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**MODELING ZINC BIOFORTIFICATION ON WINTER
WHEAT INFLUENCED BY WATER QUALITY AND
IRRIGATION LEVEL**

Master's Thesis

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.ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled “**MODELING ZINC BIOFORTIFICATION ON WINTER WHEAT INFLUENCED BY WATER QUALITY AND IRRIGATION LEVEL**” prepared by **Esther Chidinma CHUKWU**, and supervised by **Prof. Dr. Coşkun GÜLSER** and **Prof. Dr. Andon VASILEV ANDONOV** was found successful and unanimously accepted by committee members as Master thesis, following the examination on the date 17.7.2025.

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ÖZET

SU KALİTESİ VE SULAMA SEVİYESİNDEN ETKİLENMİŞ KIŞLIK BUĞDAYDA ÇİNKO BİYOFORTİFİKASYONUNUN MODELLENMESİ

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Çinko, insan vücudundaki çok sayıda biyolojik işlevde hayati bir rol oynayan temel bir mikro besin elementidir. Yine de, dünya çapındaki eksikliği, özellikle gelişmekte olan ülkelerde önemli bir küresel sağlık sorunu olmaya devam etmektedir. Buğday, mısırdan sonra en yaygın olarak yetiştirilen ikinci tahıl ürünüdür ve dünya çapında yaklaşık 2,5 milyar insan için temel bir gıda ve birincil bir diyet bileşenidir. Tahıllarda çinko konsantrasyonunu biyofortifikasyon yoluyla artırmak, insanlarda Zn eksikliğini hafifletmek için umut verici bir stratejidir. Ancak, buğdayda Zn alımı ve taşınmasının verimliliği, nem mevcudiyeti ve su kalitesi de dahil olmak üzere çeşitli toprak ve çevresel faktörlerden etkilenmektedir. Bu araştırmada, değişen Zn uygulama oranları, toprak nem içerikleri ve su kalitesi seviyelerine sahip kontrollü bir sera deneyinden elde edilen verileri kullanarak kışlık buğdayda Zn biyofortifikasyonunu simüle etmek için bir modelleme çerçevesi geliştirilmiştir. Toprak örnekleri 3 kg'lık saksılara doldurulurular, buğday tohumları ekildikten sonra saksılara beş seviyede çinko uygulaması ($ZnSO_4$ 'dan 0, 5, 10, 25, 50 kg Zn/ha) yapıldı. Sulama suyu $MgCl_2$, $NaCl$ ve $CaCl_2$ ile 0,5, 2, 4 dS/m elektrik iletkenlik değerlerinde hazırlandı. Farklı tuzluluk ve nem içeriklerinde $ZnSO_4$ uygulaması toprak özelliklerini önemli ölçüde etkilemiştir. Kuraklık ve tuzluluk stresi koşullarında orta düzeyde Zn sülfat uygulaması (5-25 mg/kg) toprak OM, P, oransal nem içeriği (RWC), su kullanım etkinliği (WUE), mikrobiyal aktivite ve klorofil içeriğini iyileştirmiş, orta ila yüksek düzeyde Zn uygulaması (10-50 mg/kg) özellikle kuraklık ve düşük tuzluluk koşullarında toprak K tutulumunu artırmış, daha yüksek tuzluluk koşullarında (4 dS/m) orta düzeyde $ZnSO_4$ uygulamaları (10-25 mg/kg) artan toprak kalsiyum bulunabilirliği ile ilişkili bulunmuştur. Tuzluluk, toprakta Na birikiminin birincil etkileyen faktördür ve Zn uygulamasıyla evapotranspirasyon (ET), sulama rejimine bağlı olarak etkisini düzenlemektedir. Toprak Zn içeriği, tuzluluk veya nemden bağımsız olarak $ZnSO_4$ uygulamasıyla orantılı olarak artmıştır. Bitki Zn alımı Zn uygulamasıyla artmış ve orta düzeyde tuzluluk (2 dS/m) altında artış göstermiştir. Zn uygulama oranı ve orta düzeyde tuzluluk, su kısıtlı koşullar altında bitki dokusunda daha yüksek Zn birikimini teşvik etmiştir. Tarla kapasitesi Zn alımını desteklemiş, ancak bitkide Zn birikimi kuraklık altında muhtemelen daha az sızma ve daha yüksek kök bölgesi Zn konsantrasyonu nedeniyle daha fazla olmuştur.

