 4.5). The highest S concentration level was found in white flour treated with MAP-ZnSO$_4$+AV. The lowest S concentration level was measured in white flour treated with Bio+HA. According to Ertl and Goessler (2018), S concentrations range between 880 and 1,900 mg kg^{-1} for grain. Sulfur uptake ranged from 273 to 498 µg pot^{-1} in white flour. The largest S uptake level was found in white flour treated with MAP-ZnO (Table 4.6).

Sulfur concentration ranged from 2,033 to 11,413 mg kg^{-1} in bran (Table 4.5). The highest S concentration level was found in bran treated with MAP-ZnSO$_4$. According to Ertl and Goessler (2018), S concentrations range between 880 and 1,900 mg kg^{-1} for grain. Sulfur uptake ranged from 12 to 70 µg pot^{-1} in the bran (Table 4.6). The highest S uptake level was found in bran treated with MAP-ZnSO$_4$, while the lowest S uptake level was measured in bran treated with ZnSO$_4$+HA. However, the interactions between Zn treatments and Co-additives for each treatment were not significant (*p*-value =0.5856, Table 4.6).

Table 4.5 Total P, S and Fe concentration stem, grain, flour , and bran (mg kg^{-1}) in wheat analyze at the end of the study as influenced by Zn fertilizer treatments, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance. *NS = no significance difference in treatments. *NS=no significant difference in treatments**.**

	Biomass			Whole grain			White flour			Bran		
Treatments	P	Fe	S	P	Fe	S	P*NS	Fe*NS	S	P	Fe	S
					……………………….mg kg^{-1}……………….							
CF	437.9fg	84.2abc	2751.3abc	2917.0bcd	39.2ab	14825cdef	4535.3	23.9	15926	978.8b	124.5ab	7771
ZnSO4	624.8cdefg	95.4abc	3511.7a	4289.3ab	54.7ab	17842ab	4198.0	22.8	17313	1025.4b	106.5abc	9601
ZnO	607.7defg	64.2bc	2806.0abc	4132.0abc	68.3ab	15480bcdef	4678.5	27.2	16169	1082.1b	95.3abc	7124
ZnSO4-MAP	417.0fg	124.2a	3058.3abc	3188.0abcd	49.8ab	16100bcdef	4786.0	24.8	16805	1022.9b	86.3bc	11413
ZnO-MAP	677.6cdef	83.9abc	3218.3abc	3689.7bcde	79.5a	16935abcd	4479.3	25.2	17677	941.1b	46.8c	8573
Bio	732.4ab	68.2abc	2677.7abc	2896.7abcd	79.8a	13323f	4533.	20.6	14098	1277.3b	108.7abc	7877
AVAIL	445.6efg	65.0abc	2362.7bc	3739.7abcd	55.1ab	14820cdef	4367.3	21.2	15255	1515.2ab	97.3abc	6351
ZnSO4-AV	958.3b	69.1abc	2947.3abc	3892.3abc	59.0ab	16224abcdef	4398.7	22.7	17431	790.7b	101.8abc	4311
ZnO+AV	882.9bcd	53.4bc	2912.3abc	3075.3bcd	70.4a	16582abcde	4819.7	23.7	16142	1863.3ab	148.9a	7502
ZnSO4-MAP+AV	1892.3a	75.6abc	3297.7ab	3012.7bcd	53.0ab	18988a	4868.3	25.7	18110	2908.3a	121.4ab	7169
ZnO-MAP+AV	668.8bcdef	73.1abc	2515.0abc	4839.7a	61.2ab	18156ab	4718.3	25.5	18064	1884.3ab	111.7abc	3048
Bio+AV	893.6ab	97.7ab	2719.3abc	3188.7bcd	32.6b	13807ef	4882.3	24.6	15622	1655.7ab	133.6ab	3668
HA	751.6bcd	67.8abc	2280.7c	2908.0bcd	78.2a	14115def	4796.0	24.7	14558	1207bb	130.4ab	3181
ZnSO4+HA	584.1defg	56.9c	2546.5abc	3238.0cde	72.9a	17337ab	3454.3	20.8	17833	786.07b	112.7abc	2033
ZnO+HA	600.0cdefg	83.2abc	2947.3abc	2611.0d	41.6abc	15979bcdef	4421.3	21.2	17123	1053.9b	95.8abc	3441
ZnSO4-MAP+HA	346.9g	59.3bc	3051.7abc	2436.7d	72.2ab	17741ab	3487.7	21.7	17193	538.8b	118.6ab	2792
ZnO-MAP+HA	598.8cdefg	81.8abc	3422.7a	2752.7cd	48.1ab	17648abc	4032.0	24.8	17252	1191.8bab	87.9bc	3941
Bio+HA	365.3b	64.6bc	2640.3abc	2866.3bcd	33.6b	14460def	4312.3	19.5	13763	1492.9ab	111.4abc	3914
P-value	**<.0001**	**0.0013**	**0.0379**	**0.0136**	**<.0001**	**0.0366**	0.4704	0.3803	0.1625	**0.0247**	**0.0136**	0.7019

Table 4.6 Total P, S and Fe uptake stem, grain, flour, and bran (µg pot^{-1}) in wheat analyze at the end of the study as influenced by Zn fertilizer treatments, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance. *NS = no significance difference in treatments.

Treatments	Biomass			Whole grain			White flour			Bran		
	P	Fe	S	P	Fe	S	P	Fe	S	P	Fe*ns	S
	……………………………………………………………………. µg kg^{-1}……………………………………………………..…………											
CF	8.8de	1.6bcde	54b	144f	1.9bc	732	85.3	0.4	300	6.3	0.84	52
ZnSO4	16.7bcde	2.7ab	84	284ab	3.6abcd	1186	124.3	0.6	485	6.5	0.77	67
ZnO	15.6bcde	1.6bcde	72	268abcd	4.4abc	1001	135.7	0.7	459	8.1	0.65	47
ZnSO4-MAP	10.6cde	4.2a	78	221.3abcde	3.4bcd	1110	109.8	0.6	412	5.8	0.53	70
ZnO-MAP	19.1bc	2.4bc	85	272.8abc	6.1a	1255	136.2	0.7	498	5.8	0.68	51
Bio	16.4bcde	1.5bcde	58	178.4cdef	4.0abcd	676	88.2	0.4	273	7.2	0.65	41
AV	8 e	1.1de	45	145f	2.6bcd	690	90.4	0.4	316	4.6	0.5	30
ZnSO4-AV	24b	1.8bcde	75	251abcde	3.8abcd	1055	110.7	0.6	440	5	0.52	22
ZnO+AV	19.4bcd	1.2de	70	182cdef	4.2abc	977	125.4	0.6	403	13.5	0.90	45
ZnSO4-MAP+AV	51.5a	2.0bcd	81	167.7def	2.7bcd	1080	111.7	0.6	421	16.6	0.71	31
ZnO-MAP+AV	18.8bcde	1.9bcde	79	313a	4.0abcd	1167	113	0.7	462	12.9	0.61	17
Bio+AV	17.8bcde	1.9abcd	53	134.6f	1.5d	647	98.7	0.5	317	8	0.63	19
HA	15bcde	1.4cde	47	155ef	3.8abcd	701d	101.2	0.5	321	7.5	0.89	20
ZnSO4+HA	8.7de	0.8e	47	209bcdef	4.8ab	1157	66.9	0.5	445	2.2	0.44	12
ZnO+HA	13.6bcde	2.0bcd	64	155.6ef	2.5bcd	959	129.8	0.6	470	4.5	0.59	21
ZnSO4-MAP+HA	8.5de	1.5cde	74	154.1ef	4.9ab	1128	103.1	0.5	431	3.3	0.71	15
ZnO-MAP+HA	15.3bcde	2.2bcd	91	181.6cdef	3.07bcd	1149	86.9	0.6	428	8.6	0.56	25
Bio+HA	9.3cde	1.4bcde	59	160.1ef	1.9cd	710	115.4	0.5	327	7.8	0.62	22
P-value	**<.0001**	**<.0001**	0.0940	**0.0046**	**0.0001**	0.9278	0.0815	0.5058	0.4426	0.0596	0.3088	0.5856

Select chemical and physical properties of soils after harvest

The physical and chemical characteristics of the soils are provided in Table 4.1. Initial soil pH ranged from 8.8, Olsen P concentration was 62 mg kg^{-1}, total organic C concentration was 1.11%. The reactive $CaCO_3$ was 5.59%. The total Zn concentrations of soils at the end of the study in untreated and treated soil are presented in Table 4.8. Total Zn, in untreated soils (CF, AV, HA), ranged from 51 to 53 with a mean of 52 mg kg^{-1} while in Zn treated soils, it ranged from 52 to 59 mg kg^{-1}. The total Zn concentration in soil at the harvest were significantly higher than control and was in the following order: MAP-$ZnSO_4$ +AV > ZnO+AV > MAP-$ZnSO_4$ > MAP-$ZnSO_4$+HA > $ZnSO_4$ > ZnO > $ZnSO_4$+AV > $ZnSO_4$+HA > MAP-ZnO > ZnO+HA > MAP-ZnO +AV > MAP-ZnO+HA > Bio > Bio+HA > AV > Bio+AV > CF > HA. Total Zn concentration below 18–20 mg kg^{-1} indicates possible Zn deficiency for some crops (Reuter and Robinson, 1997). On average, the total Zn concentration in our study was more than this potential threshold deficiency limit (Table 4.5). On the other hand, total concentration does not matter as it may or may not be available for the plant.

Table 4.7 Total Zn concentrations in soil at the end of study, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic.

Treatments	Zn mg kg^{-1}
CF	51.4
ZnSO4	58.5
ZnO	57.4
ZnSO4-MAP	58.7
ZnO-MAP	56.3
Bio	53.8
AV	52.7
ZnSO4+AV	57.0
ZnO+ AV	58.8
ZnSO4-MAP+AV	59.5
ZnO-MAP+AV	55.8
Bio+ AV	52.0
HA	51.1
ZnSO4+HA	57.0
ZnO+ HA	56.2
ZnSO4-MAP+HA	58.6
ZnO-MAP+HA	55.1
Bio+ HA	53.5

Soil pH

The pH of the soil was 8.8 initially. At the end of the study period, control soil had pH of 8.5. The pH of some treated soils was significantly lower compared to the control (Table 4.6). Soil pH is an important chemical property that affects nutrient availability and microbial activity. The availability of Zn in the soil increases with decreasing pH because the decreased pH weakens the affinity of soil to Zn by influencing the hydroxylation of Zn or the soil adsorption surface (Pardo and Guadalix, 1996; Msaky and Calvet, 1990).

Table 4.8 The effect of Zn fertilizer treatments on soil pH at the end of study, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zin oxides; $ZnSO_4$-MAP= $ZnSO_4$ incorporated–MAP; ZnO-MAP= ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; $ZnSO_4$-MAP+AV = $ZnSO_4$ incorporated– MAP with AVAIL; ZnO-MAP + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; $ZnSO_4$-MAP+HA= $ZnSO_4$ incorporated-MAP with humic; ZnO-MAP+ HA= ZnO-incorporated-MAP with humic; Bio+HA= biosolids with humic.

Treatments	pH
CF	8.5a
ZnSO4	8.4ab
ZnO	8.4ab
ZnSO4-MAP	8.3ab
ZnO-MAP	8.4ab
Bio	8.3ab
AV	8.4ab
ZnSO4+AV	8.3ab
ZnO+ AV	8.3ab
ZnSO4-MAP+AV	8.3ab
ZnO-MAP+AV	8.3ab
Bio+ AV	8.4ab
HA	8.3ab
ZnSO4+HA	8.3ab
ZnO+ HA	8.2b
ZnSO4-MAP+HA	8.2b
ZnO-MAP+HA	8.2b
Bio+ HA	8.4ab

DTPA-Extractable-Zn

The results of the DTPA extractions performed on soils collected after the wheat harvest are shown in Table 4.7. The DTPA extractable concentration of Zn in the original soil was closer to 1 mg/kg, indicating that this soil might be responsive to Zn fertilizers. The DTPA-extractable Zn concentrations were still higher with inorganic and organic Zn source treatments compared to control (Table 4.7). This could be due to its special physicochemical characteristics: a calcareous soil with a high pH and very low organic matter content. This extractant removes Zn from the labile pool of Zn in soils (Lopez and Graham, 1972). Iyengar and Deb (1977), reported that DTPA, like EDTA $(NH_4)_2$ $CO_{3,}$ could extract water-soluble, exchangeable, and complexed and portions of precipitated Zn fractions. There are many reports which support DTPA as a better extractant for assessing plant available Zn from a wide range of soils. Results showed significantly higher DTPA-extractable Zn in several different Zn treatments compared to the controls (CF, AVAIL, and HA) (Table 4.4).

Table 4.9 DTPA-extractable Zn (mg kg^{-1}) in soil at the end of the study as influenced by Zn fertilizer treatments, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; $ZnSO_4$-MAP= $ZnSO_4$ incorporated–MAP; ZnO-MAP= ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; $ZnSO_4$-MAP+AV = $ZnSO_4$ incorporated– MAP with AVAIL; ZnO-MAP + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; $ZnSO_4$-MAP+HA= $ZnSO_4$ incorporated-MAP with humic; ZnO-MAP+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic.

Treatments	DTPA-extractable Zn	% Increase/Decrease
CF	0.84 g	-
ZnSO4	3.12 ab	271
ZnO	2.64 abcd	213
ZnSO4-MAP	1.94 cdef	131
ZnO-MAP	2.10 bcde	150
Bio	1.16 efg	38
AVAIL	0.80 g	-5
ZnSO4-AV	3.16 a	275
ZnO+AV	2.87 abc	241
ZnSO4-MAP+AV	2.28 abcd	171
ZnO-MAP+AV	1.78 defg	112
Bio+AV	1.09 efg	30
HA	0.82 g	-2
ZnSO4+HA	3.19 a	280
ZnO+HA	3.26 a	288
ZnSO4-MAP+HA	2.02 cdef	140
ZnO-MAP+HA	1.90 cdef	126
Bio+HA	1.04 gf	23

The effect of Zn treatments on the distribution of Zn in different soil fractions

The Zn adsorption-desorption reactions between the solution and solid phases control Zn concentrations in soil solution and the availability of Zn to plants (Catlett et al., 2002; Lindsay, 1991). As mentioned before, this is dependent on the pH, organic matter, soil minerals, and co-existing ions, as well as the distribution into various fractions (Alloway, 2008). Chemical forms of Zn in soil were extracted following the Tessier sequential extraction procedure (Tessier et al., 1979), and results are presented in Table 4.8 and Table 4.9 as % total Zn extracted in soil. The Zn concentrations in the soil fractions depend on the different fertilizer treatments. In the original soil, the order for the different Zn fractions was as follows: Residual > Fe-Mn oxide-bound > carbonate-bound > exchangeable > organic-bound > water-soluble. It is evident from the data presented in Table 4.5 that the distribution of different fractions of Zn in soil was significantly affected by Zn sources but followed that same order.

We observed considerable increases in all Zn fractions with fertilizer treatments (Table 4.8). The residual Zn (Res-Zn) fraction was predominant and ranged from 26.91% to 30.89% of the total Zn in soil. This was followed by the Fe-Mn oxide-bound-Zn fraction, ranging from 7.1% to 10.6% of the total Zn in soil compared to control (Table 4.9). Obrador et al., (2003) found that about 92% of Zn was found as Res-Zn in calcareous soil (pH 8.3) with the Zn-EDTA amendment after a maize crop.

The distribution of Zn among various chemical forms may vary significantly in response to changing soil properties (Adhikari and Rattan, 2007). Kiekens (1980), stated that there appeared to be two different mechanisms involved in the adsorption of Zn by clays and organic matter. One mechanism operates mainly in acid conditions and is closely related to cation exchange. The other mechanism operates in alkaline conditions mainly involves with chemisorption and complexation by organic ligands. Apart from reversible adsorption by cation exchange, Zn can also be absorbed irreversibly by lattice penetration in clay minerals. The latter mechanism fixes the amount of Zn in excess of the cation exchange capacity. This may be due to the sorption of Zn in a hydrolyzed form and precipitation of $Zn(OH)_2$. This 'fixation' of Zn tends to increase over time and can affect the long-term availability of Zn fertilizers. At low pH, Zn is held in an exchangeable form at the basal planes of clay minerals, whereas at higher pH, Zn could be adsorbed by edge sites of clay minerals (McBride, 1994).

In the present study, the water-soluble Zn fraction was the smallest fraction and varied from 0.1% to 0.5% of the total Zn in soil (0.1 to 0.3 mg kg^{-1}) due to its high pH (Lindsay, 1979). The addition of Zn treatments did produce an increase in Zn in the most labile fraction (Zn-water-soluble) after the wheat harvest. This fraction is depleted by crop uptake, and the physicochemical characteristics of this soil (e.g., the high montmorillonite clay content, pH, and the presence of $CaCO_3$) can result in less lability of Zn complexes. Tiller et al., (1984) found that with soil clay containing a high proportion of montmorillonite, specific Zn sorption was still high after two weeks. In addition, Jeffery and Uren (1983), found that Zn availability decreases to very low levels at high soil pH. The exchangeable Zn fraction varied between treatments and values ranged from 0.3 to 2.1 mg kg^{-1}. The addition of Zn fertilizers to this soil had a significant effect on the Zn content in the fractions exchangeable (0.5-3.5% of the total Zn in soil). The carbonate-bound Zn fraction varied between treatments, and values ranged from 0.2 to 2.6 mg kg^{-1} (0.4- 4.6% of the total Zn in soil). The Fe-Mn oxides-bound varied over a wider range (3.3-6.7 mg kg^{-1}) (6.2- 12.2% of the total Zn in soil) among treatments. Zinc in the crystalline oxides of Fe will not be available to the wheat plant because of a strong chemical bond between the heavy metals such as Zn and oxide surfaces. The distribution of the organic-matter-bound Zn fraction was diverse from 0.1 to 0.7 mg kg^{-1} (0.1-1.1% of the total Zn in soil). Soil pH and organic matter level will markedly alter the distribution of Zn among the plant's available pools. Similar results were obtained by Adhikari and Rattan (2007) and Sharad and Sharad (2001). The results of this study indicated that Zn in water-soluble, exchangeable, and organic complexes were the fractions (pools) that influence the plant available Zn in the soil. The acid-soluble fraction shows the amount of each element that may be released into the environment if the conditions become acidic. On the other hand, the exchangeable-Zn and organic-Zn fractions are considered the most labile Zn pools in soils (Iyengar et al., 1981). The reducible fraction theoretically represents the contents of each metal bound to iron and manganese oxides that would be released if the sediment were subjected to more reducing conditions. Mahin et al., (2009) mentioned that the phytoavailability of soil micronutrients depends on soil properties, such as total micronutrient concentrations, pH, calcium carbonate ($CaCO_3$) content, OM content, soil moisture conditions, and available P. A perusal of the data (Table 4.8) revealed that Zn in water-soluble, exchangeable, and organic complexes fractions (pools) governed the plant-available Zn in the soil. Differences had previously been reported between cultivated and not cultivated soils. This was even between soils cultivated with different plants (Ahumada et al., 1999). These differences would be due to the physicochemical changes produced in the soil as a consequence of the crop. In the greenhouse experiment conducted by Lopez-Valdivia et al., (2002) it was found that Zn speciation in the soil after harvesting showed that almost all Zn was found in the residual fraction followed by metal in the water-soluble plus exchangeable fraction and metal bound to organic matter, which is in good agreement with results obtained in this study.

Table 4.10 Zinc fractions (mg kg^{-1}) in soil at the end of the study as influenced by Zn fertilizer treatments. CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated–MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. †NS=no significant difference in treatments.

	Zn mg kg-1					
Treatments	Water soluble †ns	Exchangeable	Carbonate-bound	Fe-Mn−1 oxide bound	Organic-bound*NS	Residual
Original soil	0.09†	0.34	0.46	3.5	0.5	18.5
CF	0.11b	0.39c	0.3g	3.3c	0.36	14.8ab
ZnSO4	0.17ab	0.79bc	1.6cd	5.5ab	0.4	15.8ab
ZnO	0.08b	0.74c	1.5cd	4.9ab	0.36	16ab
ZnSO4-MAP	0.13b	1.0bc	2bc	5.1ab	0.67	15.4ab
ZnO-MAP	0.12b	0.6c	1def	4.9ab	0.33	17.4a
Bio	0.11b	0.31c	0.7efg	3.8bc	0.4	15.1ab
AVAIL	0.11b	0.29c	0.2g	3.3c	0.29	15b
ZnSO4+AV	0.12b	0.73c	1.7bcd	5.2ab	0.25	15.7ab
ZnO+ AV	0.12b	0.72c	1.6cd	4.9ab	0.2	16.1ab
ZnSO4-MAP+AV	0.13b	1.86ab	2.7a	6.3ab	0.26	16.2ab
ZnO-MAP+AV	0.17ab	0.65c	1.2def	4.5ab	0.37	15.5b
Bio+ AV	0.12b	0.29c	0.5fg	4.1abc	0.31	15.8ab
HA	0.12b	0.46c	0.9ab	4abc	0.47	15.7ab
ZnSO4+HA	0.11b	0.71c	1.5cd	4.8ab	0.19	15.9ab
ZnO+ HA	0.11b	0.83bc	1.7bcd	5.5ab	0.2	15.7ab
ZnSO4-MAP+HA	0.18ab	2.06a	2.1abc	6ab	0.3	16.8ab
ZnO-MAP+HA	0.25a	0.74c	1.4cde	6.7a	0.26	15.7ab
Bio +HA	0.17ab	0.35c	0.5fg	4.9ab	0.14	15.1ab

†ns= No significant

Table 4.11 Zinc fractions as % total extracted in soil at the end of the study as influenced by Zn fertilizer treatments. CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. *NS=no significant difference in treatments.

Treatments	water soluble	exchangeable	carbonate-bound	Fe-Mn−1oxide-bound	organic-bound*NS	residual+unrecovered	∑Zn %
CF	0.2b	0.8c	0.5g	6.5c	0.7	91.4a	100.0
ZnSO4	0.3ab	1.3bc	2.7cd	9.4ab	0.7	85.5ab	100.0
ZnO	0.1b	1.3bc	2.6cd	8.6ab	0.6	86.8ab	100.0
ZnSO4-MAP	0.2b	1.7bc	3.4bc	8.7ab	1.1	84.8ab	100.0
ZnO – MAP	0.2b	1.1c	1.9def	8.6ab	0.6	87.6ab	100.0
Bio	0.2b	0.6c	1.4efg	7.1bc	0.7	90.0a	100.0
AV	0.2b	0.5c	0.4g	6.2c	0.6	92.1a	100.0
ZnSO4 + AV	0.2b	1.3c	2.9bcd	9.2ab	0.4	86.0ab	100.0
ZnO+ AV	0.2b	1.2c	2.7cd	8.4ab	0.3	87.1ab	100.0
ZnSO4 – MAP+AV	0.2b	3.1ab	4.6a	10.6ab	0.4	81.0b	100.0
ZnO– MAP+AV	0.3ab	1.2c	2.1def	8.1ab	0.7	87.6ab	100.0
Bio + AV	0.2b	0.5c	1.0fg	8.0abc	0.6	89.7a	100.0
HA	0.2b	0.9c	1.8ab	7.8abc	0.9	88.4ab	100.0
ZnSO4+ HA	0.2b	1.2c	2.5cd	8.5ab	0.3	87.2ab	100.0
ZnO+ HA	0.2b	1.5bc	3.0bcd	9.7ab	0.3	85.2ab	100.0
ZnSO4 – MAP+HA	0.3ab	3.5a	3.6abc	10.3a	0.5	81.8ab	100.0
ZnO– MAP+HA	0.5a	1.3bc	2.5cde	12.2a	0.5	83.1ab	100.0
Bio +HA	0.3ab	0.6c	1.0fg	9.1ab	0.3	88.7ab	100.0

Correlations of soil Zn concentrations with grain yield

There were weak positive correlations of Zn uptake in whole wheat and the total concentration of Zn in the soil ($R^2 = 0.27$, $P < 0.05$) (Figure 4.14**)**. This is not surprising as we know that the total Zn content of fertilizer is not enough to successfully determine the Zn uptake of a plant or crop, because the highest percentage of available Zn values were observed for MAP-$ZnSO_4$ treatments. In general, all inorganic Zn sources resulted in significantly higher available Zn values compared to the control (Figure 4.15).

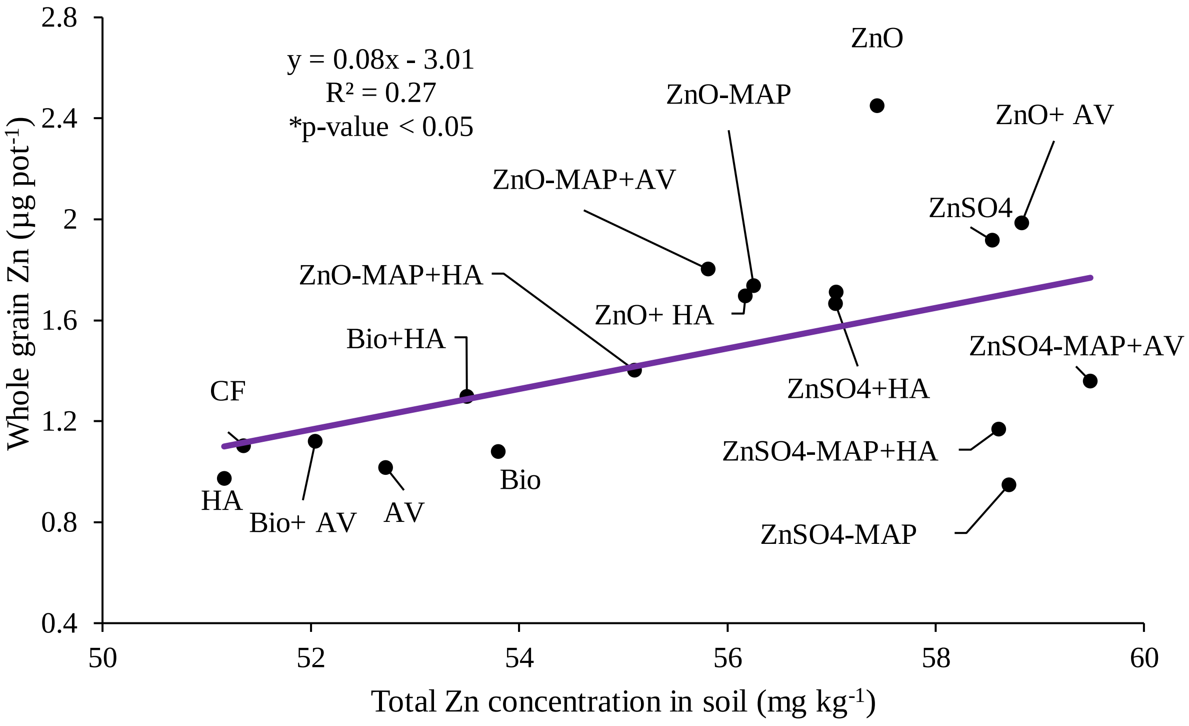

Figure 4.14 The relationship between whole grain Zn (µg pot^{-1}) and total soil Zn mg kg^{-1}. CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance. *NS=no significant difference in treatments.