Anahtar Sözcükler: Çinko, Nem içeriği, Biyofortifikasyon, Kuraklık, Tuzluluk

ABSTRACT

MODELING ZINC BIOFORTIFICATION ON WINTER WHEAT INFLUENCED BY WATER QUALITY AND IRRIGATION LEVEL

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Zinc remains an essential micronutrient that plays a vital role in numerous biological functions in the human body. Still, its deficiency worldwide remains a significant global health challenge, especially in developing countries. Wheat is the second most widely grown cereal crop after maize and serves as a staple food for approximately 2.5 billion people worldwide and a primary dietary component. Enhancing its grain Zn concentration through biofortification is a promising strategy to alleviate Zn deficiency in humans. However, the efficiency of Zn uptake and translocation in wheat is influenced by several soil and environmental factors, including moisture availability and water quality. This research will develop a modeling framework to simulate Zn biofortification in winter wheat, using data from a controlled greenhouse experiment with varying Zn application rates, soil moisture contents, and water quality levels. The soil was potted in a kg pot. Five levels of zinc application (0, 5, 10, 25, 50 kg Zn/ha from ZnSO₄) were applied to the pots after sowing the wheat seeds. The irrigation water was prepared with MgCl₂, NaCl, and CaCl₂ to an electric conductivity of (0.5, 2, 4 dS/m). ZnSO₄ application at different levels of salinity and moisture content significantly affects soil properties. Under drought and saline stress conditions moderate Zn sulfate application (5–25 mg/kg) improves soil OM, P, RWC, WUE, microbial activity and chlorophyll content, moderate to high Zn application (10–50 mg/kg) improve soil K retention, particularly under drought and low-salinity conditions, moderate ZnSO₄ applications (10–25 mg/kg) under higher salinity conditions (4 dS/m) are associated with increased soil calcium availability. Salinity is the primary driver of soil Na accumulation, and ET with Zn application modulates its effect depending on the irrigation regime. Soil Zn increases proportionally with ZnSO₄ application, regardless of salinity or moisture. Plant Zn uptake increases with Zn application and is enhanced under moderate salinity (2 dS/m). Zn application rate and moderate salinity promote higher tissue Zn accumulation under water-limited conditions. Field capacity supports Zn uptake, but Zn accumulation was more efficient under drought, likely due to reduced leaching and higher root-zone Zn concentration.


Keywords: Zinc, Moisture content, Biofortification, Drought, Salinity

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SYMBOLS AND ABBREVIATIONS

SYMBOLS

| | |
|----|------------|
| Ca | Calcium |
| K | Potassium |
| Mg | Magnesium |
| Na | Sodium |
| P | Phosphorus |
| Zn | Zinc |

ABBREVIATIONS

| | |
|-------------------|------------------------------|
| EC | Electrical conductivity |
| OM | Organic matter |
| ZnSO ₄ | Zinc sulphate |
| SBCO ₂ | Soil basal carbon dioxide |
| WUE | Water use efficiency |
| ET | Evapotranspiration |
| PdryW | Plant dry weight |
| RWC | Relative water content |
| NaCl | Sodium chloride |
| MgCl | Magnesium chloride |
| CaCl ₂ | Calcium chloride |
| Pzn cont | Plant Zinc content |
| PCA | Principal component analysis |

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1. INTRODUCTION

Zinc is a crucial micronutrient that plays a great role in growth and the immune system. It functions in cell differentiation and is an essential constituent of enzymes. 33% of the human population has been affected by its deficiency causing impaired immune function, growth retardation, and increased susceptibility to infections and chronic diseases. Worldwide, over two billion people are affected, and 116,000 deaths occur per year. In developing countries, significant economic losses are attributed to the deficiencies of Zn and other micronutrients (Sangeetha et al, 2022). These deficiencies decrease productivity and increase the cost of health care, thus affecting a nation's gross national product (GDP). A major contributing factor to this deficiency is the low Zn concentration in staple food crops, especially cereals like wheat, which form the dietary base for millions of people globally. Enhancing the Zn content of such crops through agronomic and genetic approaches is an important strategy to combat Zn deficiency.