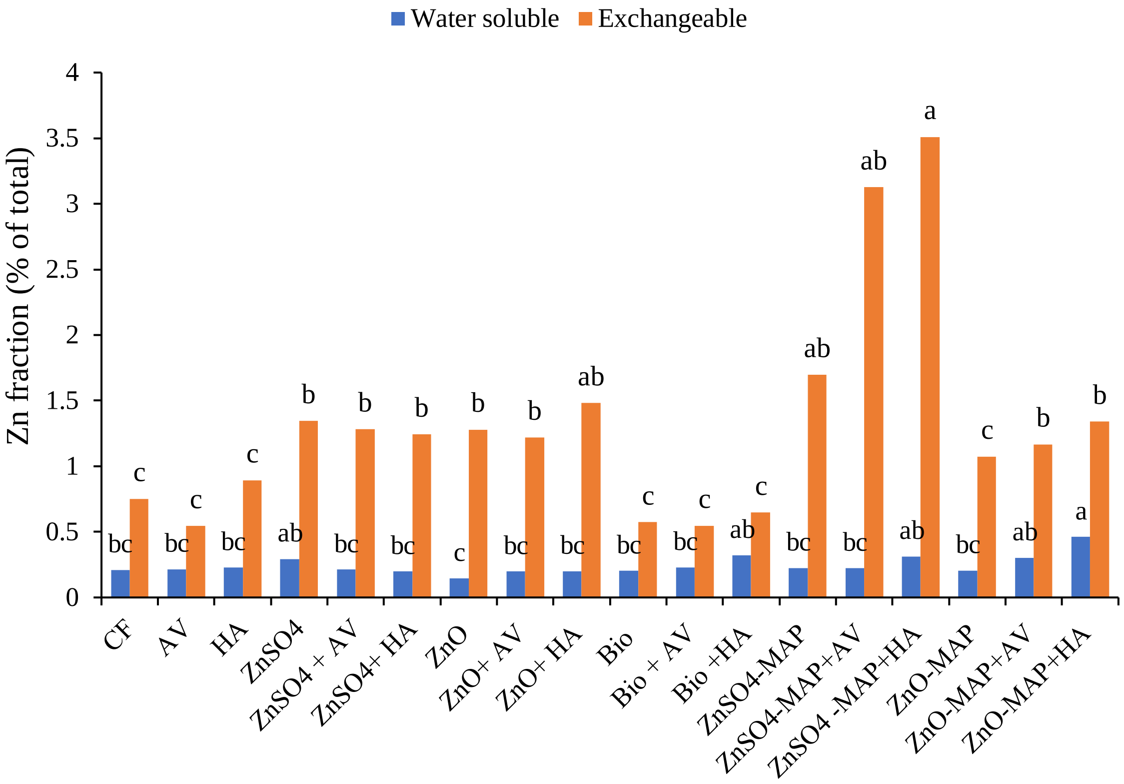

Figure 4.15 Effect of percentage most available Zn fractions in soils in the fertilizer Zn application rates. CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Bars with different letters are significantly different at the 5% level by the LSD test. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance. *NS=no significant difference in treatments.

The correlation coefficient found between whole-grain Zn uptake (µg pot^{-1}) and available Zn concentration mg kg^{-1} (sum of water-soluble and exchangeable mg kg^{-1}) in soil (Figure 4.16) was not significant. According to Guttieri et al. (2015), it was discovered that Fe and Zn concentrations were negatively correlated with grain yield. The reduced mobility and rapid adsorption of Zn by clay minerals are well-known in soils having low moisture, high pH, and low organic matter (Cakmak, 2008; Alloway, 2004). This leads to low availability of soil Zn or fertilizer Zn to roots, while our soil at all treatments had a pH of 8.8 (Table 4.1). Concentrations of Zn were very weak and negatively correlated between the water-soluble Zn and the whole grain Zn ($r= 0.0013$ $p< 0.05$) in wheat. Exchangeable-Zn is the most labile binding form and has the closest correlation with Zn uptake in plants **(**Chahal et al., 2005; Li et al., 2007). Concentrations of Zn were very weak and negatively correlated between the soil sum of fractions of Zn and the whole grain Zn ($r= 0.061$ $p< 0.05$) in wheat (Figure 4.17). Zinc concentration was negatively correlated with grain yield, similar to correlations observed in central Asian spring wheat ($r = -0.4$) (Morgounov et al., 2007). Other studies also found negative correlations of grain yield with mineral (P, Cu, Fe and Zn) concentrations (Hussain et al., 2010; McDonald et al., 2008; Fan et al., 2008; Garvin et al., 2006; Oury et al., 2006; Peterson et al., 1983). Exchangeable Zn is the most labile binding form and has the closest correlation with Zn uptake in plants (Li et al., 2007; Chahal et al., 2005). However, in our study, the Exchangeable-Zn fraction concentration had no significant correlation with Zn concentration and uptake in wheat grains. In previous research, $ZnSO_4$ was applied to soil that resulted in exchangeable-Zn concentrations higher than 1000 µg kg^{-1}, which were maintained throughout the entire wheat growth period, but had no significant effect on wheat grain Zn concentration (Lu et al., 2012).

These results indicate that the calculated Zn^{2+} activity is not always equal to the actual Zn^{2+} activity at the plasma membrane (Nyaki and Racz, 2003).

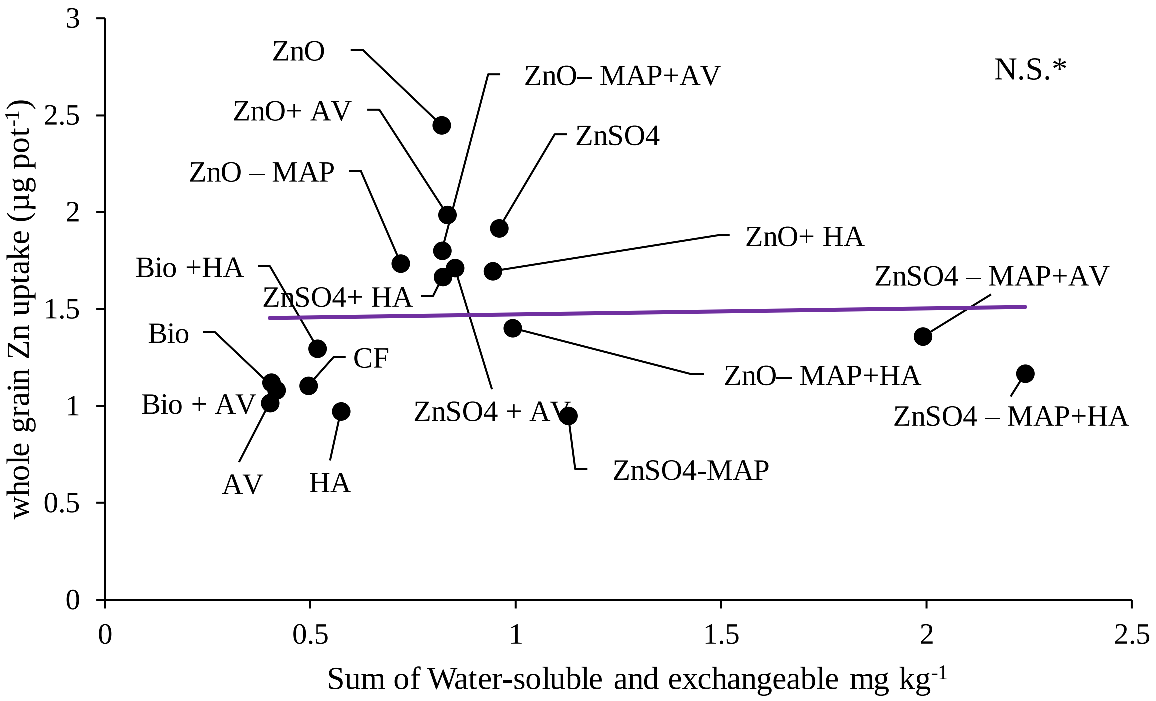

Figure 4.16 The relationship between whole grain Zn (µg pot^{-1}) and available soil Zn mg kg^{-1}. CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at P = 0.05 using Tukey's honest significance. *NS=no significant difference in treatments.

The relationship between whole grain Zn uptake (µg pot^{-1}) and the sum of water-soluble, exchangeable, and carbonate-bound mg kg^{-1} in the soil (Figure 4.17) was not significant. Plants can mainly take up Zn in the exchangeable and carbonate-bound fractions (Sungur et al. 2014). We observed that organic matter-bound Zn in soil declined significantly after harvest. This further indicates that organic matter-bound fractions were more available to the plants than the other fractions. The availability of Zn in soil increased with the enhanced transformation of Zn from tight organic matter-bound (Li et al., 2007). Guo et al. (2016) showed that the available Zn concentration was mainly influenced by exchangeable, light organic matter- and carbonate-bound fractions.

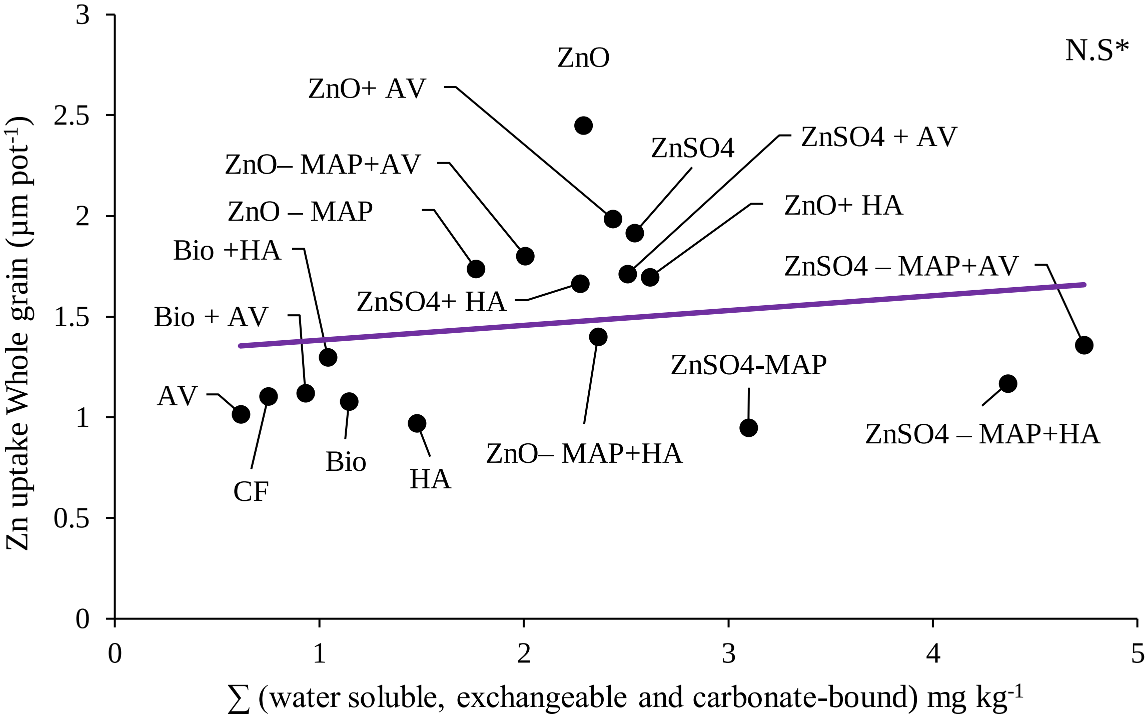

Figure 4.17 The relationship between whole grain Zn uptake (µg/pot) and sum of water soluble, exchangeable and carbonate-bound mg kg^{-1} in the soil, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO = ZnO-incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+ AV= $ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV = $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO- incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$ +HA= $ZnSO_4$ with humic; ZnO-HA= ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+ HA= ZnO- incorporated-MAP with humic; Bio+HA= biosolids with humic. Means within a Zn fertilizer treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance. *NS=no significant difference in treatments.

According to the results, there were strong positive correlations of plant Zn uptake in whole grain and Zn concentration in whole grain with DTPA-extractable-Zn mg kg^{-1} in the soil ($R^2 = 0.49; 0.38; P < 0.05$) (Figures 4.18 and 4.19**)**. On the other hand, the effects of agronomic biofortification treatments on increasing DTPA-extractable Zn were translated into significant correlations of Zn uptake and Zn concentration in whole grain with DTPA-extractable-Zn. The concentrations of DTPA-extractable-Zn in the treatments without agronomic biofortification treatments were under the critical levels. Our results demonstrated that agronomy biofortification played a significant role in altering soil Zn availability, resulting in better Zn uptake and utilization as a result of the continued Zn uptake behavior.

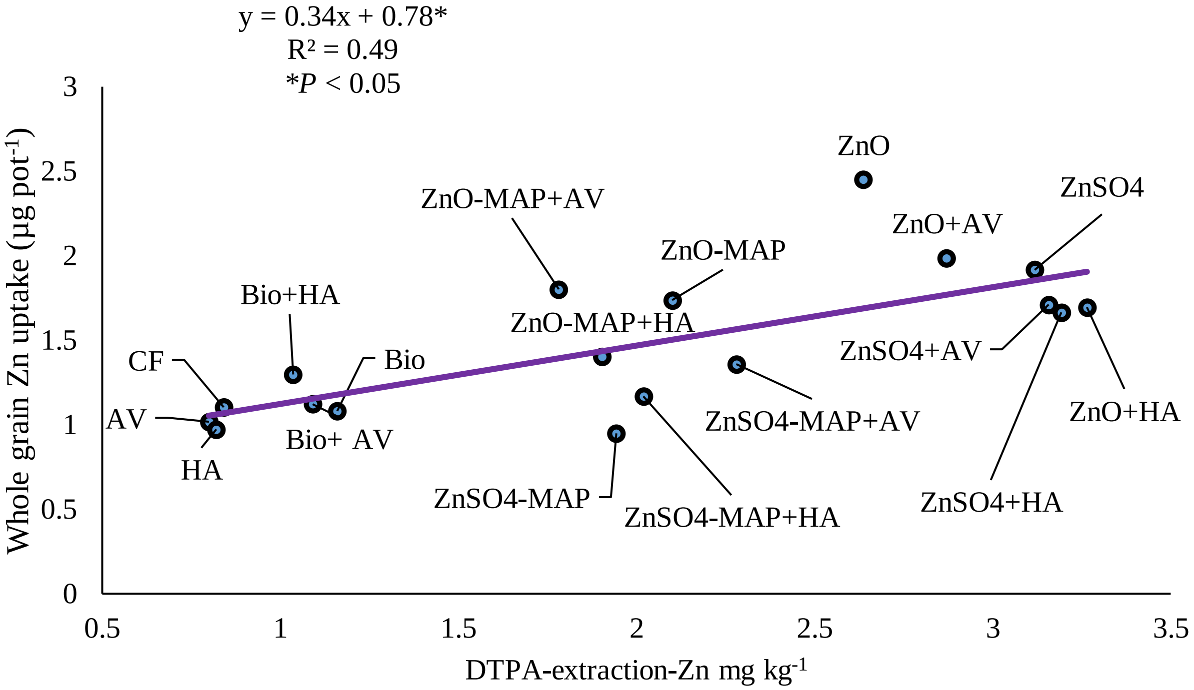

Figure 4.18 The relationship between whole grain uptake (µg pot^{-1}) and DTPA-extractable-Zn (mg kg^{-1}) in the soil, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO =ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+AV=$ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV= $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$+HA= $ZnSO_4$ with humic; ZnO-HA=ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+HA= ZnO-incorporated-MAP with humic; Bio+HA= biosolids with humic.

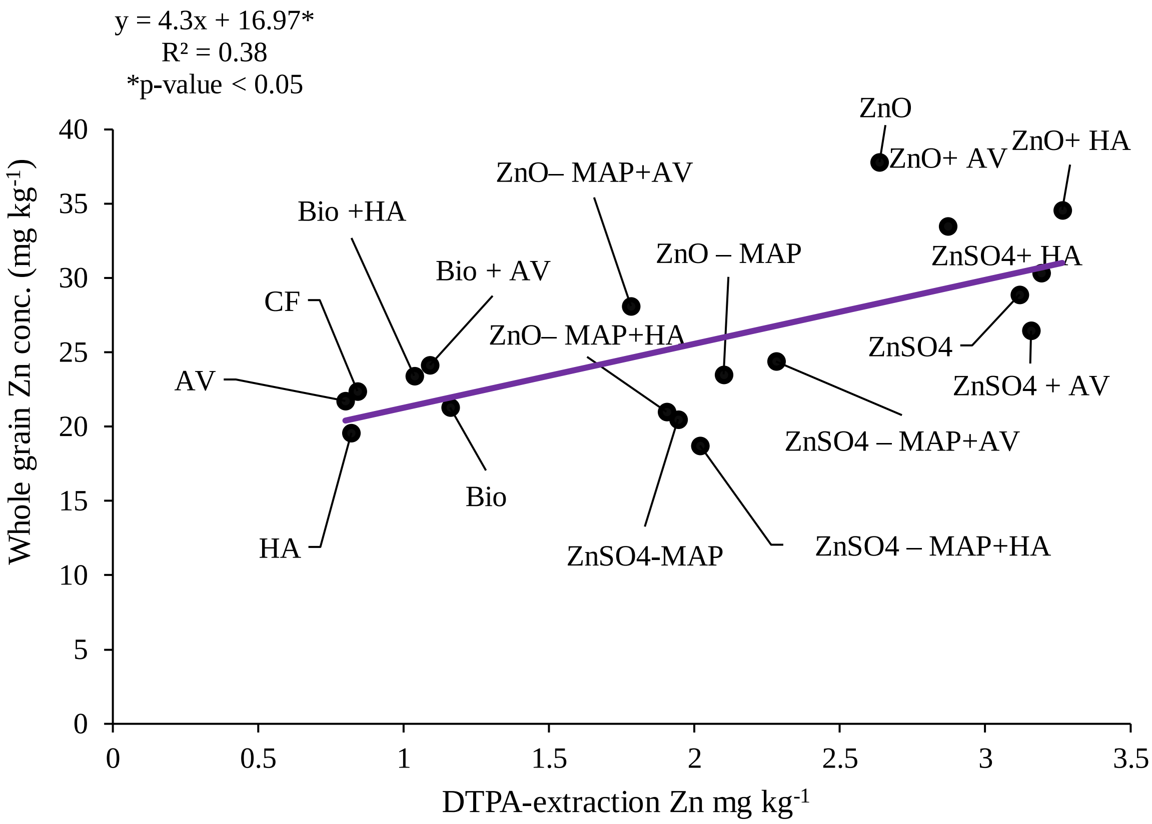

Figure 4.19 The relationship between whole grain Zn concentration and DTPA-extractable-Zn mg kg^{-1} in the soil. CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO =ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+AV=$ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV= $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$+HA= $ZnSO_4$ with humic; ZnO-HA=ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+HA= ZnO-incorporated-MAP with humic; Bio+HA= biosolids with humic.

There was a significant positive correlation between whole grain Zn uptake and stem Zn uptake ($r= 0.34$ $P< 0.05$) (Figure 4.20). An increase in stem Zn uptake substantially enhanced whole grain Zn uptake. Zinc uptake and concentration between plant parts and grain were all positively correlated. This suggests remobilization of Zn from other parts into the grain and is in agreement to the report by Stomph et al., (2014). The report states that Zn remobilized from plant parts to the grain is replaced by Zn taken up by the roots in order to maintain plant organ requirements for Zn. This shows that Zn remobilized from other organs to the grain can be compensated by root uptake. This could be explained by the positive correlations observed between plant part Zn and grain Zn, either in uptake or concentrations. Interestingly, this also showed positive correlations between whole grain concentration Zn and grain Zn uptake, with the highest correlation coefficient ($r=0.32 – 0.34$, $P<0.05$) observed between stem Zn uptake and grain Zn uptake. Under field conditions, root Zn uptake is often limited due to low water availability and reduced root activity. Consequently, continuous root uptake, shoot transport and grain deposition of Zn are limited (Waters and Grusak, 2008; Cakmak et al., 2010). Under unfavorable field conditions, levels of grain Zn are mainly affected by the pool of Zn in vegetative tissue and its remobilization into the grain. As reported previously, stem and leaf tissues represent important Zn reserves which are effectively utilized for Zn deposition into grains (Kutman et al., 2010; Haslett et al., 2001; Pearson and Rengel, 1994). The results of this study showed that the role of soil or Zn treatments in increasing grain Zn was associated with negative correlation with wheat grain yield.

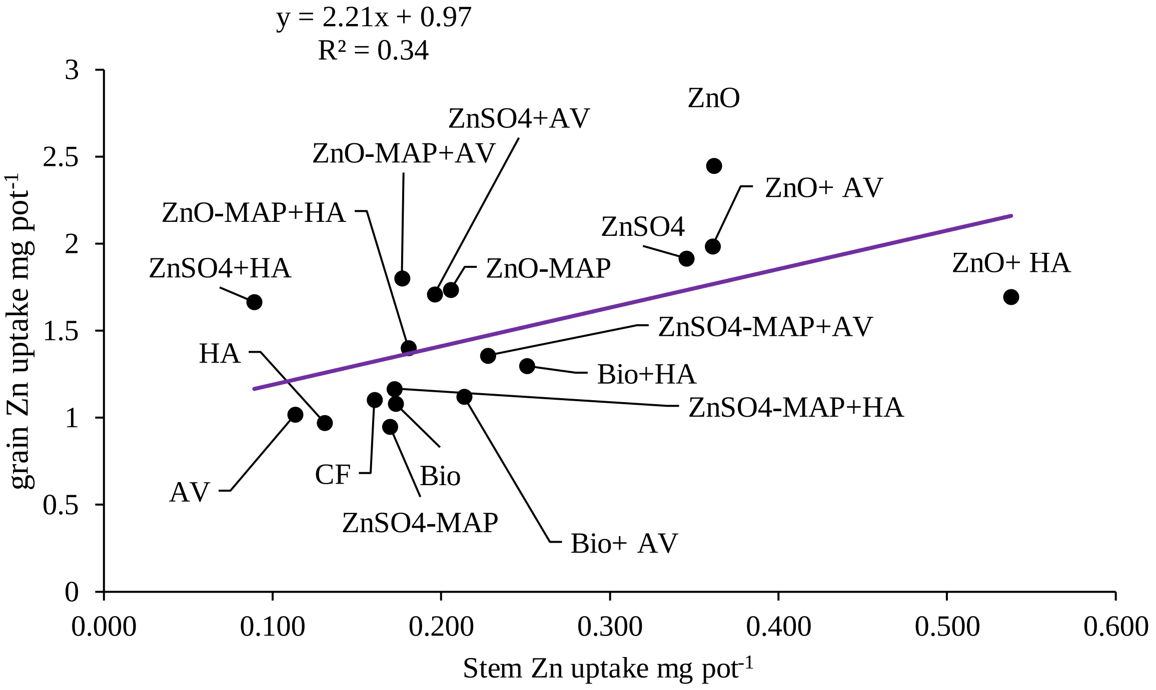

Figure 4.20 The relationship between whole grain Zn uptake and stem Zn uptake in soil, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO =ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+AV=$ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV= $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$+HA= $ZnSO_4$ with humic; ZnO-HA=ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+HA= ZnO-incorporated-MAP with humic; Bio+HA= biosolids with humic.

There was a highly significant positive correlation between total Zn uptake in aboveground biomass (µg pot^{-1}) and DTPA-extractable-Zn concentration mg kg^{-1} in the soil (R^2= 0.38 $P< 0.05$) (Figure 4.21). A study by Aghili, at el., (2014) reported that there was a significant correlation between the total Zn taken up into the aboveground biomass and the DTPA-extractable-Zn concentration in the soil (r2 =0.56 and P<0.0001). Similar observations were also made by Chahal et al. (2005), and Sahu et al., (1990).

There was not a relationship established between total above ground Zn uptake (µg pot^{-1}) and the sum of water-soluble, exchangeable, and carbonate-bound mg kg^{-1} in the soil (Figure 4.22). There was a significant positive correlation between Zn content in flour µg pot^{-1} and DTPA-extractable-Zn mg kg^{-1} in the soil, (R^2 = 0.40 and 0.23 p-value < 0.05) respectively (Figures 4.23 and 4.24). By contrast, grain Zn uptake was significantly and negatively correlated with soil DTPA-Zn after agronomic biofortification in soil.

All other fractions of Zn did not show any significant correlation with whole-grain, straw, and uptake of Zn in plants. A positive and significant correlation of DTPA-extractable-Zn with the uptake of Zn on whole grain showed that the availability of Zn to plant depends upon the amount of DTPA-extractable-Zn in soils.