Wheat is the third most important crop globally and the most important staple crop in the temperate zone. Traditional wheat-consuming areas of Western Europe (UK, Finland), West Asia and North Africa (Turkey, Egypt) are rapidly urbanizing and industrializing countries in Sub-Saharan Africa (Nigeria, South Africa) and Asia (China, India), and Mexico in which maize and wheat are staple foods (Shewry and Hey, 2015). Wheat is often considered primarily a source of energy (carbohydrate), which is important in this respect. However, it also contains significant amounts of other important nutrients, including proteins, fiber, and minor components, including lipids, vitamins, minerals, and phytochemicals, contributing about 20 % of the total dietary calories and proteins worldwide (Shiferaw et al, 2013). Concentrations of Zn in grains in wheat-cultivating regions range between 20 and 35 mg kg⁻¹ (Cakmak and Kutman 2018), whilst they can be much lower in wheat grown on Zn-deficient soil (Kalayci et al. 1999). Grain mineral density, Zn, decreased by high wheat yields over the past six decades (Curtis and Halford 2014) due to the yield dilution effect (Fan et al. 2008). Climate change-induced temperature increases are estimated to reduce wheat yields in irrigated systems by 5.3 to 7.4 % in developing countries and by 0.1 % in developed countries (Nelson et al. 2010). Extreme daytime temperatures during anthesis in high latitudes are predicted to represent threats to wheat yields (Semenov and Shewry 2011). The target range of grain Zn levels for a population

relying on a cereal-based diet is 40–60 mg kg⁻¹ (Cakmak 2008). For these reasons, Zn biofortification of wheat has become an important tool to combat Zn deficiency in humans. As a result, improving the nutritional quality of winter wheat has become a focal point in global food security and nutrition policies. Among the strategies to increase Zn levels in wheat, agronomic biofortification, particularly through soil and foliar Zn application, has gained prominence due to its rapid implementation and relatively low cost.

Agronomic biofortification involves the application of micronutrient fertilizers to crops to increase their concentration in edible parts. This approach has been shown to enhance Zn content in wheat grains, but the efficiency of Zn uptake varies considerably based on soil properties, environmental conditions, and water management practices. Soil Zn availability is influenced by pH levels, total Zn, CaCO₃, available phosphorus (P), organic matter, and moisture content. The percentage increase in grain Zn concentration due to Zn fertilization declined with rising soil Zn availability but improved with higher soil pH levels. At high pH values, the solubility of Zn in the soil is reduced by enhancing the absorptive capacity of Zn, due to forming hydrolyzed Zn forms, precipitation of iron oxides, and strong chemisorption with CaCO₃ (Xiaoli et al. 2025)

Artificial intelligence techniques find applicability in a wide range of prediction tasks, serving as fundamental elements within the framework of global precision agriculture. These models and tools leverage various methodologies, including linear regression techniques, non-linear simulations, expert systems, pattern recognition, data analysis, decision making, automation, and Artificial Neural Networks to predict agricultural components (Hirushan et al. 2024). These tools have made it possible to accurately simulate the dynamics of water movement, nutrient transport, and plant uptake. Artificial intelligence models can predict soil fertility, providing a decision-making tool capable of predicting the most suited crops to plant based on soil pH, soil nutrients, soil moisture, environmental variables, and other factors (Folorunso et al. 2023). These models help understand the spatiotemporal dynamics of Zn in the soil-plant system under different moisture regimes and irrigation water qualities, ultimately leading to optimized nutrient management practices.

Different studies have investigated the enrichment of wheat with Zn through soil or foliar application. This study aims to model Zn biofortification in winter

wheat by examining the combined influence of soil moisture content and water quality. By integrating experimental data from a greenhouse study with mechanistic and statistical models, the study explores how Zn application rates, moisture availability, and irrigation water quality interact to influence Zn uptake, translocation, and accumulation in the wheat plant. The findings are expected to contribute valuable insights into site-specific nutrient management and help inform agronomic recommendations for Zn biofortification in wheat-growing regions facing water scarcity and salinity challenges.

The specific objectives of the study are as follows:

1. To evaluate the effect of different soil moisture regimes (drought and field capacity) on Zn mobility and uptake in winter wheat. This will help determine how water availability influences Zn transport mechanisms such as diffusion and root interception.
2. To assess the impact of irrigation water quality (electrical conductivity levels of 0.5, 2, and 4 dS/m) on Zn availability, solubility, and plant uptake. This will reveal how salinity interferes with Zn bioavailability and plant absorption.
3. To simulate Zn transport and transformation in the soil–plant system under different experimental treatments.
4. To develop a model to predict Zn concentration in wheat shoots based on experimental variables. This model will help identify the most influential factors governing Zn accumulation and facilitate the development of decision-support tools for Zn biofortification.
5. To recommend optimal Zn application strategies under varying moisture and water quality conditions to enhance Zn content in wheat grains. The insights gained will guide sustainable agronomic practices, improving human nutrition and soil health.

2. LITERATURE REVIEW

2.1. Zinc as an important micronutrient for plant growth

Zinc (Zn) is an essential micronutrient that plays a vital role in various physiological and biochemical processes in plants, including enzyme activation, protein synthesis, and the regulation of growth hormones. In wheat (*Triticum aestivum* L.), Zn is particularly crucial due to its involvement in photosynthesis, nitrogen metabolism, and reproductive development (Alloway, 2008). Despite being required in small quantities, Zn deficiency significantly limits wheat productivity and quality, especially in calcareous and alkaline soils common in many wheat-growing regions.

Zn deficiency in wheat is attributed to reduced seed germination, stunted growth, delayed maturity, and poor grain filling, resulting in significant yield losses (Cakmak, 2000). Visual symptoms include interveinal chlorosis, shortened internodes, and malformed leaves. Zn influences the activity of antioxidant enzymes, improving wheat's tolerance to environmental stress conditions such as drought and salinity (Raeisi et al., 2022). Zn application can accelerate the crop yield and its components through multiple mechanisms. It can increase chlorophyll contents and stimulate photosynthetic activity for enhanced crop growth and development. In this regard, its application can efficiently boost the overall yield (Fatemeh et al. 2024). Zinc increases the utilization efficiency of macronutrients and reduces their utilization costs (Sher *et al.*, 2022). Zinc is an irreplaceable micronutrient for plant growth and plays an integral role as a structural, functional, and regulatory cofactor of various enzymes (Ma et al. 2017). Adequate zinc nutrition is important in controlling potassium accumulation by leaves and seeds (Anwar *et al.*, 2021). This increase in potassium with zinc application may be due to the synergistic relationship between zinc and potassium, resulting in greater availability of potassium and an increase in potassium flux from the root and shoot to the seed (Elshayb *et al.*, 2021). Zinc enhances plant tolerance against drought stress by regulating several physiological mechanisms (Hassan et al. 2020). Incorporation of Zn prominently promoted the physiological attributes of wheat and accelerated the Zn accumulation in wheat shoots and grains (Athar et al. 2025). With global Zn-deficient soils estimated to affect nearly 50% of cereal-growing areas, particularly in developing

countries, effective Zn management in wheat cultivation has become increasingly urgent.

Biofortification through Zn fertilization has emerged as a promising strategy to enhance wheat yield and grain Zn concentration, addressing agronomic deficiencies and human Zn malnutrition (Bouis & Saltzman, 2017). Agronomic approaches such as soil and foliar application of Zn, especially in the form of ZnSO₄, have shown positive effects on Zn uptake and translocation to wheat grains (Cakmak et al., 2010). However, Zn availability is strongly influenced by soil properties such as pH, moisture, and salinity, which affect its mobility and uptake efficiency. Therefore, understanding the soil-plant dynamics of Zn under various environmental conditions is key to optimizing wheat productivity and improving nutritional outcomes.

2.2. Zinc biofortification of wheat

It is a promising strategy to address crop productivity and human micronutrient malnutrition. Wheat is a global staple crop, yet its grains typically contain insufficient Zn to meet human dietary requirements, especially in developing countries where Zn deficiency contributes to stunted growth, weakened immunity, and poor pregnancy outcomes (Wessells & Brown, 2012). Agronomic biofortification through Zn fertilization has emerged as a scalable solution to enhance Zn concentration in wheat grains without altering consumer preferences or requiring genetic modification.

Globally, an estimated 30%–50% of agricultural soils are Zn-deficient (Alloway, 2008), particularly in regions with calcareous soils, low organic matter, and high pH, which limit Zn solubility and plant uptake. Consequently, wheat grown in these soils often has grain Zn concentrations below 25 mg/kg, whereas the target level for dietary sufficiency is 40–50 mg/kg (Cakmak, 2008). Conventional breeding alone has proven insufficient to overcome these limitations within a short time frame, leading to increased focus on agronomic approaches.