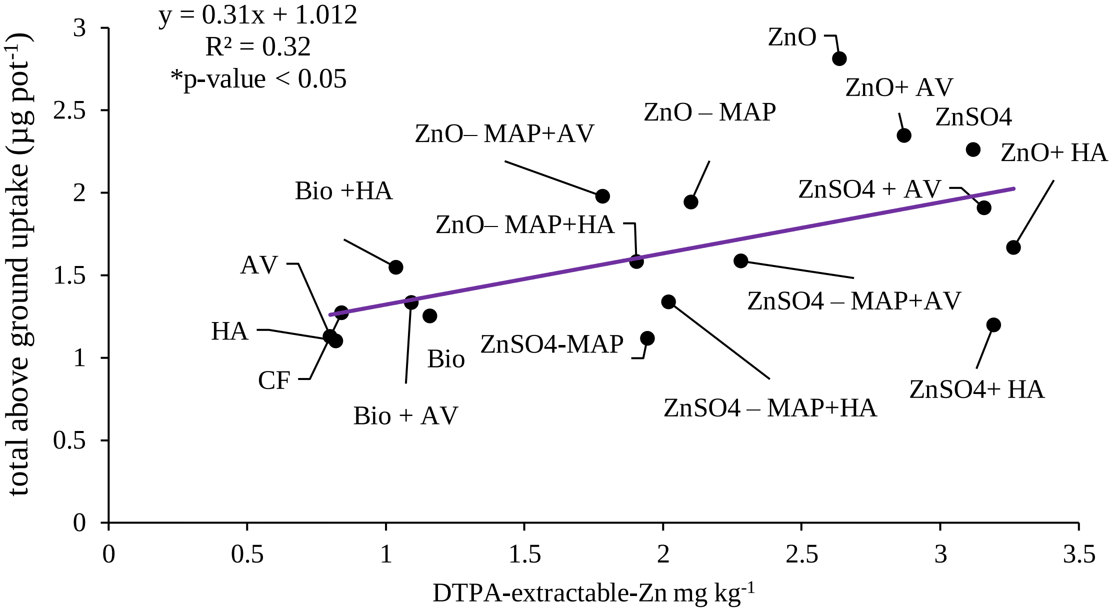

Figure 4.21 The relationship between total above ground uptake (µg pot^{-1}) and DTPA-extractable-Zn mg kg^{-1} the soil, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO =ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+AV=$ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV= $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$+HA= $ZnSO_4$ with humic; ZnO-HA=ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+HA= ZnO-incorporated-MAP with humic; Bio+HA= biosolids with humic.

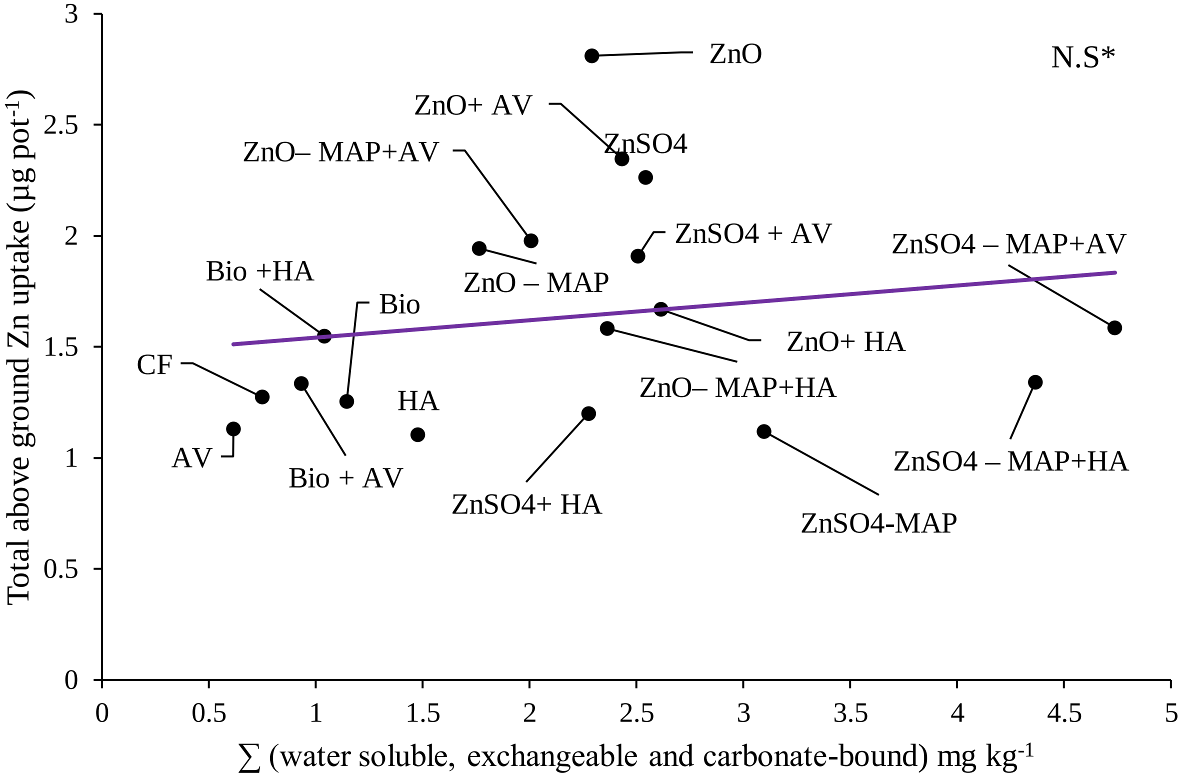

Figure 4.22 The relationship between total above ground uptake of Zn (µg pot^{-1}) and sum of water soluble, exchangeable and carbonate-bound mg kg^{-1} in the soil, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO =ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+AV=$ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV= $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$+HA= $ZnSO_4$ with humic; ZnO-HA=ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+HA= ZnO-incorporated-MAP with humic; Bio+HA= biosolids with humic.

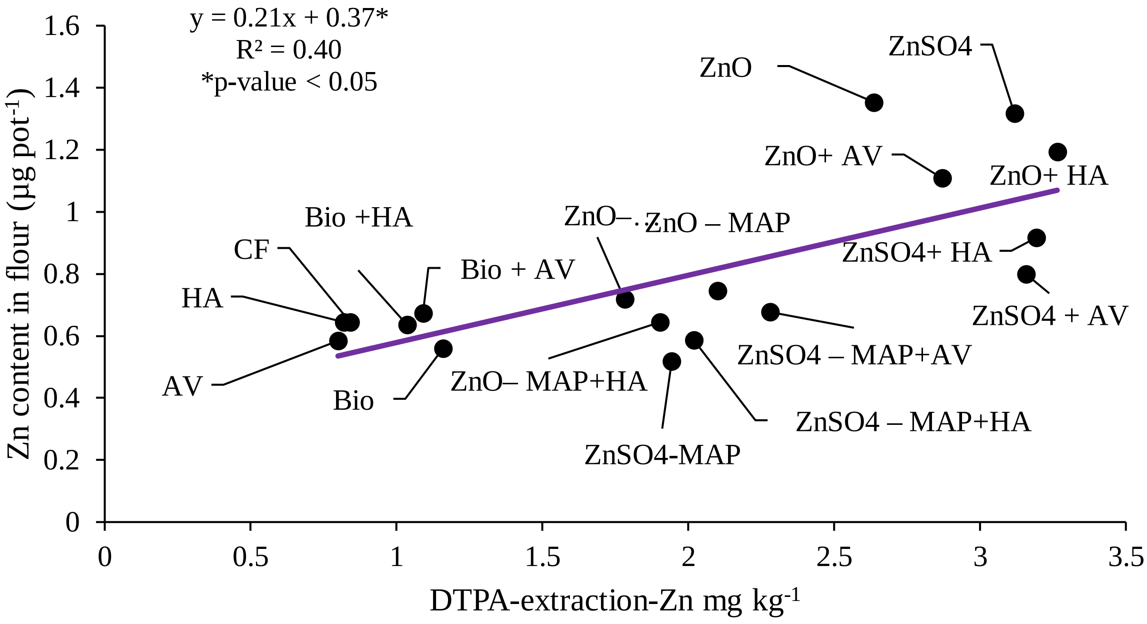

Figure 4.23 The relationship between Zn content in flour (µg pot^{-1}) and DTPA-extractable-Zn mg kg^{-1} the soil, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO =ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+AV=$ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV= $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$+HA= $ZnSO_4$ with humic; ZnO-HA=ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+HA= ZnO-incorporated-MAP with humic; Bio+HA= biosolids with humic.

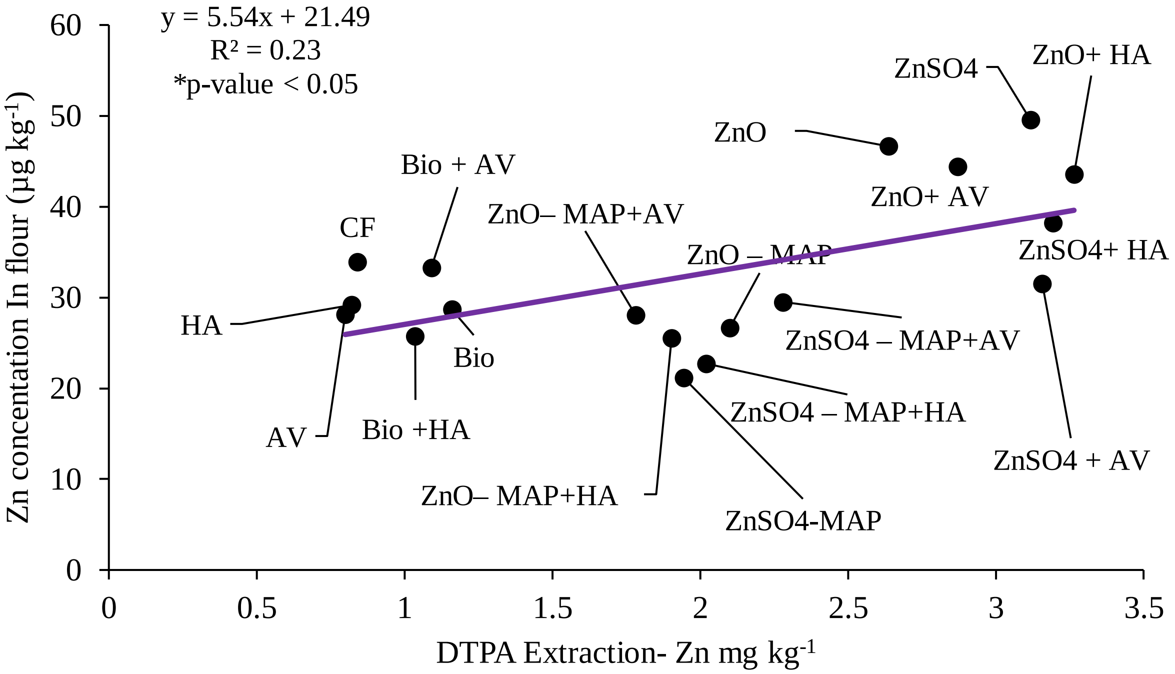

Figure 4.24 The relationship between Zn concentration in flour µg kg^{-1} and DTPA-extractable-Zn mg kg^{-1} the soil, CF= control with fertilizer; $ZnSO_4$= Zn sulfate; ZnO= Zn oxides; MAP-$ZnSO_4$ = $ZnSO_4$ incorporated–MAP; MAP-ZnO =ZnO- incorporated–MAP; Bio= biosolids; AV= AVAIL; $ZnSO_4$+AV=$ZnSO_4$ with AVAIL; ZnO+AV= ZnO with AVAIL; MAP-$ZnSO_4$+AV= $ZnSO_4$ incorporated– MAP with AVAIL; MAP-ZnO + VA= ZnO-incorporated–MAP with AVAIL; Bio+AV= biosolids with AVAIL; HA= humic acid; $ZnSO_4$+HA= $ZnSO_4$ with humic; ZnO-HA=ZnO with humic; MAP-$ZnSO_4$+HA= $ZnSO_4$ incorporated-MAP with humic; MAP-ZnO+HA= ZnO-incorporated-MAP with humic; Bio+HA= biosolids with humic.

Conclusions

Results described here indicated that the productivity of wheat was significantly affected by different Zn treatments and showed that the application of inorganic Zn sources with and without co-additives would result in increased wheat yield. Additionally, we found a significant influence of Zn treatments on Zn supply on grain and biomass. Co-additives applications lead to variable results. Results strongly suggested a benefit of agronomic biofortification to achieve the highest yield. The results of this study indicated that ZnO and/or $ZnSO_4$ application increased the grain Zn concentration in wheat more effectively than the other Zn sources in all cases. The results also suggest that ZnO was a better or equally effective Zn source than $ZnSO_4$ in increasing wheat grain Zn concentration in potentially Zn-deficient calcareous soil. Zinc treatments increased both the concentration and uptake of Zn in wheat grain and flours. Zinc treatments increased both the concentration and bioavailability of Zn in wheat flour. Although Zn concentrations in wheat bran were increased, Zn bioavailability was lower in wheat bran because of the extremely high concentrations of PA in the bran. The root uptake of soil Zn by the plant was shown by significant positive correlations between Zn concentration in multiple plant parts (e.g., stem, whole grain, and grain) and DTPA-extractable soil Zn. Fertilizers Zn application resulted in successful biofortification of wheat grain with Zn while simultaneously increasing yield.

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Chapter 5 - Understanding Reaction Pathways of Fertilizer Zinc with and without Co-Additives in Two Calcareous Soils

Abstract

Zinc (Zn) is present in soils in different chemical forms, the relative distributions of which vary with the type of soil and its physicochemical properties. Most Zn in soils is on the surfaces of clays, hydrous oxides, and organic matter, rather than in solution. Understanding the distribution of various fractions of Zn in soils is essential for the effective and efficient management of soils for optimum crop production and environmental conservation. The objective of this study was to investigate and understand differences in mobility, extractability, and reaction products of Zn as a result of the different Zn sources with and without a co-additive AVAIL, a fertilizer enhancement product) in two mildly calcareous soils from Kansas and Idaho. Fertilizers used in this study were: two liquid treatments, Zn sulfate ($ZnSO_4$), Zn oxides (ZnO), with and without AVAIL; two granular treatments, $ZnSO_4$-incorporated- monoammonium phosphate, ZnO- incorporated-MAP, with and without AVAIL; and two controls (zero fertilizer and MAP treated). Fertilizers were incubated in soils for five weeks in petri dishes, and at the end soils were sectioned in concentric rings from the point of application and analyzed. The influence of the Zn treatments on soil pH was similar for both the KS and ID soils. The Zn diffusion was higher in the ID soil, which had a coarse texture. Zinc diffusion in these calcareous soils was limited and generally did not move beyond the 0-7.5 mm section for the granular Zn and/or liquid treatments. In both soils, ZnO+AV showed the lowest percentage of Zn added in the center section, indicating that Zn diffusion from the point of application was greatest with the ZnO+AV treatment. Granular treatments, with and without AVAIL showed a minimal movement of Zn from the point of application. The energy dispersive X-ray analysis

results were in agreement with the wet chemical analyses and revealed that significant amounts of P and Zn remained in the Zn-incorporated MAP granules after 5-wk incubation in soil providing reasoning for low efficiency observed for these treatments in study 2 (Chapter. 4).

Introduction

Zinc (Zn) is present in soils in different chemical forms, the relative distributions of which vary with the type of soil and its physicochemical properties (Alvares et al., 1996). The term “availability” is commonly used to describe the ability of plants to take up nutrients from the soil; availability is governed by a dynamic equilibrium between the different nutrient forms rather than by the total concentration of the nutrients in the soil. Several factors affect the solubility of Zn in soils and its availability for plant uptake. Zinc availability for plants can be influenced by such factors, such as total soil Zn content, organic matter, soil pH, soil temperature, moisture regimes, root distribution, and rhizosphere effects (Sadeghzadeh, 2013).

Excluding boron (B), chlorine (Cl) and molybdenum (Mo), the availability of micronutrients decreases as the soil pH increases. Increasing the soil pH enhances Zn adsorption onto the surface of various soil constituents, such as metal oxides and clay minerals. This results in decreases in Zn solubility and the availability of Zn to plants. Higher pH soils will decrease the desorption of Zn from soil surfaces, which also reduces the availability of Zn to plants. Additionally, Zn can precipitate in the form of Zn hydroxide ($Zn(OH)_2$), Zn carbonate ($ZnCO_3$), and Zn silicate mineral (Zn_2SiO_4) at high pH. The Zinc concentration in the soil solution is largely dependent on pH. For example, at pH 5.0, the concentration of Zn in the soil solution is approximately 10^{-4} M, while, at pH 8.0, this concentration is 10^{-10} M (Parker and Walker, 1986). The formation of Zn precipitates also occurs where the concentration of Zn exceeds Zn adsorption maxima of the soil, and the solubility product of Zn minerals is exceeded in the soil solution (Bingham et al., 1964). Solubility calculations predict that under specific conditions of high pH (pH > 8) and very high Zn^{2+} concentration in solution (>10^{-4} M) Zn could theoretically precipitate as Zn hydroxide ($Zn(OH)_2$), smithsonite ($ZnCO_3$), Zincite (ZnO), or willemite

(Zn_2SiO_4) (Lindsay, 1979). It is still being debated whether precipitation reactions control the concentration of Zn^{2+} in soil solutions as many studies have shown that solubility of a single mineral phase cannot describe Zn solution concentrations (Degryse et al., 2011; Montalvo et al., 2016). The existence of defined Zn minerals has been identified only in Zn fertilizer soil bands or in heavily contaminated soils, e.g., smithsonite in contaminated overbank sediments (Montalvo et al., 2016; Milani et al., 2015; Hettiarachchi et al., 2008).

Singh et al. (2005) reported that the total soil Zn concentration in calcareous and non-calcareous soils is usually similar. However, Zn deficiency is frequently associated with calcareous soils. Calcareous soils (pH>7.4) with moderate-to-high organic matter content (>15g organic carbon per kg soil) are likely to be Zn deficient due to high levels of bicarbonate (HCO_3^-) in the soil solution. Liu and Tang (1999), reported as in alkaline soils with a low Zn supply, increasing the Zn application increased the Zn concentrations in plants, and reduced the deficiency symptoms. Nevertheless, plant growth was only slightly improved. Plant growth in alkaline soils was more responsive to correcting soil alkalinity than Zn deficiency.

Soils with low plant-available Zn could be treated with Zn fertilizers to provide enough Zn for crops. Several different Zn compounds are used are used as fertilizers, although sulfate ($ZnSO_4$) is by far the most widely used material. Zinc sulfate may also be used as a foliar treatment for crops, although chelated forms of Zn are usually used to foliar application (Alloway, 2001). The application rates of Zn fertilizers could also vary depending on the crop, the form of Zn applied, the application method, and the soil conditions. For soil applications, concentrations can range from 2.5-22 kg Zn ha^{-1} of inorganic Zn fertilizers, such as $ZnSO_4$, and 0.3-6 kg Zn ha^{-1} for chelated forms (Alloway, 2001).

The effectiveness of Zn fertilizers for providing plants with Zn in Zn-deficient soils depends primarily on the solubility of the Zn source in the soil. Mortvedt (1992), found a significant correlation between water-soluble fractions of Zn and Zn availability to crops from several macronutrient fertilizers with Zn oxide (ZnO) or Zn sulfate ($ZnSO_4$) incorporated. Further investigations have confirmed that water-soluble Zn, not the total Zn concentration, is the primary parameter controlling the effectiveness of Zn-enriched fertilizers for plant growth and development (Amrani et al., 1999; Westfall et al., 2005).

The organic compounds EDTA and DTPA are considered common synthetic chelating agents used for Zn. Chelation of a metal cation (Zn^{2+}), with an organic chelating agent ($EDTA^{4-}$), causes the reversion of its charge ($ZnEDTA^{2-}$), thus reducing its electrostatic attraction of the soil's adsorption sites. Consequently, when added in chelated form, the Zn is less probable to be retained by the soil colloids and has more potential to be transported through the soil to the roots. Because of these characteristics, synthetic chelates are purportedly effective Zn fertilizers. Zhao et al., (2016) reported that chelated Zn is not readily taken up by plant roots, but the enhanced uptake of Zn observed in studies is probably due to enhanced diffusion of Zn to the roots.

Ghosh (1990) and Hettiarachchi et al., (2010) reported that the movement of Zn into the soil from surface-applied fertilizer granules could be assessed, for instance, by sampling soil sections at increasing distances from the fertilizer application point (1 cm). From such experiments, two zones have been identified with marked differences in the concentration of Zn: A Zn-saturated zone immediately adjacent to the fertilizer granule (within 1 cm), and an outer area with low Zn concentrations (Ghosh, 1990). This zone of saturation can be smaller depending on soil characteristics, and it was <4 mm for a highly calcareous soil (Hettiarachchi et al., 2010). Different chemical reactions may occur in these two zones of soil. For example, Zn precipitates may form in the zone of high Zn concentration, while the area of low concentration adsorption reactions most likely controls the fate of Zn.

Studies that investigated the solid-phase reaction products that were formed, following the addition of Zn fertilizers in soils using direct identification methods (synchrotron techniques), are scarce in the literature. This could be in part due to the difficulty in the sample preparation as it can be challenging to isolate sufficient soil with high Zn concentrations necessary to obtain definitive quantitative data. Most information regarding the formation of Zn reaction products has been derived from laboratory experiments that investigated the chemical reactions between Zn compounds and macronutrient carriers. Kalbasi et al., (1978) used X-ray Diffraction (XRD) analysis to investigate the solid-phase reaction products that formed after the application of 0.1 g of Zn as $ZnSO_4$, ZnS, and Zn-EDTA in four soils. These soils were incubated for 2–32 weeks, and the soils that were used for the analyses were sampled from the zone immediately adjacent to the fertilizer placement site. Sphalerite was mostly identified in soils treated with ZnS, while no crystalline products were detected in soils treated with Zn-EDTA. In two calcareous soils, Zn carbonates were detected after the application of $ZnSO_4$. In an alkaline, non-calcareous soil $Zn(OH)_2$ was discovered shortly after the application of $ZnSO_4$ but was not detected after four weeks of incubation. The researchers were able to identify crystalline Zn precipitates in the soil adjacent to fertilizer bands of calcareous and non-calcareous soils that received $ZnSO_4$ and $NH_4H_2PO_4$. In the non-calcareous soil, hopeite was the reaction product detected; whereas, in the calcareous soil $ZnNH_4PO_4$, $ZnCO_3$ and hopeite were identified.

Degryse et al., (2015) showed that the fate of Zn fertilizers was also affected by the chemical properties of the soil. A visualization technique was used to evaluate the effect of P carriers on the diffusion of Zn from $ZnSO_4$-coated MAP and $ZnSO_4$-coated DAP granules applied to three soils of contrasting pH (soil pH in $CaCl_2$ 4.3, 6.1, and 7.7). The extent of Zn diffusion after 28 days of incubation was much less for Zn-DAP granules than for Zn-MAP in the most acidic soil, while the extent of diffusion was similar for Zn-MAP granules and $ZnSO_4$ applied alone (control treatment). For the soil with pH 6.1, the diffusion of Zn from the fertilizers followed the order $ZnSO_4$ > Zn- MAP > Zn-DAP. While in the non-calcareous soil at pH 7.7, Zn diffusion could only be visualized in the control treatment. The difference in the diffusion of Zn from MAP and DAP granules in the non-calcareous soils can be related to the higher pH induced by DAP around the granule during dissolution, which may have enhanced adsorption and promoted precipitation of Zn phosphates and hopeite ($Zn_3(PO_4)_2.4H_2O$).

Hettiarachchi et al., (2008) used a combination of bulk and focused synchrotron-based techniques and scanning electron microscopy to investigate the distribution and speciation of Zn in the soil-fertilizer reaction zones around granular fertilizers. Commercially available Zn-incorporated MAP granules were placed in the center of a Petri dish containing a calcareous soil (pH 8.5) and incubated for five weeks. Micro-X-ray fluorescence maps of the incubated granules showed that most of the fertilizer Zn was retained in the granule, and its distribution was heterogeneous. X-ray Absorption Fine Structure Spectra (XAFS) of the incubated granule showed that the Zn species present in the residual granule were scholzite [$CaZn_2(PO_4)_2 \cdot 2H_2O$)], willemite [$Zn_2(SiO_4)$], and Zincite (ZnO). In the soil section adjacent to the fertilizer granule (0-4 mm), the Zn species detected were willemite, hopeite, and ferrihydrite-adsorbed Zn. When Zn was delivered with a fluid MAP formulation instead, willemite was the dominant Zn species in the 0-4 mm soil section, but as the distance from the application point increased, ferrihydrite-adsorbed Zn and hopeite were the main Zn species in the soil. Studies related to the co-additives and their potential benefits are lacking; thus, we conducted this study. Our primary focus of this study is to understand the reaction products of different fertilizer-Zn, how reaction products of fertilizer-Zn are affected by fertilizer formulation and the co-additive, and how reaction products of fertilizer Zn affect their potential plant availability in two calcareous soils with different soil textures. The specific objective of this study was to understand the differences in mobility, extractability, and fractionation of Zn resulted from different Zn sources with and without co-additive AVAIL using wet chemical methods and SEM-EDS analysis.

Materials and Methods

This study consisted of two incubation experiments following the same preparation and treatments procedures. The first soil, hereon defined as the KS soil, a mildly calcareous surface soil, was collected from Garden City, Finney County, KS and had silt loam (SiL) texture (Table 5.1). The second soil, hereon defined as the ID soil, a mildly calcareous surface soil, was collected from Grandview, Elmore County, ID and had loam (L) texture (Table 5.1). All soils were collected to a depth of 10 cm, air-dried and sieved to < 2 mm before being shipped from their place of origin. The pH of the soil samples was measured in a 1:10 soil: water extract (Watson and Brown, 1998) and available phosphorus (P) was measured by Olsen et al., (1954). The Cation Exchange Capacity (CEC) was determined by the displacement method (Soil Survey Staff, 2011); carbonates were determined according to Allison and Moodie (1965); and total P, Zn and Calcium (Ca) were determined according to Zarcinas et al., (1996) then were modified to use a digestion block instead of a microwave. The Maximum Water Holding Capacity (MWHC) determination was made using the protocol from Jenkinson and Powlson (1976).

Table 5.1 Selected initial properties of soils

Soil†	Particle size distribution			Texture	pH	$CaCO_3$	CEC†	TOC	Available Zn	Total Zn
	Sand	Silt	Clay		(1:10)	%	cmol Kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
KS	24.9	52.4	22.7	SiL	8.8	5.59	18.28	1.11	1.3	52.1
ID	40.3	41.4	18.3	L	8.7	6.48	28.09	0.59	0.3	45.65

† KS: Garden City soil, Kansas State; ID: Grandview, Idaho State; CEC (cmolc kg $^{-1}$) Cation Exchange Capacity; $CaCO_3$: Calcium Carbonate; TOC: Total organic carbon; pH: (1:10) in water; Soil texture: SiL = Silt loam, L = Loam.

Petri dish experiments were carried out for this study. First, each soil was pre-moistened with 10% of the total distilled water needed for 60% MWHC (KS soil) and 50% MWHC (ID soil). Soils were packed into Petri dishes (87x11 mm) at a bulk density of 1.1 g cm^{-3} for both (KS and ID soil. Preliminary work indicated that these bulk densities were ideal for completely filling the volume of the Petri dishes without unnecessary compaction. There were ten treatments with five replications. Two additional sets of Petri dishes were prepared for Scanning Electron Microscope (SEM)-Energy Dispersive Spectroscopy (EDS) and X-ray absorption spectroscopy analyses. The remaining distilled water was added to bring the soil to 60% MWHC for the KS soil and 50% for the ID soil by carefully dripping the water on the soil packed in each plate. The plates were then sealed using Para-film M® (Bemis Flexible Packaging, Neenah, WI), wrapped in aluminum foil and left to equilibrate overnight at room temperature (~24°C) for at least 24 hours. The plates were then unwrapped, and the treatments were introduced by placing the fertilizer just below the soil surface in the center of the dish and covering with soil as follows:

1) Unfertilized control soil sample.
2) Monoammonium phosphate (MAP) granular (11-52-0; 11% N - 52% P2O5 - 0% K2O by weight). A 42± 0.05 mg, (~9.76 mg P) standard grade MAP granule.
3) Zinc sulfate ($ZnSO_4$) liquid 5 µL.

4) Zinc oxides (ZnO) liquid 5 µL.
5) $ZnSO_4$-incorporated-MAP (40 Rock 12-40-0-6.5S-1 Zn) with 0.42 mg Zn (42± 0.05 mg).
6) ZnO- incorporated-MAP (MES 12-40-0-10S-1 Zn) with 0.42 mg Zn (42± 0.05 mg).
7) Zinc sulfate ($ZnSO_4$) with AVAIL 5µL.
8) Zinc oxides (ZnO) with AVAIL 5 µL.
9) $ZnSO_4$- incorporated-MAP (MAP-ZnSO4 12-40-0-6.5S-1 Zn) with 0.42 mg Zn-coated with AVAIL.
10) ZnO- incorporated-MAP (MAP-ZnO 12-40-0-10S-1 Zn) with 0.42 mg Zn-coated with AVAIL.

All fertilizer treatments received equivalent amounts of Zn, approximately 0.42 mg Zn per Petri dish. After adding all the treatments, the Petri dishes were sealed with Parafilm M® and wrapped in aluminum foil to minimize water loss while maintaining aeration. Soils were incubated (Precision Low Temp Incubator, Waltham, MA) in the dark at 25°C for five weeks. This particular incubation time was chosen because at five weeks' root development would be limited, and this is considered one of the critical P uptake periods for cereal crops (Hettiarachchi et al., 2010; Williams, 1948).

Following incubation, the dishes were excavated and placed into four concentric circular sections with radii of 0-7.5 mm, 7.5-13.75 mm, 13.75-25 mm, 25-43.5 mm, from the point of fertilizer application by using metal cylinders that were pushed gently into the soil to the bottom of the plate, starting with the smallest cylinder in the center and carefully collecting the soil samples from the circular sections as described by Lombi et al., (2004). Each concentric section's soil sample was placed in a separate plastic specimen container (Fisher Scientific, Waltham, MA) with the soil weight recorded. The samples were then fast dried immediately at

40°C (Fisher Scientific Drying Oven, Waltham, MA). After drying, the weight of the soil sample was recorded, and the lid was placed to avoid any contamination. Two of the seven replicates from the granular treatments were utilized for scanning by electron microscopy coupled with energy dispersive X-ray analysis (SEM-EDX). For these samples, the fertilizer granules were carefully removed using a small metal spatula and tweezers, placed in separate labeled containers, and dried at 40°C. The samples were sealed and stored in the refrigerator until the SEM-EDX analysis.

Soil samples were taken from each dish and analyzed for the total elemental concentration in the soil, which was determined using the aqua regia digestion procedure. The procedure is as follows: Air-dried samples were finely ground with an agate mortar and pestle. Next, 0.5 g of soil was added to a digestion tube while adding 5 mL of aqua regia solution (1:3 (v/v) HNO3/HCl) to it. Pretreated samples were allowed to react overnight at room temperature. The soils were digested by slowly increasing the temperature from 75°C for 30 minutes (min), to 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 #42 Whatman filter paper. All samples were digested in duplicate. In each batch of digestion, two blanks and two samples of standard reference soil material (NIST 2711a - Montana soil) had included as a QA/QC control. The analysis was done using the inductively coupled plasma emission spectrometry (ICP-OES, Varian 720-ES, Santa Clara, CA).

Sequential extraction procedure of Tessier et al., (1979) was performed by using 1 g of soil and shaken with water for 3 days (see Table 5.2) and water-soluble-Zn from the remaining four sections for all treatments, then the soil was extracted initially with 12.5 mL of 1 M $MgCl_2$ for 1 hour (h) (exchangeable). Then with 12.5 mL of 1 M $NaC_2H_3O_2$ (pH 5.0) for 5 h (carbonate-bound) The Fe-Mn^{-1} oxide-bound fraction was determined by adding 20 mL of 0.04 *M* $NH_2OH(HCl)$ in 25% (v/v) $HC_2H_3O_2$ solution for 6 h at 96 °C. The organic-bound fraction was determined by adding 3 mL of 0.02 *M* HNO_3 and 5 mL of 30% H_2O_2 and heating at 85°C for 2 h. An additional 3 mL of H_2O_2 was then added, followed by heating for an additional 3 h. After cooling, 5 mL of 3.2 *M* $NH_4C_2H_3O_2$ in 20% (v/v) HNO_3 and 5 mL of deionized water were added and shaken for 30 min. The remaining soil material was treated with 10 mL of 4 *M* HNO_3 for 2 h at 80°C to determine the residual fraction from the remaining two sections for all treatments (0-7.5 to 7.5-13.5). In between these steps, the tubes were centrifuged for 10 min, the supernatant was decanted and stored until analysis, and the tubes were weighed to allow for correction for the metals remaining in the entrained solution prior to the addition of the next reagent.

The SEM-EDS (scanning electron microscope – energy dispersive X-ray spectroscopy) analysis was performed on the original and extracted Zn fertilizer granules incubated five weeks in the KS soil to look for morphological changes within the granule as well as the remaining components of the granule. After neatly extracting the residual Zn granule from the petri dish, the attached soil particles were carefully removed using a soft paintbrush and tweezers. The samples were then fast dried immediately at 40°C (Fisher Scientific Drying Oven, Waltham, MA). All fertilizer granules were neatly fractured using a stainless steel knife to cross sections, and mounted on an Al pin SEM stub. The samples were coated with 30 nm of platinum by sputter coater (Denton Desk II) to provide electrical conductivity and analyzed at accelerating potential of 15 kV under Hitachi S-3500N SEM (Hitachi Science Systems, Japan) equipped with a Backscattered electron detector (Robinson, Australia) and X-ray Si(Li) Detector (Oxford Instruments Microanalysis Limited, England).

Statistical Analysis

Soil data were statistically analyzed using the Proc MIXED Procedure in SAS (SAS 9.4, 2011). The experimental design was a Completely Randomized Design (CRD). The data were analyzed using ANOVA with Zn fertilizer treatment as the main treatment and dish sections as subplot treatments. The Tukey Pairwise Method was used for a comparison of all treatments at a 0.05 level of significance.

Results and Discussion

Physical and chemical properties of soils

Table 5.1 summarizes the properties and chemical characteristics of the soils. Both KS and ID soils had an initial soil pH of 8.7. Both soils were mildly calcareous; the KS soil contained 5.59% reactive $CaCO_3$, and the ID soil contained 6.48% reactive $CaCO_3$. Their texture was different; the KS soil was a silt loam texture (SiL), and the ID soil was a loam texture (L).

Soil pH

Soil pH measurements at the end of the incubation period for each soil section are shown in Table 5.2. For both soils, Zn treatments had a similar effect on soil pH. The greatest decrease in pH, regardless of the Zn source, was in the 0-7.5 mm section with the soil pH increasing as the distance increased from the center section to a point relatively similar in pH of the control treatment by the 25-43.5 mm section. In both soils, MAP-ZnO, MAP-$ZnSO_4$, MAP-ZnO + AV and MAP-$ZnSO_4$+AVAIL treatments had significantly lower pH compared to the control and liquid Zn treatments with and without AVAIL in the 0-7.5 mm section. One explanation is acidification caused by granular P fertilizer. When the soil pH is greater than MAP fertilizer pH, protons are released into the solution upon application of $H_2PO_4^-$ based P fertilizers (Weeks, 2019). In the 7.5-13.75 mm section, Both Zn granule (MAP-$ZnSO_4$ and MAP-ZnO) treatments had the lowest pH. Consistent with the report from Hettiarachchi (2010), the results concluded that acidification caused by granule fertilizer addition extended to at least 13.5 mm in both soils. Treatment with MAP-$ZnSO_4$ + AV had the next lowest pH. The MAP when added to soil it does not use up any H^+ ions in the soil solution, therefore, the full acidifying effect of the nitrification process impacts soil pH. The pH captured from nitrification dissolution of MAP was represented in reduction tendency. This was because hydrogen ions were continuously released, and another possibility was the production of hydrogen sulfide alongside nitrification. This tendency was affecting the nitrification rate because the pH value has ever been below.

Overall, the ZnO treatment and the $ZnSO_4$ treatment had the highest soil pH. The strong pH buffering of these soils likely resulted in a high pH at the soil fertilizer interface for both ZnO and $ZnSO_4$. Zinc solubility was likely controlled by precipitates at very low concentrations. Over the five-week incubation period, all Zn treatments had a lower pH than the control. An important soil property in relation to Zn availability is soil pH. Generally, soil pH is recognized as the main factor influencing Zn solubility and mobility across a wide range of soils (Lindsay, 1972; Mattiello et al., 2017). Other factors that play important roles are organic matter content, type, and content of clay minerals, and soil moisture (Mattiello et al., 2017). Although in the ID soil, Zn diffusion were much higher than the KS soil, granular treatments had the largest effect on pH of both soils. Hettiarachchi et al., (2010) reported that soil pH should return to its original value given enough time. This is due to the acid-neutralizing power of free $CaCO_3$ in the calcareous soil and pH-buffering power of the non-calcareous soil.

Table 5.2 Soil pH after a five-week incubation of three Zn fertilizer sources applied to two different soils, KS and ID, alone or in combination with a fertilizer enhancement product at four distances from the point of fertilizer placement.

	Distance from the point of fertilizer placement (mm)			
treatments†	0-7.5	7.5-13.5	13.5-25.5	> 25
KS soil				
Control	8.7a	8.7ab	8.8a	8.8ab
MAP-$ZnSO_4$	7.6c	8.3d	8.5c	8.5e
MAP-ZnO	7.6c	8.3d	8.4c	8.5e
MAP-$ZnSO_4$+AV	7.8b	8.6b	8.7a	8.7bcd
MAP-ZnO+AV	7.5c	8.4cd	8.6b	8.6d
$ZnSO_4$	8.6a	8.7ab	8.7a	8.7abc
ZnO	8.6a	8.8a	8.8a	8.8ab
$ZnSO_4$+AV	8.6a	8.7ab	8.7a	8.8ab
ZnO+AV	8.6a	8.7ab	8.8a	8.8a
MAP	7.7b	8.4c	8.6b	8.7cd
ID soil				
Control	8.5bc	8.5bcd	8.5c	8.5c
MAP-$ZnSO_4$	7.5de	8.2ef	8.5c	8.5bc
MAP-ZnO	7.5de	8.3cde	8.5c	8.6ab
MAP-$ZnSO_4$+AV	7.6d	8.3de	8.6bc	8.6ab
MAP-ZnO+AV	7.5d	8.4bcd	8.5c	8.6a
$ZnSO_4$	8.4bc	8.5abcd	8.5c	8.5c
ZnO	8.6a	8.7a	8.7a	8.7a
$ZnSO_4$ +AV	8.4c	8.5abc	8.6bc	8.6abc
ZnO+AV	8.5ab	8.6ab	8.7ab	8.6ab
MAP	7.4e	8.1f	8.3d	8.4d

† MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; MAP-$ZnSO_4$ + AV = ZnSO4- incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

Percent of Zn Added

In the KS soil, regardless of Zn source, almost all of the added Zn remained within the 0-7.5 mm section of the dish (center section) for both Zn liquid ($ZnSO_4$ and ZnO) and granular treatments (Figure 5.1). However, the lowest percent Zn added in the center section of the ZnO+AV treatment indicated a significant diffusion of Zn from the point of application to other sections. In fact, all liquid treatments (ZnO+AV, $ZnSO_4$, $ZnSO_4$+AV, and ZnO) showed a significant enhancement of added Zn in the second section. This was not observed for the granular treatments. Phosphate dissolved from the MAP granules could rapidly reduce the release of Zn^{2+}. This is a result of precipitation from Zn phosphate-like species adjacent to the MAP granule (Zhang et al., 2012). Despite significantly low soil pH, which may promote the dissolution of ZnO to Zn^{2+}, no significant movement of Zn was observed from the granular P-Zn fertilizers. Additionally, the large pH buffering capacity of the alkaline calcareous soil may limit the solubility and diffusion of Zn around the MAP granules (Bertrand et al., 2006). There are multiple possible explanations for the overall reduction in diffusivity observed with all Zn treatments in KS soil. First, the combination of the impediment of the Zn fertilizer movement outward by the silt loam texture could be a contributor. Also, Zn precipitation ($ZnCO_3$) and the hygroscopic movement of soil water toward the Zn granules in the center section. Hettiarachchi et al. (2006; 2008) and Lombi et al. (2004) reported that restricted movement and diffusion of the nutrient in calcareous soil is the result of quick precipitation and sorption reactions with Ca in soil solution, solid calcium carbonates, and other colloids. Further, a possible mechanism restricting Zn diffusion could be the flow of water from the soil towards the moisturizing fertilizer granule in the reverse direction. This may be due to the restriction of distribution or movement of nutrients with the possibility of reacting with excessive P concentrations at the point of addition. The latter would then participate in reducing the lability of fertilizer.

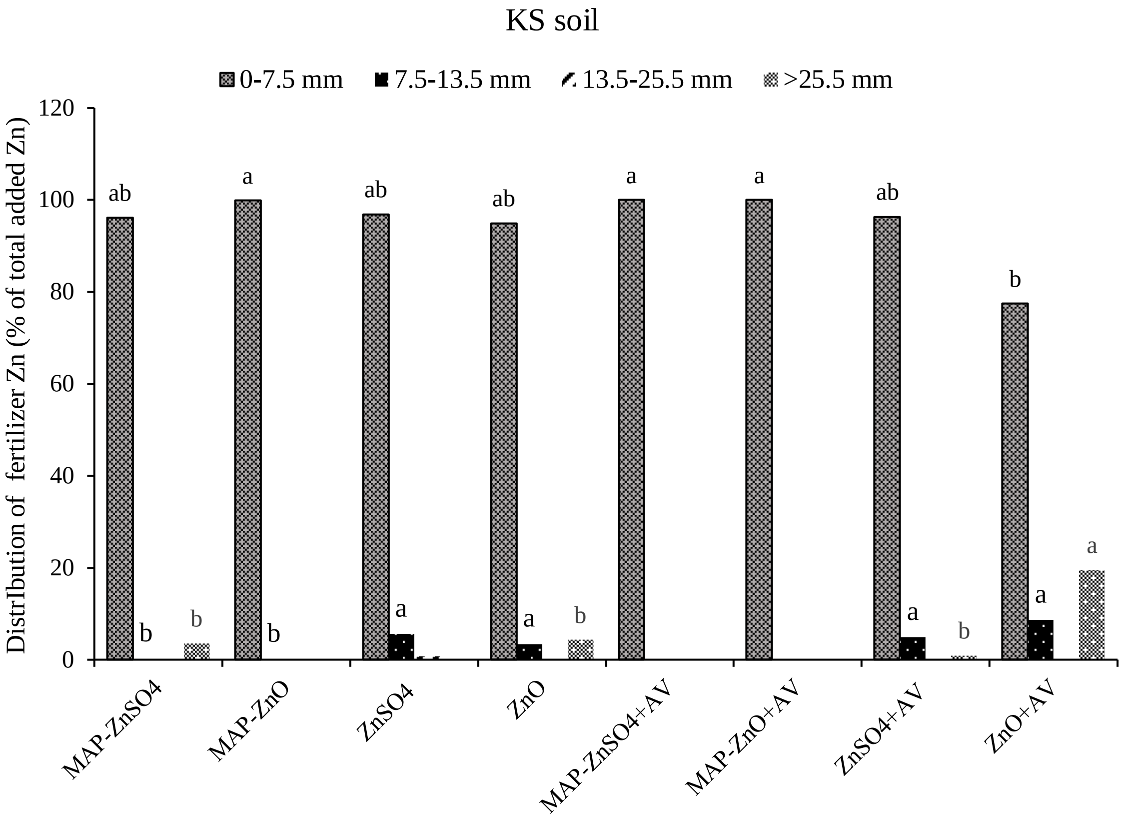


Figure 5.1 Total Zn (mg kg^{-1}) collected at different distances from the point of fertiliser application in Kansas soil. MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO-incorporated – MAP; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; MAP-$ZnSO_4$ + AV = ZnSO4-incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at P = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

Similarly, almost all of the added Zn remained within the 0-7.5 mm section of the dish for both Zn liquid ($ZnSO_4$ and ZnO with and without AVAIL) and granular Zn (MAP-$ZnSO_4$ and MAP- ZnO with and without AVAIL) treatments in ID soil (Figure 5.2). The ZnO+AV treatment showed the greatest diffusion from the point of application and gave the lowest percent Zn added in the center section. Percent Zn added values in 7.5-13.75 mm sections for all treatments were low. Although the MAP-$ZnSO_4$+AV treatment had a greater amount of Zn added than MAP-$ZnSO_4$ in the 7.5-13.5 mm section, all granular treatments showed only a small amount of added Zn in the 7.5-13.5 mm section. Ghosh (1990) observed that most of the dissolved Zn remained within 1 cm of the granular placement site and only moved a maximum of 4 cm, for ordinary superphosphate and MAP). The leading possible cause is a large amount of chemically reactive sorption sites close the point of fertilizer application. Additionally, the high clay texture of the soils slows radial migration via diffusion and/or mass flow. Weeks et al. (2019) and Pierzynski (2016) reported similar observations for the diffusion of P.

The Zn fertilizer diffusion in KS soil and ID soil was greatly affected by the different textures despite the similar $CaCO_3$ content. The silt loam texture of KS soil allows it to retain more water and nutrients. Compared to the ID soil, KS soil most likely retained more Zn due to its increased surface area and cohesiveness properties.

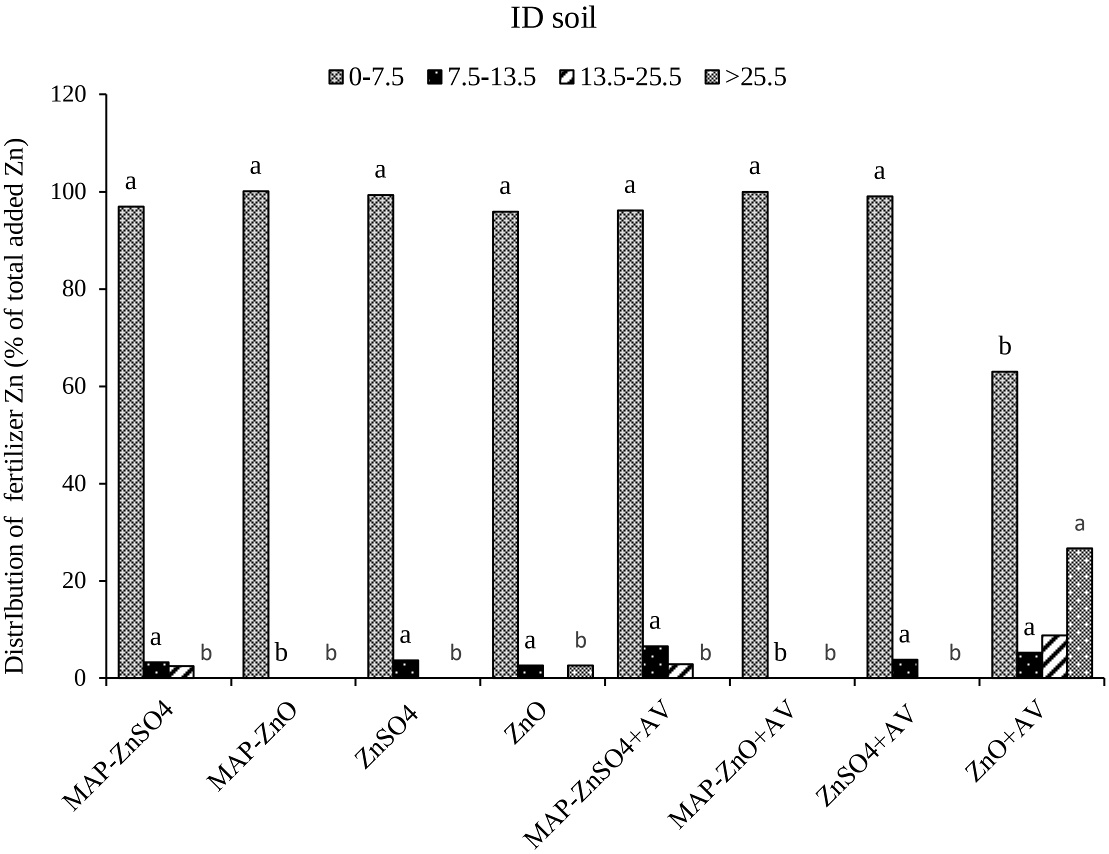


Figure 5.2 Total Zn (mg kg^{-1}) collected at different distances from the point of fertiliser application in Idaho soil., MAP-ZnSO$_4$ = ZnSO$_4$- incorporated – MAP; MAP-ZnO = ZnO-incorporated – MAP; ZnSO$_4$ = Zn sulfate; ZnO = Zn oxide; MAP-ZnSO$_4$ + AV = ZnSO4-incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; ZnSO$_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

Analysis of incubated MAP-Zn fertilizer granules

Figure 5.3 shows the scanning electron microscopy image of the external surface and cross-sectional view of non-incubated granular MAP-$ZnSO_4$ with and without the AV coating. The EDAX spectrum collected from the cross-section of non-incubated MAP-$ZnSO_4$ with/without AV coating (Figure 5.4) showed significant amounts of P, O, Al, and S. This is not surprising as these granules did contain ~ 5.5% of S as $(NH_4)_2SO_4$.

Scanning Electron Microscopy (SEM) technique is also used to study the morphology of the external surface as well as the cross-sectional view of MAP-$ZnSO_4$ fertilizer granules with/without AVIL coating, incubated five weeks in the KS soil (Figure 5.5). The EDAX spectra collected from selected spots of the cross-section of the granules and their elemental compositions are also reported (Figure 5.6). The EDAX results revealed that the remaining granules still containing significant amounts of P while concentrations of Zn, Al, and Fe in the granules were enhanced. This directly implies that the concentration of Zn in the incubated granules increased following incubation in soil, possibly due to the decreased concentration of the major nutrient elements upon granule dissolution (Hettiarachchi et al., 2008). These observations are in agreement with the earlier findings from Hettiarachchi et al., (2008) in a highly calcareous soil from South Australia and the current study showed that even in mildly calcareous soils when Zn is incorporated into P granules most of the original Zn present in the granules remained within the granules for quite a long time.