The most widely used method for Zn biofortification is soil or foliar application of Zn-containing fertilizers, primarily zinc sulfate (ZnSO₄·7H₂O). Soil application during rice production enhances root uptake, and it is often applied at 5–25 kg Zn/ha, while foliar sprays (0.5%–1% ZnSO₄) are more effective in directly translocating Zn to the grain during the reproductive stage (Shivay et al., 2015). A

meta-analysis by Dimkpa & Bindraban (2016) found that Zn fertilization can increase grain Zn concentration by 25%–80 %, depending on soil type, wheat genotype, and Zn form. Cakmak et al. (2010) demonstrated that foliar Zn application at the early grain-filling stage is particularly effective in increasing grain Zn without significantly affecting yield.

The efficiency of Zn biofortification is influenced by soil properties (pH, organic matter, texture), wheat genotype, moisture availability, and the timing/method of Zn application. In alkaline and calcareous soils, Zn forms insoluble compounds, reducing bioavailability. Chelated Zn (e.g., Zn-EDTA) or integrated use with organic amendments like farmyard manure (FYM) improves Zn solubility and uptake (Raeisi et al., 2022).

Genotype also plays a significant role. Some wheat varieties exhibit greater root uptake and translocation to grain. Yilmaz et al. (1997) identified genetic variation in Zn use efficiency and grain loading capacity among wheat genotypes, which can be exploited alongside agronomic measures. Drought and salinity can reduce Zn uptake due to impaired root growth and transport mechanisms. However, Zn application has also alleviated stress symptoms, helping maintain Zn translocation under suboptimal conditions (Umair et al., 2020).

Integrating soil + foliar application or Zn fertilization + biochar/FYM can maximize biofortification outcomes. However, economic and logistical barriers remain, especially for smallholder farmers. Cost of Zn fertilizers, lack of awareness, and limited access to Zn-enriched seeds or fertilizers hinder widespread adoption. Additionally, there is a need for improved Zn fertilizer formulations, such as Zn-coated urea, slow-release Zn fertilizers, and nano-Zn particles, which enhance uptake efficiency and reduce environmental losses (Rehman et al., 2012).

2.3. Zinc fertilization and soil properties

Zinc is an essential micronutrient that contributes to wheat growth and development and interacts with multiple soil properties and plant physiological traits. Zn application has been widely studied for its influence on soil chemical properties and plant health indicators, such as chlorophyll content. Understanding how Zn interacts with soil macronutrients (Ca, Mg, Na, K), soil reaction (pH), salinity (EC), organic matter (OM), and plant chlorophyll levels is critical for optimizing nutrient management and maintaining soil-plant health balance during wheat cultivation.

2.3.1. Zinc Application and Soil Calcium (Ca) and Magnesium (Mg)

Zinc (Zn) is an essential micronutrient for plant growth; moreover, its application can influence the behavior and availability of other cations in soil, particularly calcium (Ca) and magnesium (Mg), affecting their interaction, which is largely influenced by soil properties, fertilizer form, and environmental conditions. Due to competitive uptake and soil sorption dynamics, Zn interacts with calcium (Ca) and magnesium (Mg). These interactions are critical in biofortification programs and sustainable fertilizer management. A balanced approach to Zn fertilization should consider its potential to alter the dynamics of macronutrients like Ca and Mg, which are crucial for structural and enzymatic functions in plants.

Zinc and calcium are divalent cations (Zn^{2+} and Ca^{2+}) that can compete for similar sorption sites in soil and plant root transporters. Excessive application of Zn fertilizers can lead to antagonistic interactions with Ca, reducing its availability and uptake (Alloway, 2008). This antagonism is more pronounced in acidic soils where cation exchange dynamics are more active. For instance, Kopittke et al. (2010) showed that Zn^{2+} application could suppress Ca^{2+} uptake in root cells due to competitive inhibition of calcium transport channels.

Furthermore, Zn reduces soil pH, and plants' uptake of ions such as Ca^{2+} and Mg^{2+} increases along with pH, while micronutrient availability increases with a decrease in soil pH (Khaidem and Thounaojam 2018). However, in calcareous soils, high levels of $CaCO_3$ may buffer these effects, leading to reduced Zn bioavailability. This demonstrates the bidirectional nature of Zn-Ca interactions based on initial soil conditions.

Magnesium and Calcium share similar physicochemical properties with Zn^{2+} , making them susceptible to interaction. Zn application can displace Mg from exchange sites, particularly in sandy or low-Cation Exchange Capacity (CEC) soils (Rene et al., 2017). A study by Guo et al. (2021) suggests that low Zn supply ($< 1 \mu M$) promoted the absorption of potassium (K), magnesium (Mg), and manganese (Mn) in wheat seedlings, while high Zn supply ($> 1 \mu M$) significantly inhibited the absorption of these elements, suggesting competitive displacement from the soil colloids. Moreover, the antagonistic effect of Zn on Mg uptake is influenced by plant genotype and application time. In cereals such as wheat and maize, simultaneous deficiency of Zn and Mg has been reported under intensive Zn fertilization regimes. Conversely, moderate Zn application in Zn-deficient soils has enhanced root

biomass, indirectly promoting Mg uptake due to increased root-soil contact (Abdul et al., 2022). However, excessive Zn fertilization may decrease exchangeable Ca and Mg in soil due to cation competition on soil colloids. Balanced Zn application is therefore important to avoid nutrient antagonism and ensure adequate Ca and Mg availability.

Soil pH, organic matter content, and the presence of other cations modulate Zn–Ca–Mg interactions. In soils with high sodium or salinity stress, Zn application may mitigate Na toxicity, thus indirectly promoting Ca and Mg uptake due to improved membrane integrity and ionic balance (Cakmak, 2008). Additionally, chelated forms of Zn, such as Zn-EDTA, are less antagonistic compared to ZnSO₄, as they release Zn more slowly and reduce competitive binding to soil colloids (Dhaliwal et al., 2021). Studies have shown that Zn application can reduce the excessive uptake of Ca and Mg by maintaining cation balance in plant tissues, which is particularly beneficial in calcareous soils where Ca is abundant (Cakmak, 2008).

2.3.2. Zinc Application and Soil Sodium (Na) and Potassium (K)

Zinc (Zn) interactions with soil sodium (Na⁺) and potassium (K⁺) are often overlooked. Understanding these interactions is critical in optimizing nutrient use efficiency and improving soil health, particularly in saline and nutrient-deficient soils. Zinc's influence on Na and K availability and uptake can be antagonistic or synergistic, depending on the soil environment, fertilizer form, and crop type. Soil sodium is a major concern in saline or sodic soils, where excess Na⁺ causes poor soil structure, high pH, and reduced nutrient uptake. Zinc application has been shown to mitigate some of these adverse effects by improving plant tolerance to salinity and reducing Na accumulation in plant tissues. According to Cakmak and Kutman (2018), Zn plays a crucial role in maintaining membrane integrity, which reduces the passive influx of Na⁺ into root cells, thereby decreasing Na toxicity under saline conditions. Fatemeh et al. (2024) observed that Zn application (as ZnSO₄) lowered exchangeable Na⁺ by enhancing cation exchange with Zn²⁺ and improving overall ionic balance. This is particularly beneficial in reclaiming salt-affected soils. Moreover, the expression of Na⁺ transport-regulating genes, which aid in Na exclusion and compartmentalization, can be improved by Zn as reported in rice studies (Zhang et al., 2019). In alkaline soils, Zn application can also enhance the

leaching of Na^+ by modifying soil structure and increasing the soil's aggregation potential, indirectly improving aeration and water infiltration (Riaz et al., 2020).

Potassium (K^+), as a vital macronutrient, is involved in osmotic regulation, enzyme activation, and stomatal function. Zn and K interactions are complex due to potential competition at root absorption sites and effects on membrane transporters. Alloway (2008) notes that Zn deficiency can impair K^+ uptake, while adequate Zn levels enhance root development and K absorption. Khan et al. (2023) found that foliar or soil-applied Zn significantly increased K uptake in wheat grown under Zn-deficient conditions. This positive correlation was attributed to improved root growth and increased transpiration, which enhanced K mobility and uptake from the soil. However, in Zn-excess conditions, there may be antagonistic interactions where high Zn^{2+} concentrations displace K^+ from exchangeable sites, especially in soils with low cation exchange capacity (CEC) (Rene et al., 2017). Additionally, Zn affects the soil microbial community, influencing the mineralization of organic matter and release of exchangeable K. Studies have shown that Zn application stimulates microbial activity, indirectly promoting K release from clay minerals and organic complexes (Khan et al., 2023). In saline or saline-sodic soils, Zn application is particularly crucial due to the complex interaction among Na^+ , K^+ , and Zn^{2+} . Excess Na^+ can reduce K^+ uptake due to similar ionic radii and competitive inhibition. Zn helps restore this balance by reducing Na^+ influx and promoting selective K^+ absorption. This was confirmed in field studies on rice and wheat, where Zn-treated plots had lower $\text{Na}^+ : \text{K}^+$ ratios, leading to improved growth and yield under saline irrigation (Fatemeh et al. 2024). In saline or sodic soils, sodium (Na) accumulation can impair wheat growth. Zn supplementation has been reported to alleviate sodium toxicity by improving cell membrane integrity and ion selectivity, reducing Na uptake and enhancing K^+/Na^+ ratios in plant tissues (Fatemeh et al. 2024). Zn may also enhance potassium (K) availability and uptake by improving root growth and transporter activity, particularly under abiotic stress conditions. While the effect on soil K content is not always direct, Zn promotes better K utilization and redistribution in wheat plants.