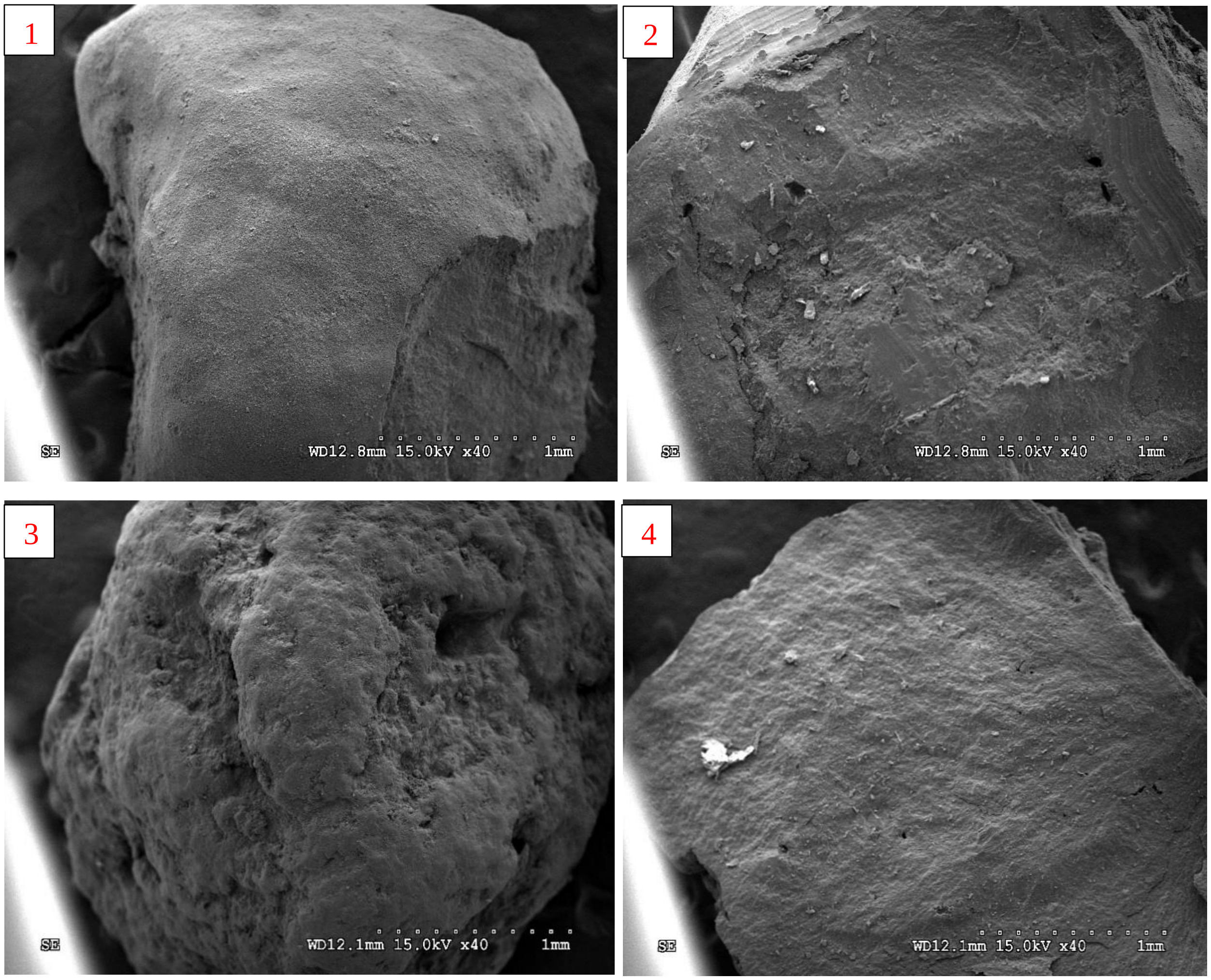
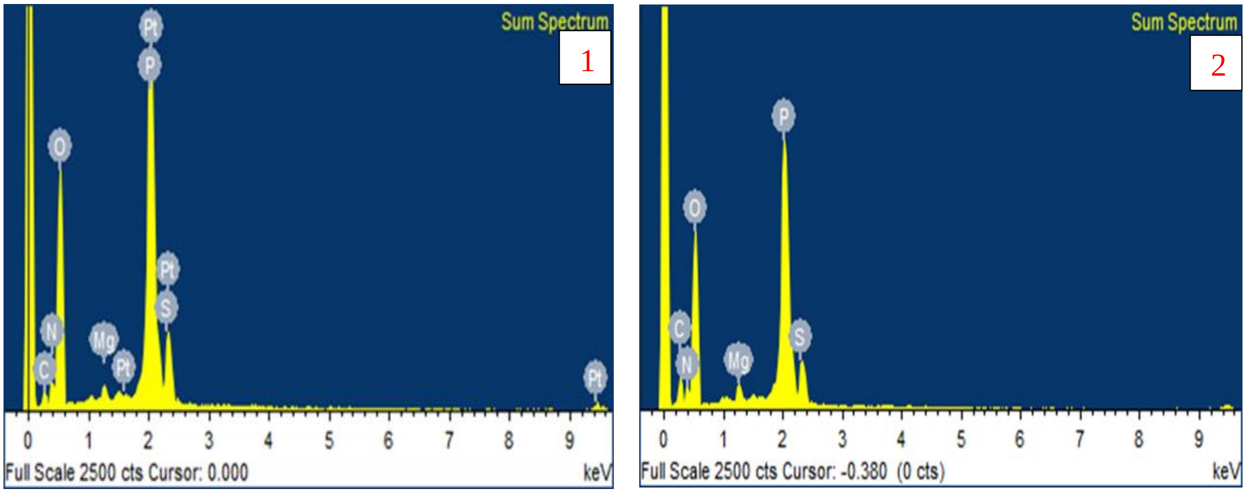


Figure 5.3 Scanning electron micrographs of the original (non-incubated) $ZnSO_4$ incorporated-MAP granules, with and without AVAIL coating. (1) MAP-ZnSO4 Non-incubated, external surface, (2) MAP-ZnSO4 Non-incubated, cross section, (3) MAP-ZnSO4 Non-incubated coated, external surface, (4) MAP-ZnSO4 Non-incubated coated, cross section.



Treatments	C	O	N	Mg	P	S	Zn	Al	Ca
	……………...% by weight……………………								
MAP-ZnSO4 Nonincubated, external surface	--	--		--	--	--	--	--	--
MAP-ZnSO4 Nonincubated, cross section	5.9	41.7	10.8	0.8	19.3	5.7	--	--	--
MAP-$ZnSO_4$ Nonincubated,coated external	5.6	52.5	11.9	1.2	19	7.7	1.6	0.4	--
MAP-$ZnSO_4$ Nonincubated, coated cross section	16	45.5	10.7	1.5	20.2	5.2	--	--	0.84

Figure 5.4 Energy dispersive-X ray analysis (EDXA) of $ZnSO_4$ incorporated-MAP granules, original (non-incubated) granules cross section and elemental analysis by energy dispersive-X ray analysis. (1) MAP-$ZnSO_4$ Non-incubated, external surface, (2) MAP- $ZnSO_4$ Non-incubated, cross section, (3) MAP-$ZnSO_4$ Non-incubated coated, external surface, (4) MAP- $ZnSO_4$ Non-incubated coated, cross section.

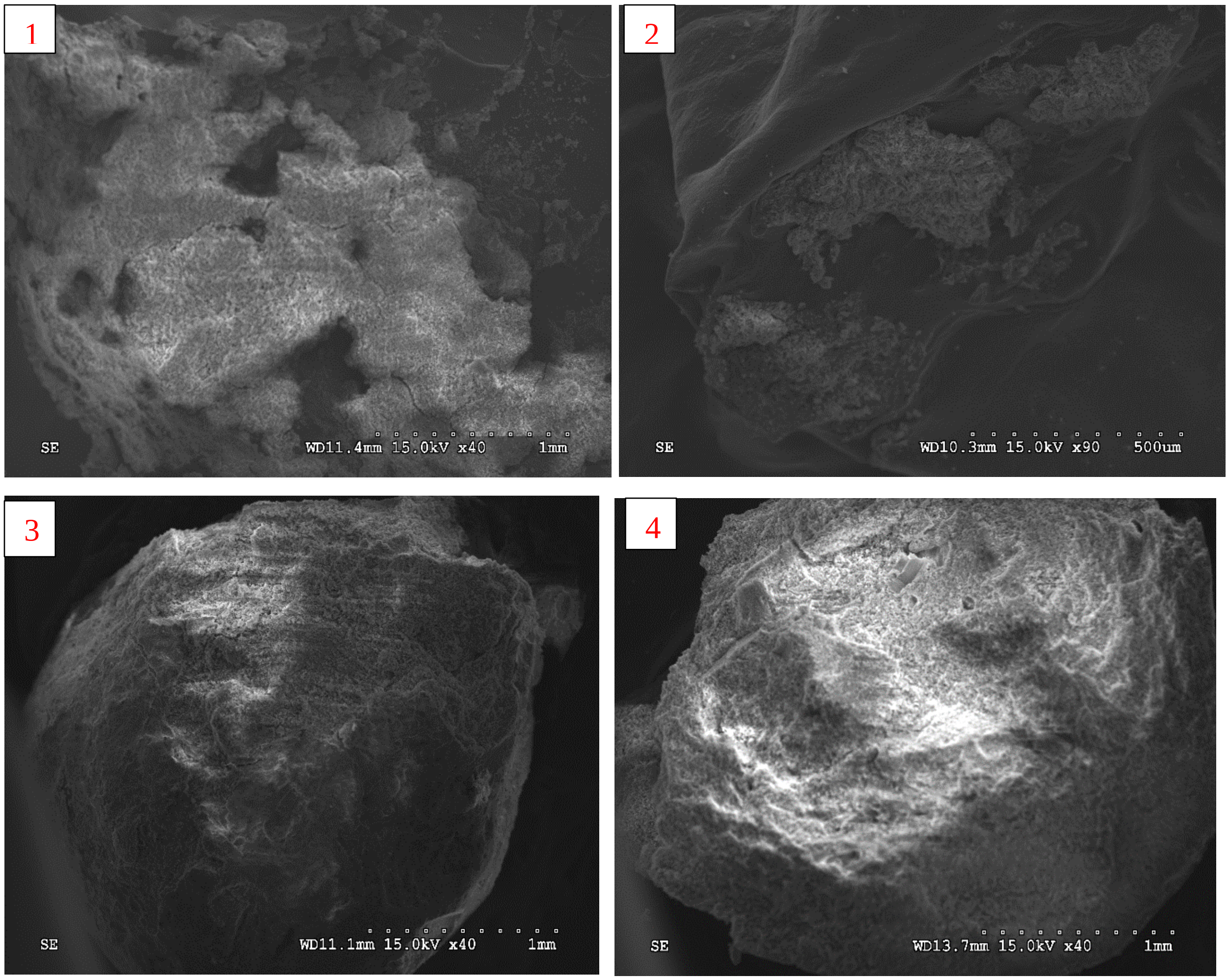
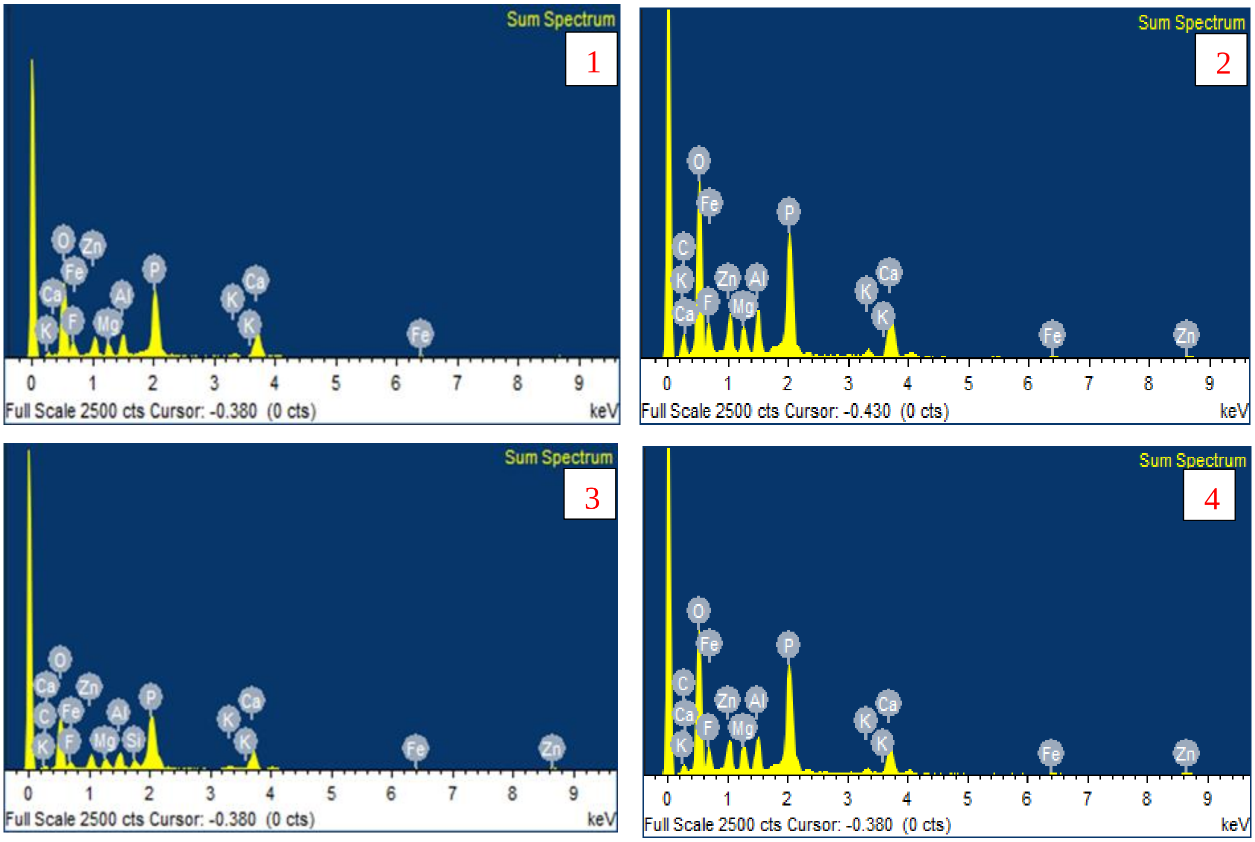


Figure 5.5 Scanning electron micrographs of $ZnSO_4$- incorporated-MAP granules incubated in the KS soil and elemental analysis by energy dispersive-X ray analysis. (1) MAP-$ZnSO_4$ incubated, external surface (2) MAP-$ZnSO_4$ incubated, cross section (3) MAP-$ZnSO_4$ incubated coated, external surface (4) MAP-$ZnSO_4$ incubated coated, cross section.



Treatments	C	O	Mg	P	Zn	Al	Fe	Ca	K
	……………………...% by weight…………………								
MAP-$ZnSO_4$ incubated, external surface	--	40.6	3.2	14.5	6.9	4.7	2.5	10.7	1.3
MAP-$ZnSO_4$ incubated, cross section	9.9	41.7	2.6	12.8	5.56	3.9	1.9	8.8	0.9
MAP-$ZnSO_4$ incubated-coated, external	5.1	38.7	2.7	17.9	11.9	4.4	5.3	10.1	2.8
MAP-$ZnSO_4$ incubated-coated, cross section	5	43	3.5	14.5	6.8	4	1.5	7.9	1

Figure 5.6 Energy dispersive-X ray analysis (EDXA) of $ZnSO_4$ incorporated-MAP granules incubated in the KS soil and energy dispersive-X ray analysis. (1) MAP-$ZnSO_4$ incubated, external surface (2) MAP-$ZnSO_4$ incubated, cross section (3) MAP-$ZnSO_4$ incubated coated, external surface (4) MAP-$ZnSO_4$ incubated coated, cross section.

The SEM image of the external surface and cross-sectional view of non-incubated MAP-ZnO granules, with and without AVAIL coating. are shown in Figure 5.7. The EDAX spectra of the cross-section electron images of non-incubated MAP-ZnO granules, with and without AVIL coating (Figure 5.8), showed peaks with P, O, and S together with C, Ca and other elements. Milani et al. (2015) reported that the coatings of granules mainly consisted of P, Zn, and O, followed by N, whereas the elemental composition of inner granules is made up of P, O, and N.

The figure (5.9) shows an SEM image of the external surface and its cross-section of incubated MAP-$ZnSO_4$ granules, with and without AVAIL coating, inner granule, and the coated surface of MAP-ZnO granule, and their elemental compositions. The EDAX spectra of the cross-sectional image for the incubated MAP-ZnO granules with and without AVAIL showed that the granules still contained significant amounts of P and S while the concentration of Zn, Ca, Al and Fe were enhanced (Figure 5.10). The original MAP-ZnO granules do contain 5% of S as $(NH4)_2SO4$ and 5% as elemental S. We collected EDXA spectra of the brighter spots scattered in the backscattered electron image of the remaining granule, and found mainly a peak of S, suggesting that elemental S is remaining as is in the incubated granules. It was evident from the SEM images that both the granules became more porous following their incubation in soil. Structural changes were probably caused by the partial dissolution of the granules and the diffusion of soluble nutrients into the surrounding soil (Nascimento et al., 2018 and Hettiarachchi et al., 2006). Overall, we believe that Ca moved into the granules from the soil, while Zn, Fe, and Al present in the granules became more concentrated in the remaining granules as soluble nutrients (such as N) moved out of the granules upon incubation.

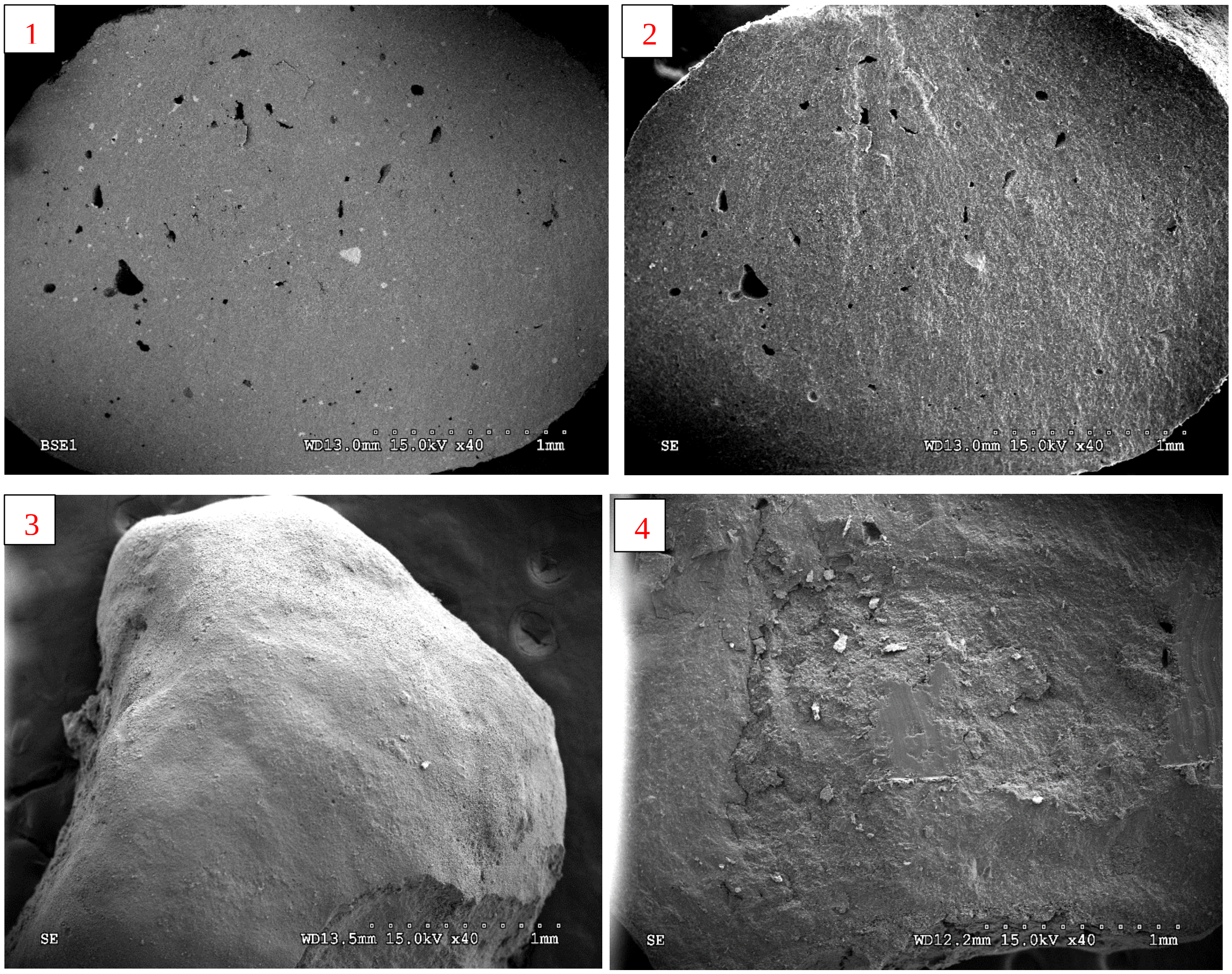
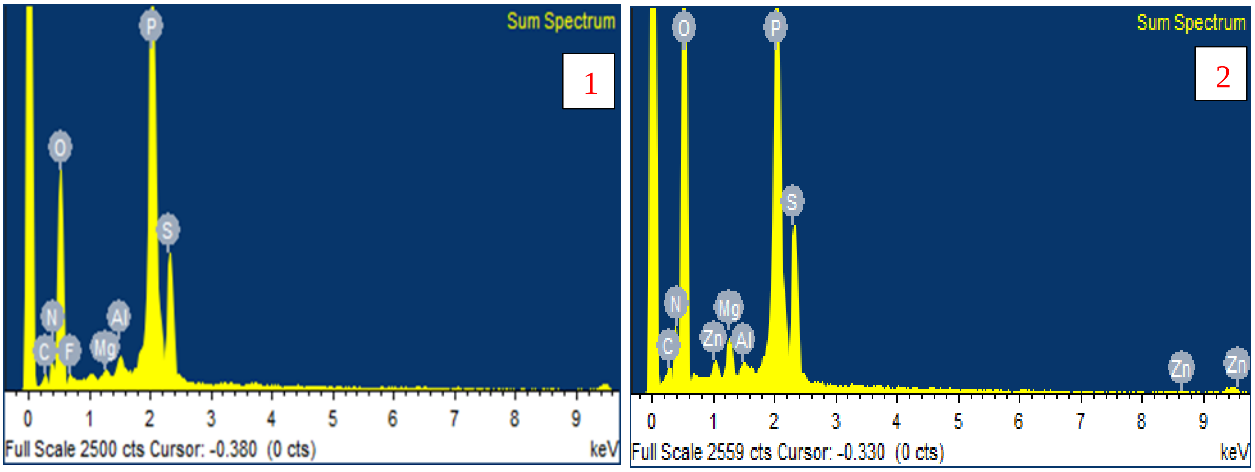


Figure 5.7 Scanning electron micrographs of the original (non-incubated) ZnO incorporated-MAP granules, with and without AVAIL coating. (1) MAP-ZnO Non-incubated, external surface, (2) MAP- ZnO Non-incubated, cross section, (3) MAP-ZnO Non-incubated coated, external surface, (4) MAP- ZnO Non-incubated coated, cross section.



Treatments	C	O	N	Mg	P	S	Zn	Al	Ca
	………………………...% by weight…………………								
MAP-ZnO Nonincubated external surface	--	--		--	--	--	--	--	--
MAP-ZnO Nonincubated, cross section	10	42.2	10	0.4	25.3	10.9	--	0.84	--
MAP-ZnO Nonincubated-coated external	6.8	43.9	15.9	1.3	10.5	21.7	--	--	--
MAP-ZnO Nonincubated-coated, cross, section	3.9	54.1	13.6	2	17.1	9.7	1.2	0.28	--

Figure 5.8 Energy dispersive-X ray analysis (EDXA) of original ZnO incorporated-MAP granules and elemental analysis by EDXA. (1) MAP-ZnO Non-incubated, external surface (2) MAP-ZnO Non-incubated, cross section (3) MAP-ZnO Non-incubated coated, external surface (4) MAP-ZnO Non-incubated coated, cross section.