Overall, Zn application modifies the dynamics of both Na and K in soil by influencing ion exchange processes, plant physiological responses, and microbial-mediated nutrient cycling. These interactions are critical in managing saline soils and enhancing plant nutrient efficiency in Zn-deficient regions.

2.3.3. Effect of Zinc on Soil pH and Electrical Conductivity (EC)

Soil pH affects Zn availability significantly, with Zn being more available in acidic soils and less so in alkaline or calcareous conditions due to precipitation as insoluble Zn compounds. Conversely, Zn application can cause slight shifts in soil pH depending on the fertilizer form and soil buffering capacity. Studies have shown that ZnSO₄ (zinc sulfate) may slightly acidify the rhizosphere, particularly in neutral to alkaline soils. According to Alloway (2008), the acidifying effect of Zn fertilizers occurs due to the hydrolysis of Zn salts and subsequent proton release. This localized acidification can enhance micronutrient solubility in the short term but may be temporary depending on soil buffering capacity. Pranita et al. (2017) observed a decrease in soil pH from 7.7 to 7.5 after repeated ZnSO₄ applications in a wheat-based cropping system, attributing this to the cumulative acidifying effect of sulfate and Zn²⁺ ions. However, in soils with high organic matter and carbonate content, the change in pH is often negligible.

Moreover, Rene et al. (2017) reported that chelated forms of Zn (e.g., Zn-EDTA) have a minimal effect on soil pH due to their neutral or slightly basic nature, making them more suitable for pH-sensitive soils.

Electrical conductivity is a measure of the total soluble salt concentration in soil, which is influenced by fertilizer application. Zn application, especially in sulfate or chloride form, can temporarily increase soil EC due to the addition of soluble salts. This increase is more pronounced in soils with poor drainage or high initial salinity. In a study by Rehman et al. (2018), ZnSO₄ application in wheat fields led to a measurable increase in soil EC from 0.62 to 0.85 dS/m, particularly under limited leaching conditions. The increase in EC is primarily due to the sulfate anion accompanying Zn, which contributes to the total dissolved salt content. With irrigation and microbial activity, EC levels tend to stabilize over time as Zn ions bind to soil colloids or are taken up by plants. However, repeated high-dose applications in arid or semi-arid soils can lead to long-term salinity issues, especially when compounded with other saline fertilizers. Moreover, Zn application can also indirectly influence EC by modifying soil microbial activity and organic matter decomposition, leading to fluctuations in ionic concentrations in the soil solution.

The effects of Zn on pH and EC are not uniform across all soil types. In acidic soils, Zn availability is high, and application has minimal effect on pH or EC due to strong cation exchange buffering. In contrast, alkaline and saline soils show

more pronounced shifts in EC and localized pH due to Zn addition. Additionally, soil moisture status, organic matter, and the form of Zn fertilizer determine the magnitude and duration of these changes. Chelated Zn forms are more stable across varying pH and EC levels, while inorganic salts (e.g., ZnSO₄ or ZnCl₂) are more reactive.

Overall. Zn application can lead to minor modifications in soil pH, particularly in the rhizosphere. Zinc sulfate (ZnSO₄), a commonly used Zn fertilizer, can lower rhizosphere pH slightly, especially in alkaline soils, by stimulating proton exudation from roots and microbial activity (Alloway, 2008). This acidification enhances Zn solubility and the availability of other nutrients like phosphorus and iron. Regarding electrical conductivity (EC), Zn fertilizers themselves may temporarily increase soil EC, especially when sulfate-based sources are applied. However, this increase is usually moderate and short-lived unless Zn is applied in excessive doses or with saline irrigation water. Khalilzadeh et al. 2018 found that under salinity stress, plant membranes are subject to changes often associated with increases in permeability and loss of integrity, but Zn application under such conditions decreased EC (Kheirizadeh et al. 2016).