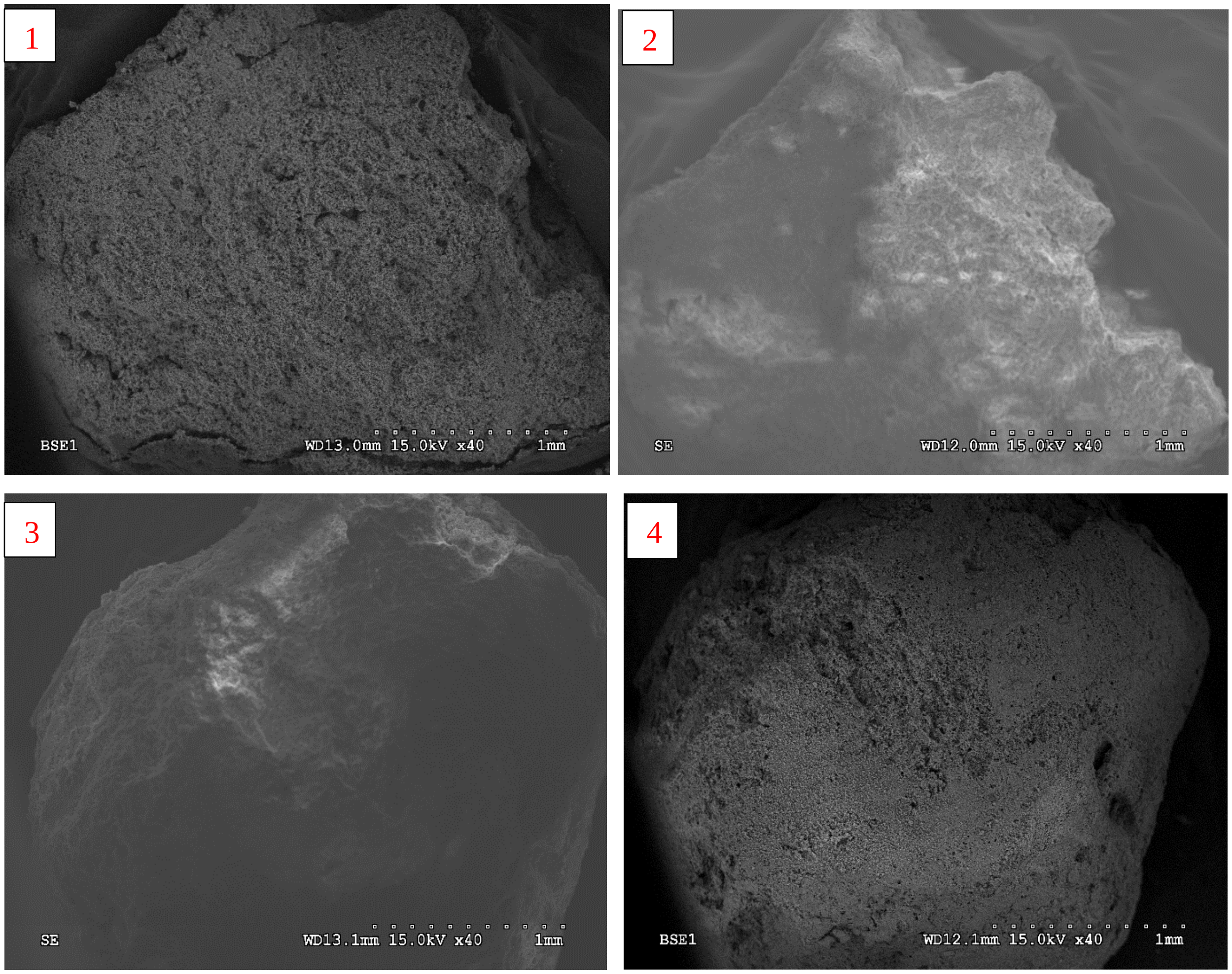
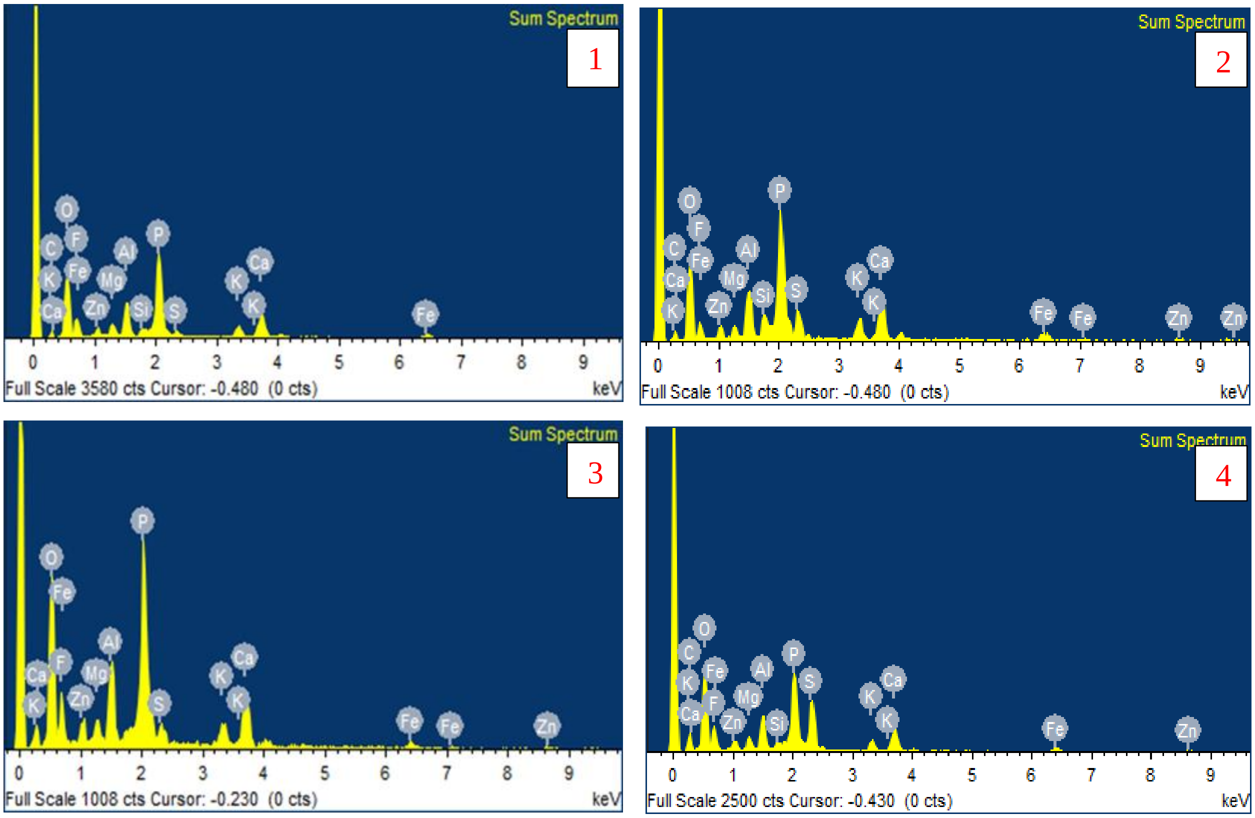


Figure 5.9 Scanning electron micrographs of the original (incubated) ZnO incorporated-MAP granules, with and without AVAIL coating. (1) MAP-ZnO incubated, external surface, (2) MAP-ZnO incubated, cross section, (3) MAP-ZnO incubated coated, external surface, (4) MAP- ZnO incubated coated, cross section.



Treatments	C	O	Mg	P	S	Zn	Al	Fe	Ca	K
	………………..	%, by weight	…………………							
MAP-ZnO incubated, external surface	7.5	33.3	1.9	16.6	1.4	3.9	5.7	4.3	8.5	3.8
MAP-ZnO incubated, cross section	9.1	29.2	1.6	16.1	4.5	7.5	5.2	6.5	9.3	4.1
MAP-ZnO incubated-coated external	57	36.5	1.8	16.6	2.1	5	6.1	3.5	9	3.1
MAP-ZnO incubated-coated, cross section	12	32.9	1.8	11.3	8.8	4.2	4.4	3.8	5.8	2.7

Figure 5.10 Energy dispersive-X ray analysis (EDXA) of ZnO incorporated-MAP granules incubated in the ID soil and elemental analysis by energy dispersive-X ray analysis. (1) MAP-ZnO incubated, external surface (2) MAP-ZnO incubated, cross section (3) MAP-ZnO incubated coated, external surface (4) MAP-ZnO incubated coated, cross section.

The effect of Zn treatments on the distribution of Zn in different soil fractions

Fractionation of Zn in soil was performed following the Tessier Sequential Extraction Procedure (Tessier et al., 1979), and the corresponding results are presented in Table 5.2. This technique is widely used by environmental scientists in research dealing with organic or inorganic treatments. Fractionation schemes have not been standardized yet, and each researcher uses his own scheme or a slight modification of one developed by another researcher. However, the main interest of the Zn fraction study focused on understanding reaction pathways and mechanisms of fertilizer Zn sequestration in calcareous soils when Zn is added as Zn-incorporated P granules.

Water-Soluble-Zn

At the end of the incubation, the percentage of the water-soluble Zn fraction of the soils differed on a mean value basis. Water-soluble Zn ranged from 0.2 to 0.48 mg kg-1 and comprised 0.15 to 0.32% of total soil Zn in the KS soil. This was followed by the ID soil that ranged from 0.30 to 0.44 mg kg-1 and comprised 0.19 to 0.28 % of total soil Zn (as shown in Figures 5.11 and 5.13).

For the KS soil, MAP-ZnO, MAP-$ZnSO_4$+AV, and MAP-ZnO+AV had significantly higher water solubility in the 0-7.5 mm section when compared to other treatment groups (Figure 5.11 and Figure 5.12). Increased water solubility in the 0-7.5 mm section for MAP-ZnO and MAP-$ZnSO_4$ both granule treatments is suggestive of the importance of the granular fertilizer for Zn dissolution, and diffusion from the granular, either with or without additives (Figure 5.11). This means that granular fertilizers are more useful in increasing Zn availability, which is an important observation for soil specific Zn nutritional management. On the other hand, soluble Zn fertilizer MAP-ZnO and MAP-ZnO+AV provided higher Zn diffusion in the high-pH soil than other fertilizer treatments, followed by MAP-$ZnSO_4$ in 0-7.5 mm section. Also, soluble Zn fertilizer (ZnO) exhibited lower Zn diffusion in the high-pH soil when compared to other Zn fertilizer treatments with and without additives. Soluble Zn fertilizer ($ZnSO_4$) provided higher Zn diffusion in the high-pH soil than the ZnO based fertilizers (Mattiello et al., 2017). Santos et al. (2019) reported that the greater diffusion of Zn^{2+} from $ZnSO_4$ could be attributed to the higher Zn concentration promoted by such soluble sources. Also, to some extent, Zn diffusion can benefit from the ionic interaction between Zn^{2+} and SO_4^{2-} in the soil solution. This is in agreement with other reports where they demonstrate that Zn diffusion in the soil is affected by the accompanying anions in the soil solution, Cl^- being more effective than SO_4^{2-}. There were no significant differences in water solubility for MAP-$ZnSO_4$, $ZnSO_4$, and $ZnSO_4$+AV in the 0-7.5 mm section. The water solubility for the MAP-$ZnSO_4$ and $ZnSO_4$ treatments was greater when compared to $ZnSO_4$+AV treatment. Also, there were no significant differences in water solubility for ZnO and ZnO+AV in the 0-7.5 mm section; the water solubility for the ZnO+AV treatment was greater than the ZnO treatment. Additionally, a comparison of the distribution of Zn across in 7.5-13.5 mm sections showed little difference between all Zn treatments. Zinc fixation is increased in the soils with more than 7.0 pH due to the enhanced concentration of carbonates. Compared to other fractions, water-soluble Zn was the least fraction in the soil.

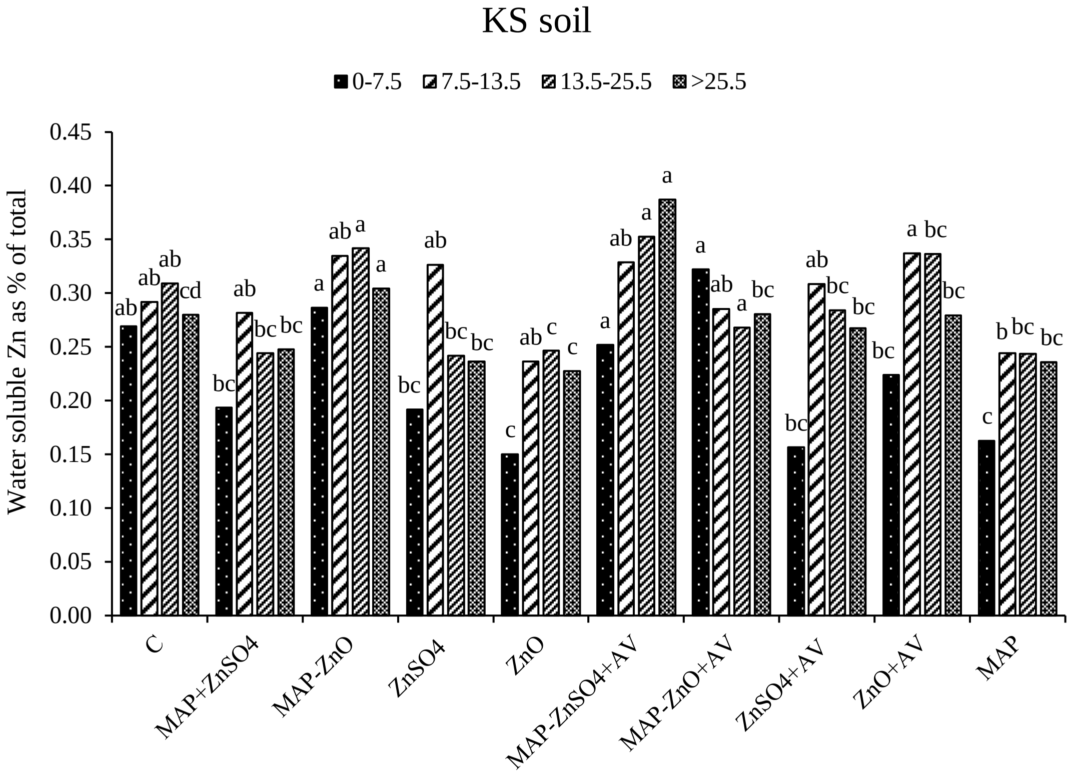


Figure 5.11 Water-soluble Zn expressed as a percent of total Zn in all soil sections collected from the point of fertiliser application, 35-days after fertilizer application in Kansas soil. C= control; MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; MAP-$ZnSO_4$ + AV = ZnSO4- incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance test. *NS = no significant difference in section.

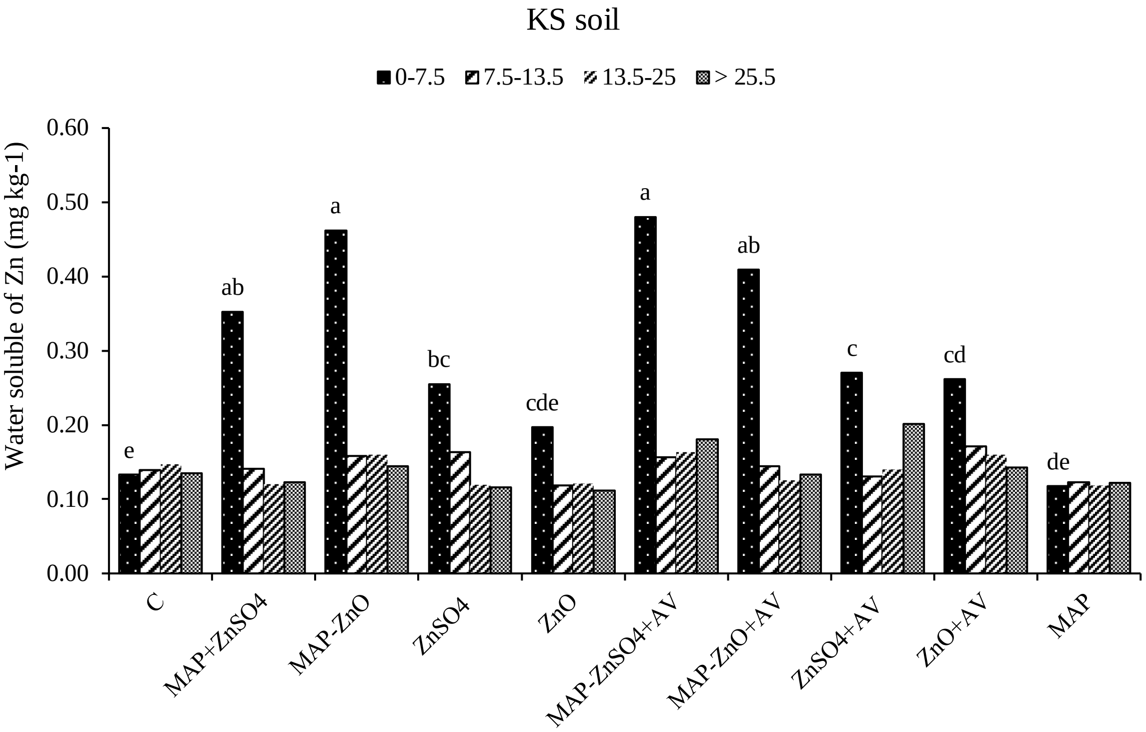


Figure 5.12 Water-soluble Zn (mg kg^{-1}) in all soil sections collected from the point of fertiliser application, 35-days after fertilizer application in Kansas soil. C= control; MAP-ZnSO$_4$ = ZnSO$_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; ZnSO$_4$ = Zn sulfate; ZnO = Zn oxide; MAP-ZnSO$_4$ + AV = ZnSO4- incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; ZnSO$_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

The water soluble results from ID soil indicates that MAP-ZnO, $ZnSO_4$, and ZnO+AV had significantly greater water solubility in the 0-7.5 mm section than all other treatments (Figure 5.13 and Figure 5.14). Santos et al., (2019) reported that the greater diffusion of Zn^{2+} from $ZnSO_4$ might be attributed to the higher Zn concentration, which in turn promoted by such soluble sources. Also, to some extent, Zn diffusion can benefit from the ionic interaction between Zn^{2+} and SO_4^{2-} in the soil solution, as it has been demonstrated that Zn diffusion in the soil is affected by the accompanying anions in soil solution, Cl^- being more effective than SO_4^{2-}. There were no significant differences in water solubility for MAP-$ZnSO_4$, MAP-$ZnSO_4$+AV and $ZnSO_4$+AV in the 0-7.5 mm section. The water solubility for the MAP-$ZnSO_4$+AV treatment was greater than the MAP-$ZnSO_4$+AV treatment. There were no significant differences in water solubility for ZnO and MAP-ZnO+AV in the 0-7.5 mm section, the water solubility for the MAP-ZnO+AV treatment was greater than the ZnO treatment. This might be attributed to the granular fertilizer composition of insoluble nutrient sources. These insoluble sources do promote retarded nutrient release into the soil solution because of their small reaction surface area, which affects plant nutrition (Santos et al., 2019). Also, water soluble Zn in $ZnSO_4$+AV exhibited lower Zn diffusion than other all fertilizer treatments. Additionally, comparison of the distribution of Zn across the soil sections (7.5-13.5 mm) showed little difference between all the fertilizers as compared to the control.

In the present study, the concentration and percent contribution of water soluble-Zn fraction to total Zn was lowest among all the Zn fractions. The high buffering capacity of these soils resulted in a low amount of water soluble-Zn. Similar findings were alsoreported from earlier studies by Deb (1997), Ghane (2011), and Ramzan et al., (2014), who also obtained the least Zn concentration in the water soluble fraction. Kabata-Pendias and Pendias (1999) reported that the concentration of water soluble Zn in soils ranges from 0.004-0.27 mg kg^{-1}, which is very low compared to the average total concentrations of about 50-80 mg kg^{-1}. The low concentration of water soluble Zn, when compared with other fractions, could be partially due to losses from leaching and plant uptake. This is the fraction which is more mobile and bioavailable in the soil. Filgueiras et al., (2002) and Alloway (2008) reported that when soils are rich in rapidly decomposable organic matter, Zn may become more available due to the formation of soluble organic Zn complexes which are mobile and probably capable of absorption into plant roots. This fraction is very important for plant nutrition, as it represents most available Zn water and clay surface sorbed.

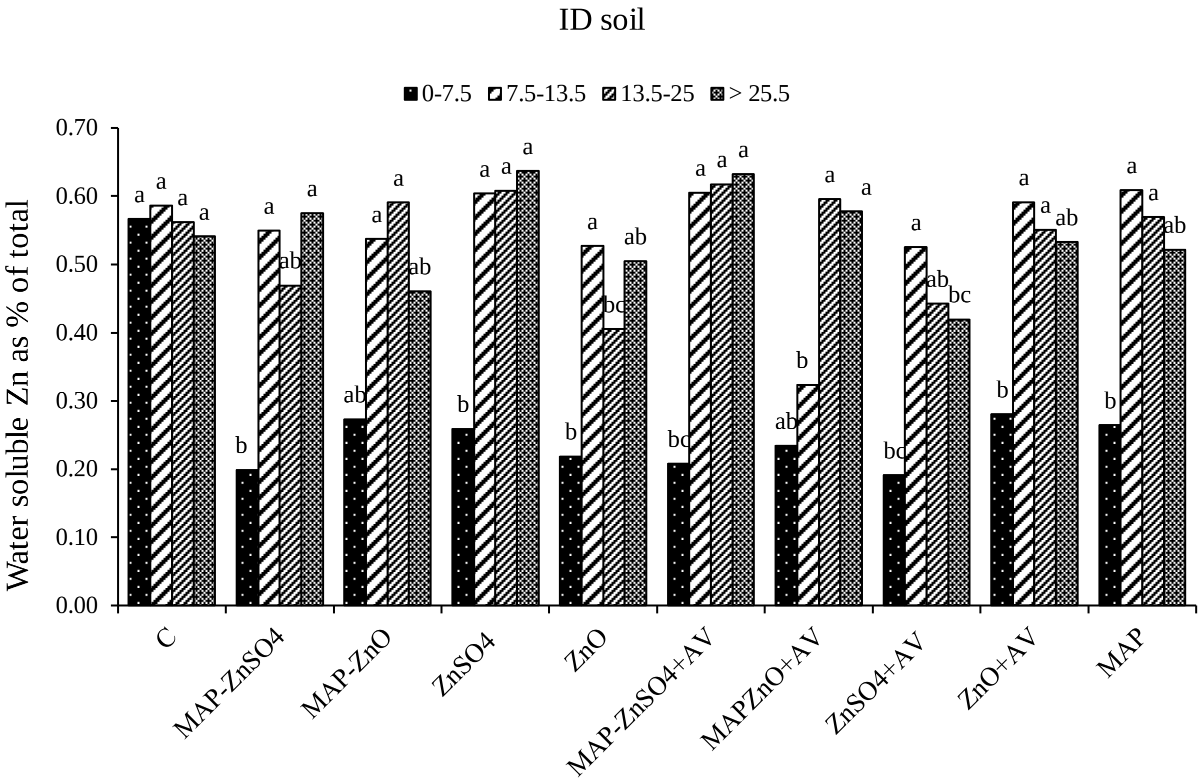


Figure 5.13 Water-soluble Zn expressed as a percent of total Zn in all soil sections collected from the point of fertiliser application, 35-days after fertilizer application in Idaho soil. C= control; MAP-ZnSO$_4$ = ZnSO$_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; ZnSO$_4$ = Zn sulfate; ZnO = Zn oxide; MAP-ZnSO$_4$ + AV = ZnSO4- incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; ZnSO$_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance test. *NS = no significant difference in section.

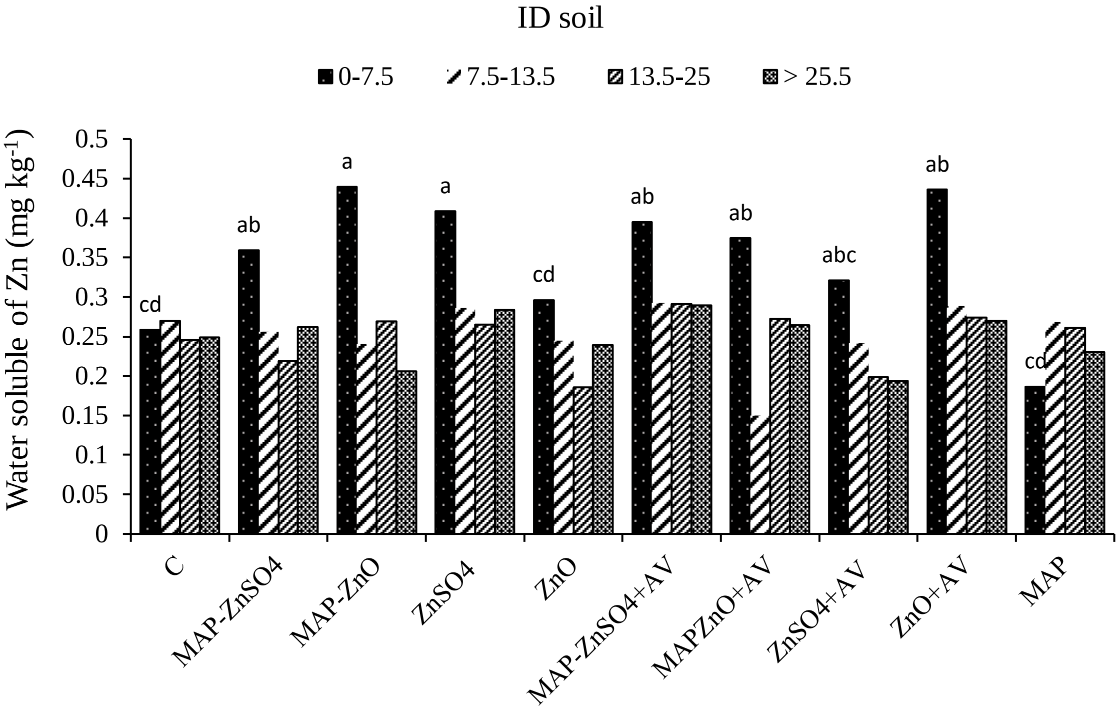


Figure 5.14 Water-soluble Zn expressed as (mg kg^{-1}) in all soil sections collected from the point of fertiliser, 35-days after fertilizer application in Idaho soil. C= control; MAP-ZnSO$_4$ = ZnSO$_4$-incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; ZnSO$_4$ = Zn sulfate; ZnO = Zn oxide; MAP-ZnSO$_4$ + AV = ZnSO4- incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO-incorporated – MAP+ AV; ZnSO$_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

Exchangeable-Zn

The exchangeable Zn fraction was higher than the water-soluble Zn and ranged from 0.2 to 5.2 mg kg^{-1}. It makes up to 0.1 to 2.9% of total Zn in the KS soil. The exchangeable Zn fraction varied from 4.69 to 6.85 mg kg^{-1} and comprised 2.7 to 3.9% of total Zn in the ID soil. Exchangeable Zn was very low in soils mainly because of the sandy nature of some of the soils, presence of $CaCO_3$, unfavorable high pH, and low organic matter content in soils (Hussain et al., 2011).

For the KS soil, results on the exchangeable Zn indicates that MAP-$ZnSO_4$ and MAP-ZnO without additives had significantly greater exchangeable in the 0-7.5 mm section when compared to all other treatments (Figure 5.15). Increased exchangeable Zn in the 0-7.5 mm section for both granular treatments may partly be due to the lower pH of granule dissolution in the 0-7.5 mm section, allowing for greater Zn solubility. The ZnO treatments contained significantly less exchangeable in the 0-7.5 mm section as compared to ZnO+AV. Decreasing exchangeable Zn in the 0-7.5 mm section for $ZnSO_4$, ZnO, and MAP-ZnO+AV was noticed across all other treatments. This is probably due to the rate of adsorption or fixation is usually rapid initially and decreases sharply with time (Lindsay and Norvell, 1969). However, MAP-$ZnSO_4$ and MAP-ZnO showed greater more exchangeable Zn in 7.5-13.5 mm section as compared to MAP-$ZnSO_4$+AV and MAP-ZnO+AV. Additionally, the concentration of Zn in the exchangeable and organic fractions increased as pH decreased, which may reflect either an increase in solubility of Zn minerals (Lindsay, 1972) or a decrease in specifically adsorbed Zn (Brümmer et al., 1983), with decreasing pH. It is possible that at a lower pH, a larger proportion of total soil Zn has been retained in the exchangeable and organic fractions because the percentage of Zn in the exchangeable and organic fraction increased as pH decreased. The Zn concentration in the exchangeable Zn fraction of the 7.5-13.5 mm section might be governed by the solubility of $Zn(OH)_2$ and see a decrease in Zn concentration with time, probably as a result of increasing pH with increasing distance from the center section.

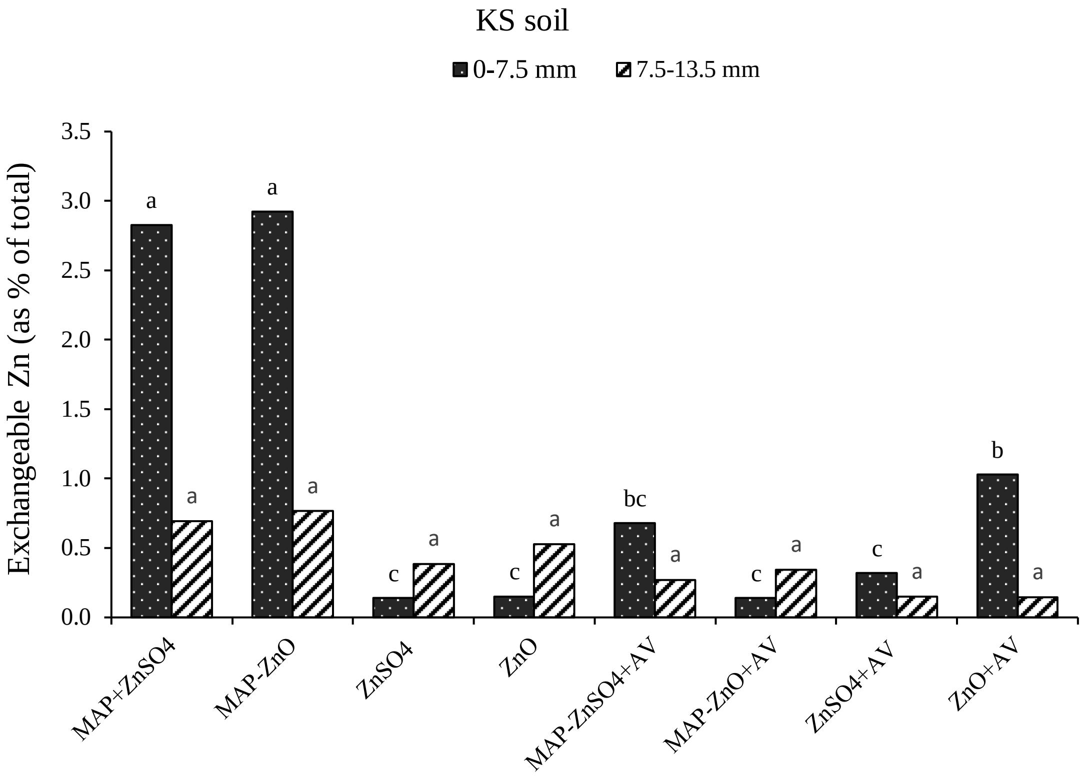


Figure 5.15 Exchangeable Zn expressed as a percent of total Zn by section, 35-days after fertilizer application in Kansas soil. MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; MAP-$ZnSO_4$ + AV = ZnSO4-incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

In the ID soil, regardless of Zn source, the exchangeable results across all of the Zn treatments had greater exchangeability in the 0-7.5 mm section (Figure 5.16). There were no significant differences in all treatments. The $ZnSO_4$ values suggest greater exchangeable of Zn out in the 0-7.5 mm section for the MAP-ZnSO4, $ZnSO_4$, MAP-$ZnSO_4$+AV, and $ZnSO_4$+AV respectively. Increased exchangeable Zn in the 0-7.5 mm section for both granular and liquid treatments may partly be due to the lower pH of granule dissolution in the 0-7.5 mm section, and the texture is loam (L) possibly indicating a general increase in Zn mobility as compared to the KS soil, allowing for greater Zn solubility. There were increased values in 7.5-13.5 mm section for $ZnSO_4$, MAP-$ZnSO_4$+AV, compared to all other Zn treatments. Both the ZnO and ZnO+AV treatments had lower values exchangeable in the 7.5-13.5 mm section as a comparison to all other treatments. This is probably due to Zn form by these fractions in the complex with other components. Therefore, even accounting for physicochemical differences between this theoretical perspective and a real soil solution, including ionic force and the presence of other ions, this data demonstrates that it is possible to have a higher Zn concentration at this soil.

Although the addition/inclusion of Zn with fertilizers, there were no significant differences between treatments in exchangeable Zn concentrations in the 7.5-13.5 mm section. Overall, treatments of Zn fertilizer in the 7.5-13.5 mm sections were much lower those in the 0-7.5 mm section regardless of Zn source, which may be the result of decreased fertilizer Zn diffusion with distance as a result of both precipitation and adsorption reactions. This was due to the extremely high levels of $CaCO_3$, which could reduce Zn solubility and free $CaCO_3$ in calcareous soil as one of the major sinks for Zn at high pH (Hettiarachchi et al., 2010; Wenming et al., 2001). Other reasons could be a high buffering capacity of soils and adsorption of Zn on soil surfaces (Robarge, 2018). The alkaline calcareous soils have hydroxyl and oxide groups, and insoluble Ca compounds which convert available Zn to unavailable forms as $Zn(OH)_2$ and $CaZnO_2$ (Mahmoud Soltani et al., 2015). Clays were dominant in this fraction as compared to other soil-textural class groups, mainly due to high surface area and high cation exchange capacity (CEC) (Khoshgoftarmanesh et al., 2018).

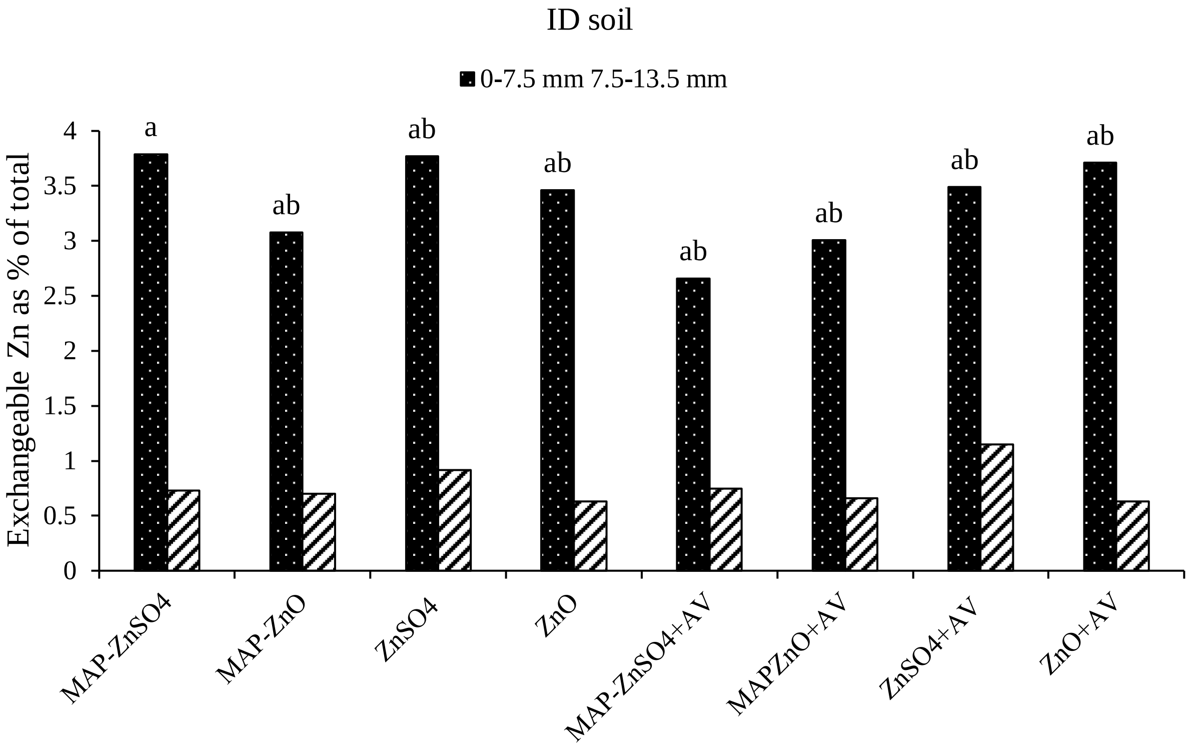


Figure 5.16 Exchangeable Zn expressed as a percent of total Zn by section, 35-days after fertilizer application in Idaho soil. MAP-ZnSO$_4$ = ZnSO$_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; ZnSO$_4$ = Zn sulfate; ZnO = Zn oxide; MAP-ZnSO$_4$ + AV = ZnSO4-incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; ZnSO$_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

Carbonate-bound Zn

The carbonate-bound Zn varied between soils (Figures 5.7 and 5.8), and the values ranged from 23.06 to 60.75 mg kg^{-1} comprising up to 17.3 and 34.1% of total Zn in the KS soil and from 30.99 to 46.78 mg kg^{-1} comprising 19.24 and 34.5% of total Zn in the ID soils, respectively. This may be due to carbonate (calcite), which is an important sorbent for Zn (Zacchara et al., 1988).

For the KS soil, Zn sources had significantly greater carbonate-bound Zn for both MAP-$ZnSO_4$ and MAP-ZnO treatments, with and without additives, in comparison to the two Zn liquid treatments in the 0-7.5 mm section (Figure 5.17). This is due to the higher pH of the soils as increasing pH increases the concentration of carbonate-bound Zn (Ramzan et al., 2014). Although the addition of Zn fertilizer treatments, either in granular or liquid forms, had not significantly increased the carbonate-bound Zn in the $ZnSO_4$, ZnO, and ZnO+AV treatments. There were no significant differences between treatments in exchangeable Zn concentration in the 7.5-13.5 mm section.

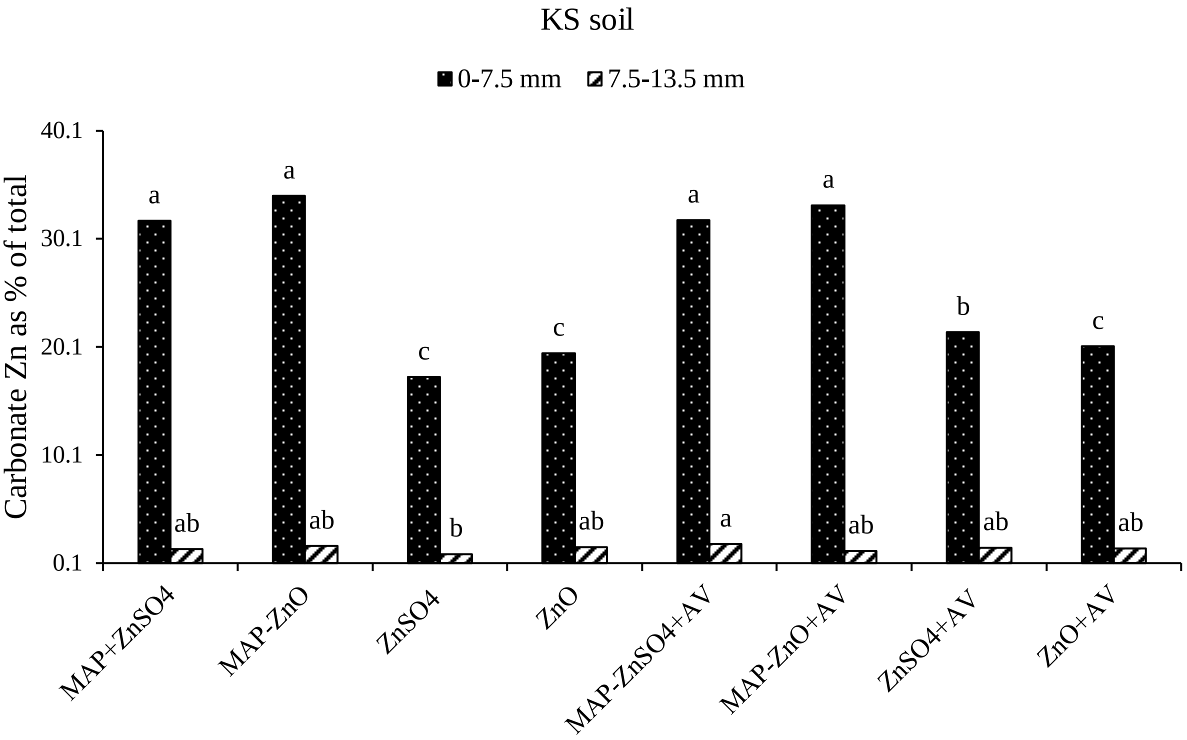


Figure 5.17 Carbonate Zn expressed as a percent of total Zn by section , 35-days after fertilizer application in Kansas soil. MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO-incorporated – MAP; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; MAP-$ZnSO_4$ + AV = ZnSO4-incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance test. *NS = no significant difference in section.

The results for the ID soil were very different from the KS soil in that all treatments had not significantly contained more carbonate-bound Zn in the 0-7.5 mm section (Figure 5.18). Carbonate-bound Zn was the highest fraction in this soil. This could be due to the high carbonate content of the soil, the parent material from which the soils were derived (Hiller, 2006), as well as the higher pH of the soils, as increasing pH increases the concentration of carbonate-bound Zn (Ramzan et al., 2014). Ramzan et al., (2014) and Rajakumar (1994) noted that carbonate-bound Zn is usually seen in soils with high pH and lime content. Although, the addition of Zn with fertilizers, either in granular or liquid forms, had increased the carbonate-bound Zn in 7.5-13.5 mm section for all treatments; we did not notice any significant differences between treatments in the 7.5-13.5 mm section.

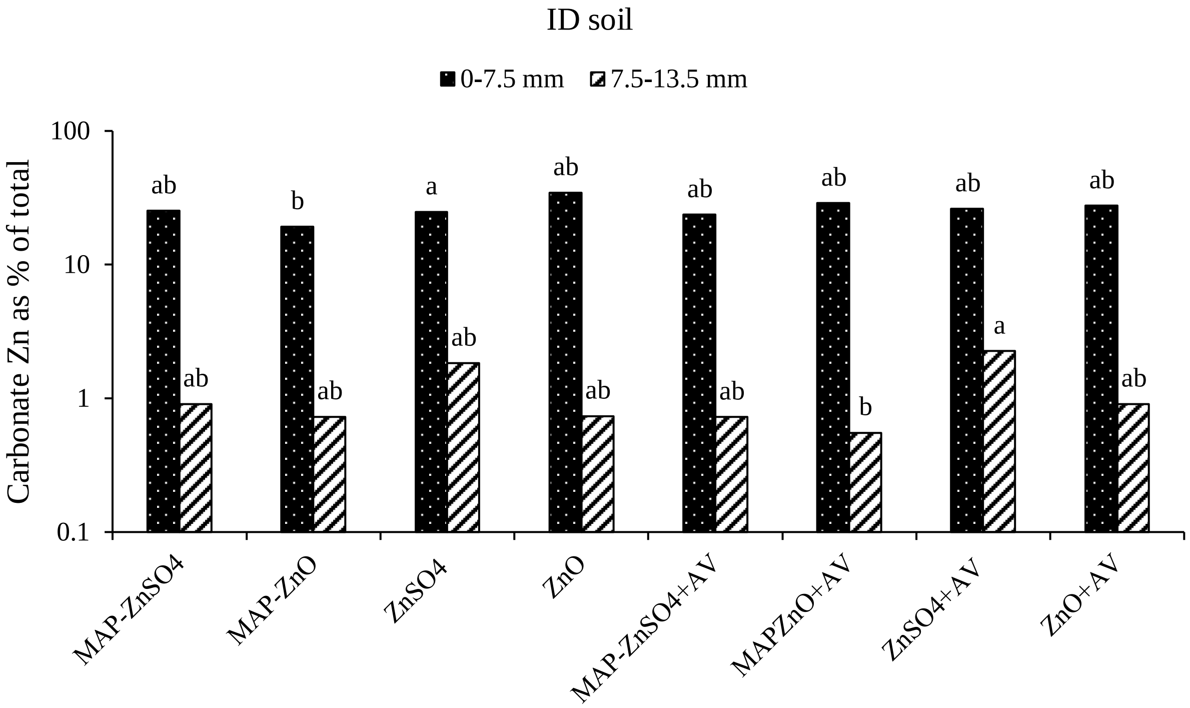


Figure 5.18 Carbonate Zn expressed as a percent of total Zn by section, 35-days after fertilizer application in Idaho soil. MAP-ZnSO$_4$ = ZnSO$_4$- incorporated – MAP; MAP-ZnO = ZnO-incorporated – MAP; ZnSO$_4$ = Zn sulfate; ZnO = Zn oxide; MAP-ZnSO$_4$ + AV = ZnSO4-incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; ZnSO$_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

Iron-Manganese (Fe-Mn) oxide-bound Zinc fraction

The iron-manganese oxide-bound Zn fraction, being among the non-residual fraction (Figures 5.19 and 5.20), was found to be a significant Zn fraction, which ranged from 36.5 to 64.6 mg kg^{-1}, forms about 26.8 to 40.6% of the total in KS soil. The same fraction ranged from 1.3 to 64.3 mg kg $^{-1}$ and comprised of 1 to 33.9% of the total ID soil. This can be attributed to higher amounts of Zn adsorption on the surface of the oxides. The Zn may be occluded and co-precipitated with hydrous oxides of manganese (Mn) and iron (Fe), which in turn help these oxides to form a principal matrix that holds abundant Zn metal (Wijebandara, 2007). At the higher pH (7.5−8.5) of calcareous soils, the negative charge of the Fe oxides is high, which enhances the formation and stability of the Zn surface complexes (Sacristán et al., 2019).

For the KS soil, regardless of Zn source (Figure 5.18), all of Zn treatments had a greater Fe-Mn oxide-bound Zn fraction in the 0-7.5 mm and 7.5-13.5mm section. This may be attributed to the predominance of the crystalline Fe-oxide content of the soils (Pal et al., 1997). Also, we observed the lowest Fe-Mn oxide-bound Zn was measured in the ZnO treatment. This may be due to the increased pH of the fertilizer. The capacity of these oxides to adsorb trace cations from solutions is controlled by their pH and crystallinity (Tessier et al., 1979). In contrast, the highest Fe-Mn oxide-bound Zn was measured in the MAP-ZnO+AV treatment. This may be due to a more retentive capacity of the amorphous sesquioxides due to its larger specific surface area (Prasad et al.,1996). Mandal and Mandal (1986) reported that higher amounts of amorphous sesquioxide-bound Zn, rather than the crystalline sesquioxide-bound Zn, was attributed to the acidic environment, which favored the formation of amorphous sesquioxides in soils. Significantly greater Zn-bound values in the 7.5-13.5 mm section for MAP-$ZnSO_4$, MAP-ZnO, MAP-$ZnSO_4$, and ZnO+AV were noted. Also, Fe and Mn are usually present in the clay fraction of soils.

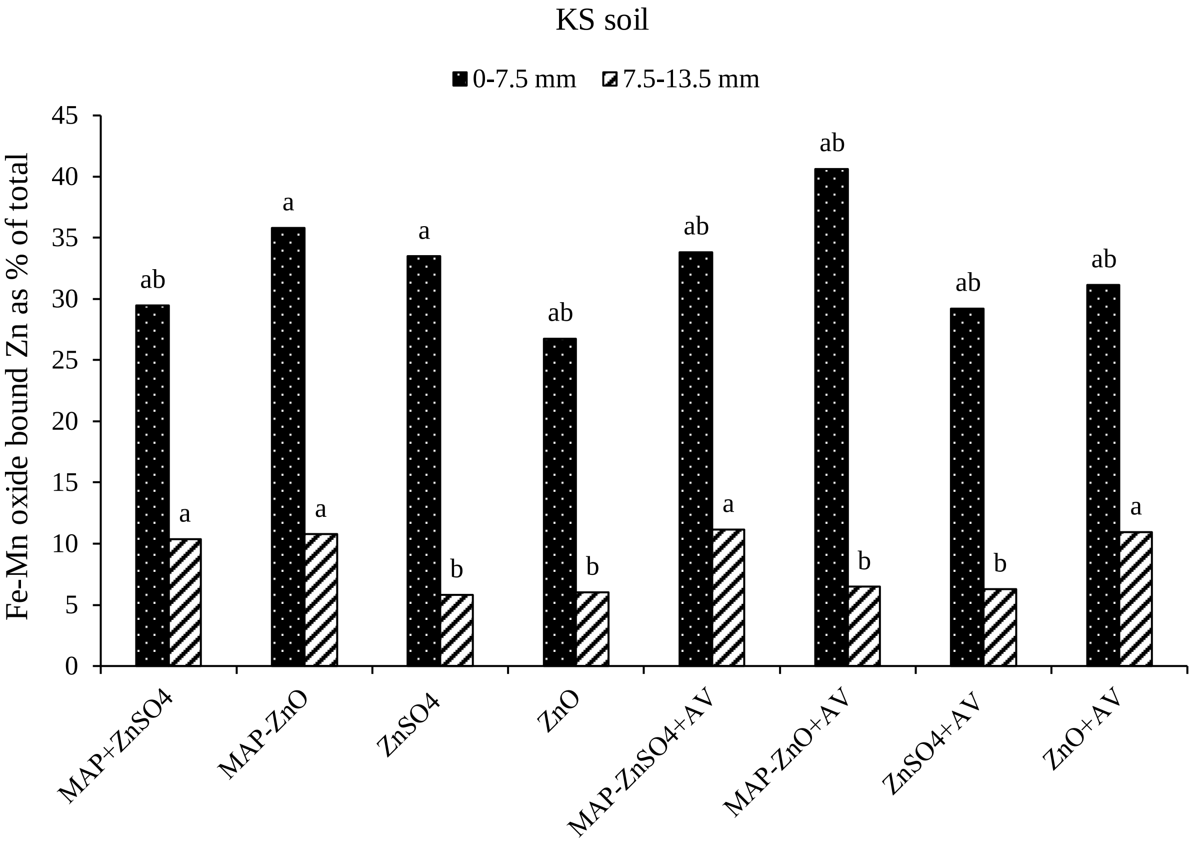


Figure 5.19 Fe-Mn oxide-bound Zn expressed as a percent of total Zn by section, 35-days after fertilizer application in Kansas soil. MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; MAP-$ZnSO_4$ + AV = ZnSO4-incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

For the soil from ID, the results were very different from the KS soil. The treatments with granular fertilizers with additives had significantly greater Fe-Mn oxide-bound Zn in the 0-7.5 mm section than other granular without additives treatments (Figure 5.20). The lowest values for Fe-Mn oxide-bound Zn was measured in the ZnO treatment. In contrast, the highest Fe-Mn oxide-bound Zn was measured in the MAP-$ZnSO_4$+AV treatment. This could be due to the ability of amorphous sesquioxide to adsorb Zn. The higher content of amorphous sesquioxide-bound Zn than crystalline-bound Zn could be attributed to the greater ability of amorphous sesquioxide to adsorb Zn because of their high specific surface area (Davis and Lecki, 1978). The Fe oxides play a central role in Zn adsorption by soils (Uygur and Rimmer, 2000).

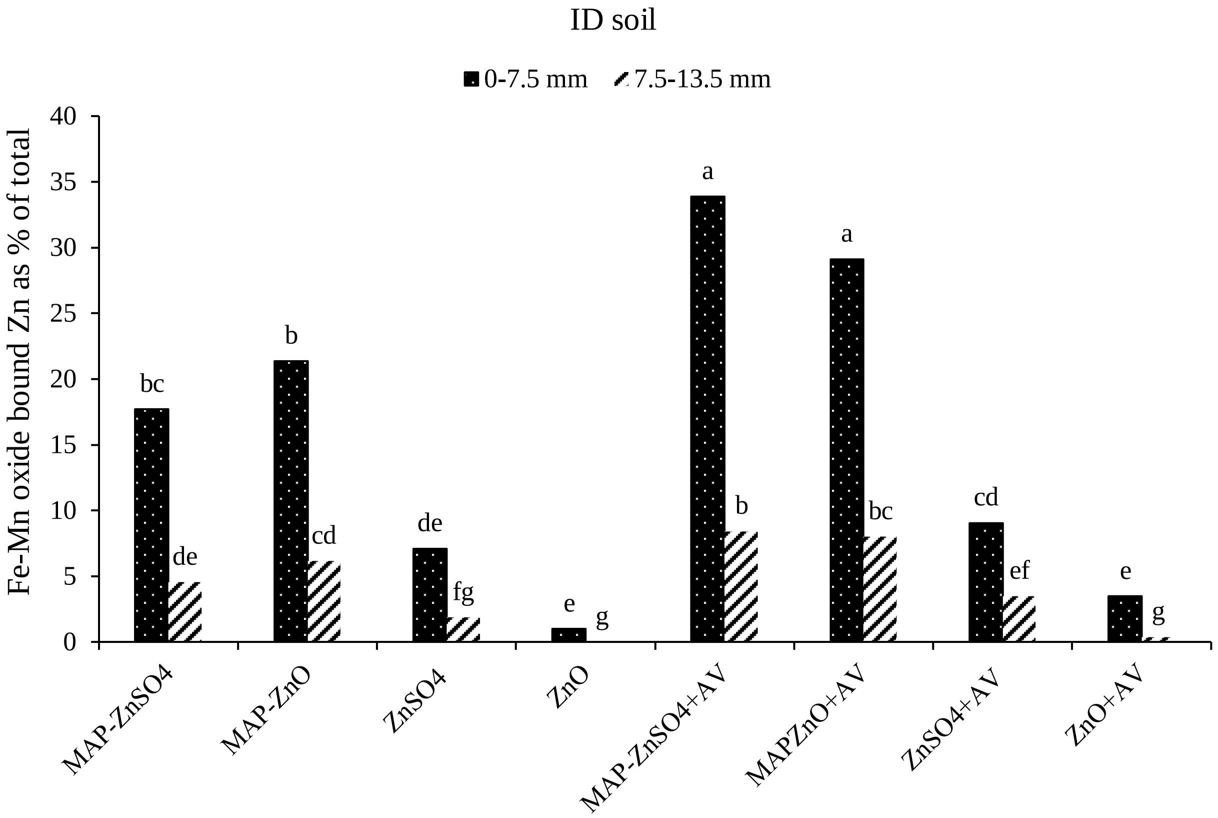


Figure 5.20 Fe-Mn oxide-bound Zn expressed as a percent of total Zn by section, 35-days after fertilizer application in Idaho soil. MAP-ZnSO$_4$ = ZnSO$_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; ZnSO$_4$ = Zn sulfate; ZnO = Zn oxide; MAP-ZnSO$_4$ + AV = ZnSO4- incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; ZnSO$_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance test. *NS = no significant difference in section.

Organic-bound Zn

The content of organic-bound Zn fraction in both soils (Figures 5.20 and 5.22) had a range from 1.3 to 3.1 mg kg^{-1} with 1 to 1.7% of total Zn in the KS soil, and it ranged from 2.5 to 18.3 mg kg^{-1} with 1.8 to 11.6% of total Zn in ID soils. Organic-bound Zn fraction was lower in the soils primarily due to lower soil organic matter contents, and a high decomposition rate of organic matter due to the elevated temperature of the region (Błońska and Lasota, 2017). In both soils, the application of deferent Zn sources in soil caused variation in the organic-Zn, which increased during the incubation period. Examination of the data further revealed that organic-bound Zn in both soils was less than all fractions except for the water-soluble of Zn.

For the KS soil, the organically-bound Zn fraction followed a similar distribution trend with water-soluble Zn, which contributed to very less to total Zn, and this could be due to low to medium organic matter content of the experimental soils. All Zn sources had low values with the organic-bound Zn fraction in the 0-7.5 mm section (Figure 5.21). This may be due to the sorption of Zn on the surface of the clay, and organic matter also increases as the soil pH (Jahiruddin et al., 1986). Similar results were also reported by Singh et al. (1988); lower content of organically bound Zn was mainly due to the low organic carbon status of the soils. However, Zn treatments in the 7.5-13.5 mm section for all treatments did not increase the Fe-Mn oxide fraction. Due to a higher rate of conversion of the amorphous sesquioxide-bound Zn and subsequent low recovery of organic fraction of Zn occurred at the later period of incubation. This could be due to greater microbial immobilization as well as the formation of insoluble complexes with soil organic matter (Das, 1996).

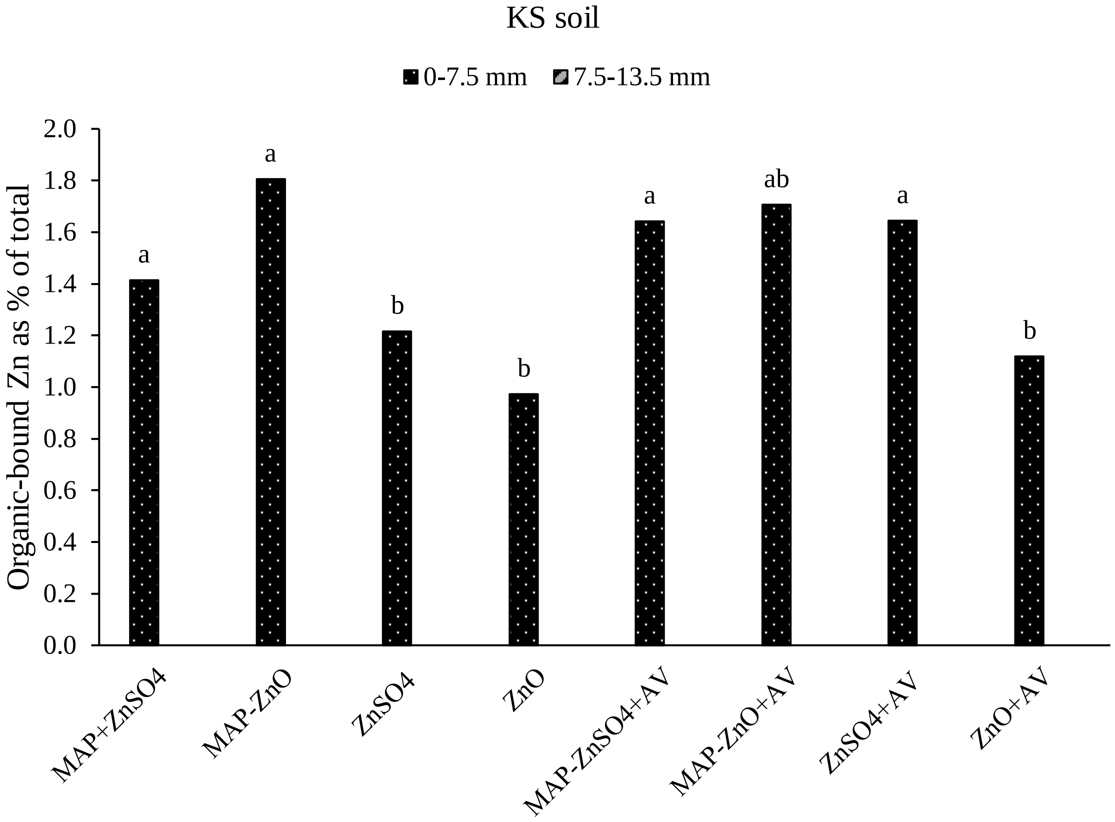


Figure 5.21 Organic-bound Zn expressed as a percent of total Zn by section, 35-days after fertilizer application in Kansas soil. MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; MAP-$ZnSO_4$ + AV = ZnSO4- incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

The ID soil Zn sources had significantly greater organic-bound Zn for both MAP-$ZnSO_4$ and MAP-ZnO treatments, with and without additives, in the 0-7.5 mm section when compared to the two Zn liquid treatments (Figure 5.22). The lowest organic-bound Zn was measured in the ZnO treatment. In contrast, the highest organic-bound Zn was measured in the treatment of MAP-ZnO+AV treatment (11.6 %).

There were no significant differences between treatments in the 7.5-13.5 mm section. The concentration of Zn in the organic fractions was increased with outer ring distance, which may reflect either an increase in solubility of Zn minerals or a decrease in specifically adsorbed Zn with an increasing pH. Such an increase in the content of the treatments receiving fertilizers may be due to the higher organic carbon content in these treatments. Zinc is known to form strong complexes with organic matter in the soil (Priyanka and Meena, 2017). The conjoint use of chemical fertilizers increased the fractions significantly in comparison to the control. This may be due to the conversion of added Zn, and the transformation of native Zn into this fraction.

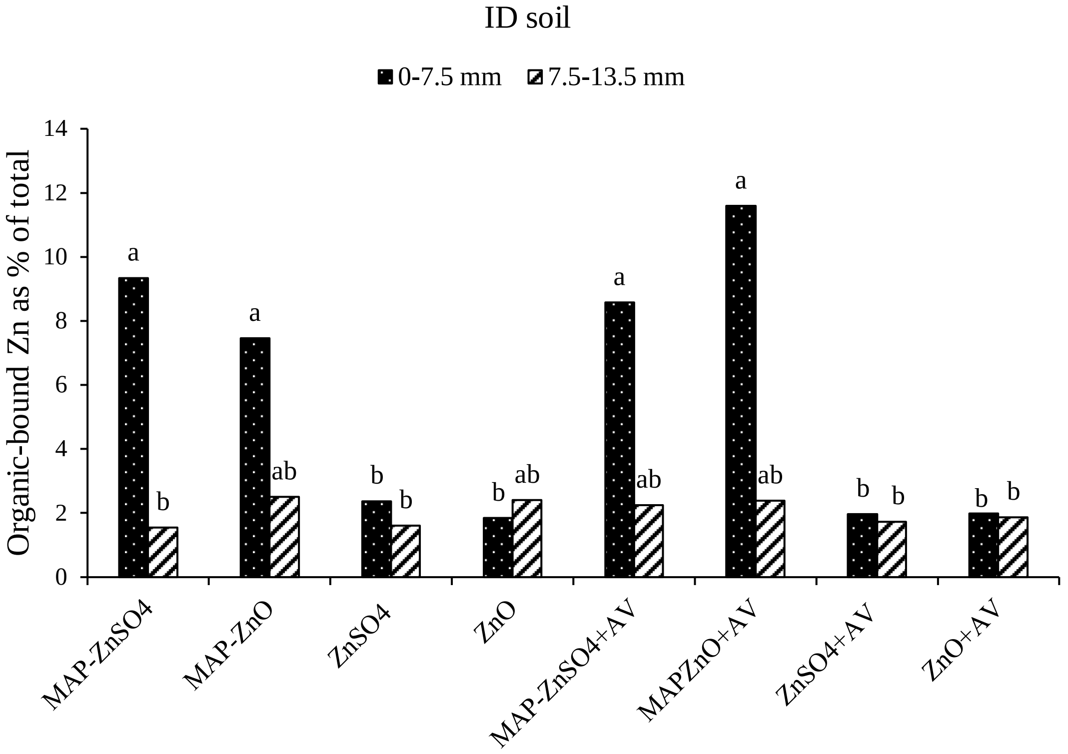


Figure 5.22 Organic-bound Zn expressed as a percent of total Zn by section , 35-days after fertilizer application in Idaho soil. MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; MAP-$ZnSO_4$ + AV = ZnSO4-incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance test. *NS = no significant difference in section.

Residual-Zn

Residual-Zn dominated most of the soils, which forms the major fraction of residual Zn (Figures 5.23 and 5.24) ranging from 32.6 to 44.8 mg kg^{-1} comprising of 28.5 to 59.2% of total Zn in the KS soil and from 23.4 to 42.1 mg kg^{-1} comprising of 31.4 to 64.9% of total Zn in the ID soil. This reversible increase in this fraction may be due to the release of other fractions of Zn from the total Zn is lesser, and it will remain as residual Zn in the soil and vice versa. Residual Zn is referred to as the Zn fraction occluded in the crystal structures of silicates and resistant minerals after the removal of extractable Zn fractions (Chao, 1984).

For the KS soil, all Zn sources had greater values of residual Zn fraction (residual+ unrecovered) in the 0-7.5 mm section (Figure 5.23). The percent contribution of this fraction from total Zn was observed to be higher than other fractions with 28.5, 32.9, 35.7, 41.1, 44.8, 46.3, 52.6, and 59.2% at MAP-ZnO, MAP-$ZnSO_4$+AV, MAP-$ZnSO_4$, $ZnSO_4$, $ZnSO_4$+AV, ZnO+AV, ZnO, and MAP-ZnO+AV, respectively. This may be due to the type of dominant clay minerals, which is the reason for variation in the amount of residual Zn fraction. This result indicated that the highest concentration of Zn was in the residual fraction. This means a greater percentage of Zn in the residual fraction in most of the soils will become unavailable to plants (Ramzan et al., 2014). Indeed, the most abundant soil Zn fraction is the residual fraction, which is the least important for plant uptake (Regmi, 2010).

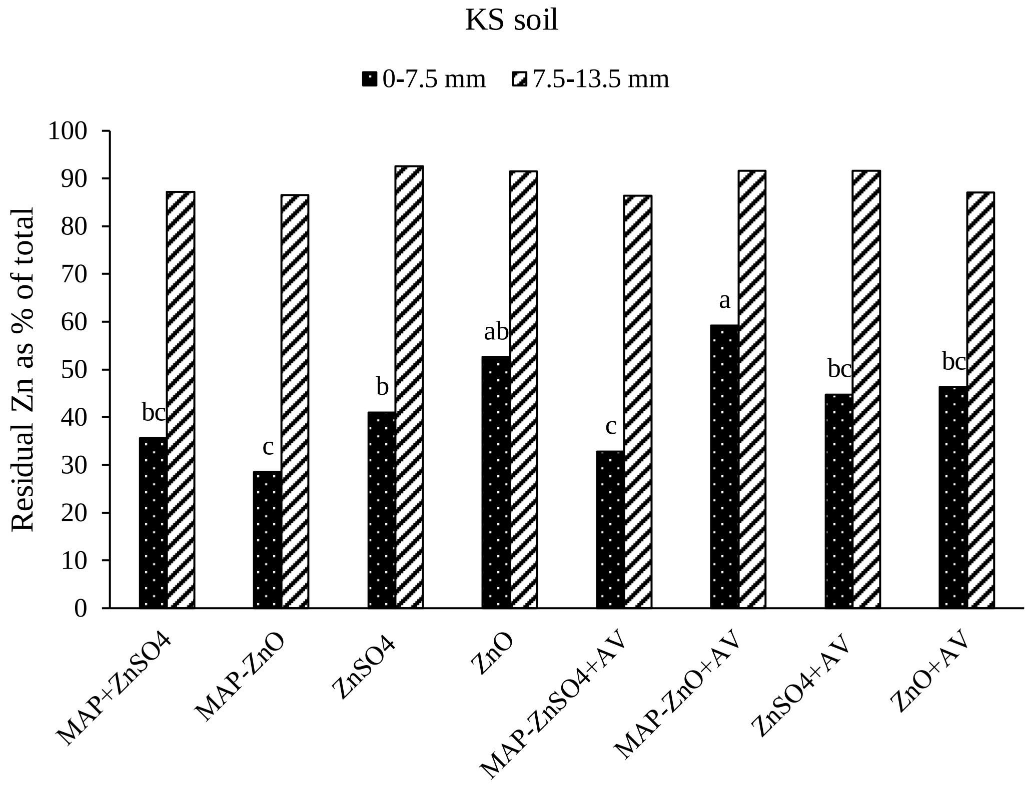


Figure 5.23 Residual Zn (residual+ unrecovered) expressed as a percent of total Zn by section, 35-days after fertilizer application in Kansas soil. MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; MAP-$ZnSO_4$ + AV = ZnSO4- incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey’s honest significance test. *NS = no significant difference in section.

Results from our study also showed that the residual fraction of Zn (residual+ unrecovered) in soil was greater with Zn treatments as MAP-ZnO+AV and ZnO in the 0-7.5 mm section than all other treatments, due to greater fixation and adsorption processes. These results are also supported by Karak et al. (2005), who reported that the relatively higher maintenance of Zn in the soil is due to applied chelated-Zn. This might be attributed to the very little or no interaction among soil components, thus preventing various harmful reactions occurring in the soil as compared to soil treated with $ZnSO_4$, which enhances greater fixation, adsorption, and precipitation. This is resulting from the greater interaction between soil components. The residual fraction of Zn was found to be dominant in the soil under this study, which contributed to more than 80% of total Zn in control. The maximum amount of residual + unrecovered Zn found in MAP-ZnO+AV treatment. Due to soils having excessive P concentrations, phosphate dissolved from the MAP granule could rapidly reduce the release of Zn as the result of the precipitation of Zn.

The results for the ID soil, all Zn sources had significantly greater percentages in MAP-Zn granular, with and without additives in the 0-7.5 mm section compared to other treatments (Figure 5.24). Due to this soil having excessive P concentrations, phosphate dissolved from the MAP granule could rapidly reduce the release of Zn as a result of precipitation of Zn. The percent contribution of this fraction (residual+ unrecovered) from total Zn was observed to be higher than other fractions with 31.4, 40.4, 42.4, 49.4, 50.2, 51.6, 55.3, and 64.9% at MAP-ZnO+AV, MAP-$ZnSO_4$+AV, $ZnSO_4$+AV, MAP-$ZnSO_4$, ZnO, MAP-ZnO, ZnO+AV, and ZnSO4. It is considered as the primary form of the native Zn and associated with soil mineral fractions. This may be due to the type of dominant clay minerals, which is the reason for variation in the amount of residual Zn fraction. Residual Zn is referred to as the Zn fraction occluded in the crystal structures of silicates and resistant minerals after the removal of extractable Zn fractions (Chao, 1984; Osakwe and Okolie, 2015). The greatest percentage of Zn in the residual fraction was associated with mineral lattice, which reflects the greater tendency of Zn became unavailable in ID calcareous soils.

The residual fraction of Zn (residual+ unrecovered) in soil was greater with ZnSO4, followed by ZnO+AV due to greater fixation and adsorption processes, as shown in our study. This is because the residual-bound fraction represents metals that were incorporated into the crystalline lattices of clays and appear inactive (Kabala and Singh, 2001). The greatest amount of residual+ unrecovered Zn was found in the $ZnSO_4$ treatments, due to $ZnSO_4$ is more soluble in calcareous soil, and it will dissolve quickly. It Zn^{2+} will be sorbed onto $CaCO_3$ with low solubility.

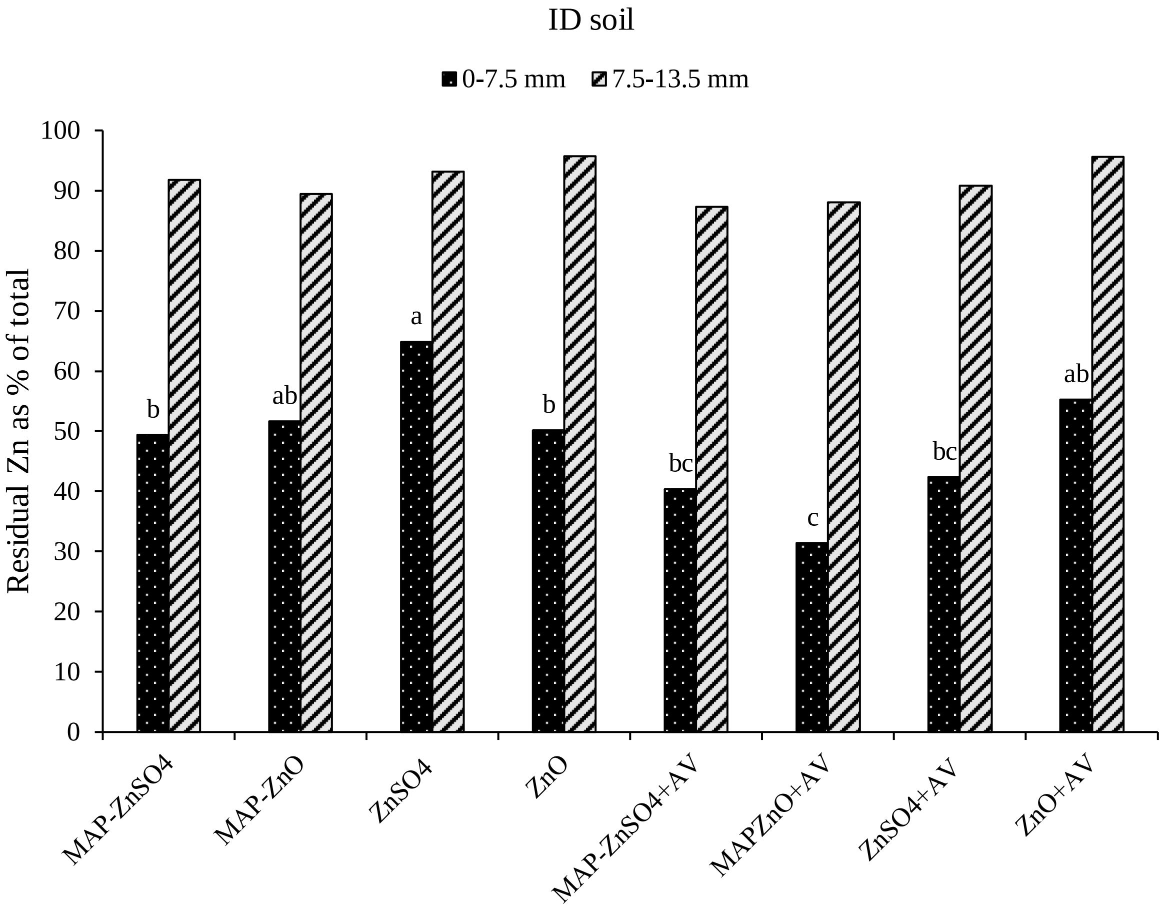


Figure 5.24 Residual Zn (residual+ unrecovered) expressed as a percent of total Zn by section, 35-days after fertilizer application in Idaho soil. MAP-$ZnSO_4$ = $ZnSO_4$- incorporated – MAP; MAP-ZnO = ZnO- incorporated – MAP; $ZnSO_4$ = Zn sulfate; ZnO = Zn oxide; MAP-$ZnSO_4$ + AV = ZnSO4- incorporated – MAP + AVAIL; MAP-ZnO + AV = ZnO- incorporated – MAP+ AV; $ZnSO_4$+AV= Zn sulfate+ AVAIL; ZnO+ AV= Zn oxide+ AVAIL; MAP = monoammonium phosphate. Means within a soil section for each treatment containing the same letter are not significantly different at *P* = 0.05 using Tukey's honest significance test. *NS = no significant difference in section.

Conclusion

The influence of the Zn treatments on soil pH was similar for both calcareous soils, KS, and ID. The MAP-incorporated Zn sources had the greatest decrease in pH in the 0-7.5 mm section. Zinc diffusion in these calcareous soils was limited and generally did not move beyond the 0-7.5 mm section for both the granular Zn and or liquid treatments, although the diffusion was, in general, greater for the liquid treatments. The co-additive AVAIL did increase Zn diffusion in one liquid treatment but not in the other. Zinc diffusion was much higher in the mildly calcareous ID soil than in the mildly calcareous KS soil indicating the role of soil texture on the fate of nutrients in soils. Scanning electron microscope – energy dispersive X-ray spectroscopy agreed with the wet chemical analyses. It revealed that the remaining Zn in -MAP granules (MAP-$ZnSO_4$ and MAP-ZnO) after incubation in soil, still contained significant amounts of P and Zn. The results of this study indicated that the Zn fractionations were varied between the soils. Zinc distribution decreased in the order of residual > Fe-Mn oxide-bound > carbonate-bound > exchangeable > organic-bound > water-soluble in the KS soil, while residual > carbonate-bound > Fe-Mn oxide-bound > organic-bound > exchangeable > water-soluble in ID soil. This study finding suggests that the liquid Zn sources would be superior to the co-granulated Zn-P fertilizers in mildly calcareous soils.

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Chapter 6 - Summary and Conclusions

As a global environmental problem, salinization threatens over 20% of the world's agricultural land. Plant systems may provide a significant capacity to remediate fringe waters through several phytoremediation processes, including uptake, accumulation, and assist with biotransformation of inorganic and organic compounds. The halophyte, *salicornia europaea,* could be a suitable plant to capitalize on its salt-tolerance potential for treating marginal waters. Our first research study mainly focused on investigating the ability of *salicornia europaea,* to accumulate and removal of salts and potentially toxic trace elements such as B and Se, in both saltwater and wastewater. To achieve this, *salicornia europaea* was grown in a soil irrigated with FGD wastewater high salt, B and Se in a greenhouse, and in FGD wastewater and brackish water hydroponic systems. *Salicornia europaea* showed the ability to remove excess trace elements (e.g., Se and B) and salts (e.g., Na); therefore, it has the potential to be used for precleaning the marginal waters. Our results suggest that *Salicornia europaea* might be a potential new candidate plant species for effective phytoremediation of Se and B contaminated saline soils. This study also showed that due to enhanced biomass production of *Salicornia europaea* when growing in highly saline marginal waters, they can also provide valuable stock for biofuel and bio-based products from marginal waters.

Zinc deficiency occurs in a wide range of soil types around the world. Soils that are frequently associated with Zn deficiency are calcareous soils, sandy soils, weathered tropical soils, saline soils, waterlogged soils, and heavy cracking clay soils. Agronomic biofortification is an effective way to increase micronutrient concentrations in grain crops. The aims of the second study were to investigate the effectiveness of various Zn sources (inorganic and organic) with and without fertilizer additives on biofortification of wheat with Zn in a mildly-calcareous soil;

and to determine distribution (stems/leaves, whole grain, bran and flour) and bioavailability of Zn in different plant parts (bran and flour). All Zn sources with and without additive treatments increased the biomass production up to 43% over compared to the control pots. Zinc oxide resulted in the largest increase in whole grain Zn, followed by ZnO + AVAIL and $ZnSO_4$. There was no difference in Zn uptake by ZnO and $ZnSO_4$, while $ZnSO_4$ treatments showed the largest increase in soil Zn extractability. Although Zn biofortification increased Zn bioavailability in wheat flour, it did not increase the bioavailability of Zn in wheat bran. Results showed DTPA extractable Zn in soil was superior to the soil Zn fractionation in predicting plant uptake of Zn. Our results suggested agronomic biofortification can be done successfully, and it also helps to obtain a better yield. The results in this study suggest that ZnO was a better Zn source than $ZnSO_4$ in increasing wheat grain Zn concentration in potentially Zn-deficient calcareous soil.

Zinc is present in soils in different chemical forms, the relative distributions of which vary with the type of soil and its physicochemical properties. Most Zn in soils is on the surfaces of clays, hydrous oxides, and organic matter, rather than in solution. Understanding the distribution of Zn among various fractions in soils is important for the effective and efficient management of soils for optimum crop production and environmental conservation. The objective of this study was to investigate and understand differences in mobility, extractability, and reaction products of Zn as a result of the different Zn sources with and without co-additives. We compared the effects of the different Zn sources, with and without a co-additive, AVAIL, in two mildly calcareous soils. The influence of the Zn treatments on soil pH was similar for the two types of soils sampled from Kansa (KS) and Idaho (ID). The Zn concentration and diffusion were much higher in the ID soil owing to its larger sand-size particles. The fertilizer (MAP-Zn) source had the greatest decrease in pH in the 0-7.5 mm section; the Zn ($ZnSO_4$ and ZnO) source was also increased proportionately with the increasing distance from the center section. Zinc diffusion in these calcareous soils was limited and generally did not move beyond the 0-7.5 mm section for the granular Zn and or liquid treatments. The results of this study indicated that the concentration of Zn fractions varied among the soils. For both soils, the addition of ZnO treatments increased the rate of Zn aging when compared to ZnSO4 treatments, with or without additives.

Scanning electron microscope – Energy dispersive X-ray Spectroscopy were in agreement with the wet chemical analyses and revealed that the remaining Zn-incorporated monoammonium phosphate (MAP-$ZnSO_4$ and MAP-ZnO) granules after incubation in soil, contained significant amounts of P and Zn. All Zn sources had significantly greater carbonate-bound Zn in the 0-7.5 mm section as compared to the control. This is due to the higher pH of the soils, as increasing pH increases the concentration of carbonate-bound Zn in both soils. All Zn sources had significantly greater Fe-Mn oxide-bound Zn fractions in the 0-7.5 mm section indicating a possible role of Fe and Mn minerals in added Zn availability in calcareous soils. Soil Zn concentrations in different fractions are influenced by the colloidal properties of the soils such that soils with higher pH and those with lower organic matter were more likely to have lower Zn concentration in the available fractions.