2.3.4. Effect of Zinc Application on Soil Organic Matter (OM)

Zinc can influence the decomposition and stabilization of soil organic matter (OM) by affecting microbial enzyme activity and biomass. Zinc (Zn) is a crucial micronutrient required for plant metabolic processes, but its application can significantly influence soil organic matter (SOM) dynamics, particularly through its interactions with microbial communities, enzymatic activities, and nutrient cycling. Soil organic matter is a central component of soil fertility, influencing nutrient retention, water holding capacity, and microbial health. Understanding the relationship between Zn application and SOM behavior is essential for optimizing sustainable soil management practices. Zinc interacts with SOM in both chemical and biological ways. Chemically, Zn can form stable complexes with functional groups in humic and fulvic acids, affecting SOM structure and nutrient-binding properties (Alloway, 2008). The binding of Zn²⁺ to carboxyl and phenolic groups in organic matter can reduce Zn mobility but also influence the solubility of SOM components, particularly in acidic and low-buffer soils. The sorption of Zn to dissolved organic matter (DOM) may decrease the bioavailability of Zn while

altering the decomposition rate of organic residues. This dynamic equilibrium affects the long-term stability and mineralization of SOM.

Biologically, Zn affects microbial communities that are responsible for the decomposition and turnover of organic matter. At optimal levels, Zn serves as a cofactor in many microbial enzymes (e.g., dehydrogenases, carbonic anhydrase), which participate in SOM mineralization and nutrient cycling. However, excessive Zn can become toxic to microorganisms, leading to suppressed microbial respiration, enzyme activity, and carbon mineralization (Lv et al., 2022). Zn application increased basal respiration and microbial biomass in Zn-deficient soils due to improved enzyme activation. Conversely, Liu et al. (2020) reported a reduction in microbial biomass carbon (MBC) and carbon-use efficiency in soils treated with high Zn concentrations, particularly in contaminated or poorly buffered soils. Furthermore, Zn application can alter the community composition of soil microbiota. Zn-sensitive microbial groups such as Actinobacteria may decline, while resistant groups (e.g., some Proteobacteria) may become dominant, leading to shifts in SOM degradation pathways and nutrient cycling processes (Da et al., 2023). The presence of organic matter significantly influences Zn availability. Organic materials like compost, farmyard manure (FYM), and biochar can form Zn-organic complexes that buffer Zn release and reduce toxicity while maintaining availability for plant uptake. Zn combined with FYM increased SOM content and microbial activity in wheat rhizospheres, leading to improved Zn use efficiency. Rene et al. (2017) also found that organic matter improved Zn solubility through chelation and pH modification, particularly in alkaline soils. These effects not only enhance Zn uptake but also promote SOM stabilization by stimulating microbial-derived binding agents such as glomalin and polysaccharides. The effect of Zn application on SOM is context-dependent. In low-Zn or degraded soils, moderate Zn application may enhance microbial activity and carbon cycling. However, excessive or repeated Zn inputs, especially in contaminated areas or through industrial sources, can reduce SOM decomposition and biological functions. Hence, Zn application should be managed based on soil type, initial organic matter levels, and environmental risks. Integrating Zn fertilization with organic inputs and monitoring microbial indicators (e.g., basal respiration, enzymatic activity) can optimize SOM preservation and improve overall soil health. Adequate Zn availability stimulates microbial communities and enzyme systems, such as dehydrogenase and phosphatase, which play critical roles in OM

turnover (Lv et al, 2022). In Zn-deficient soils, microbial activity tends to decline, leading to reduced OM mineralization. Therefore, Zn fertilization indirectly enhances OM dynamics, contributing to improved nutrient cycling and soil structure over time.

2.3.5. Effect of Zinc Application on Chlorophyll Content in Wheat

Zinc is essential for the synthesis of chlorophyll and the stability of chloroplast membranes. It plays a key role in protein and auxin synthesis, which are directly linked to leaf greenness and photosynthetic activity. Zinc (Zn) is an essential micronutrient required for the optimal growth and physiological function of plants, particularly in photosynthesis, enzyme activation, and protein synthesis. One of the key physiological indicators of Zn efficiency in wheat (*Triticum aestivum* L.) is its effect on chlorophyll content, which directly influences photosynthetic efficiency, biomass production, and ultimately, yield. Zinc is involved in the biosynthesis of chlorophyll and carotenoids by acting as a cofactor for several enzymes such as carbonic anhydrase, which plays a role in CO₂ metabolism, and superoxide dismutase, which protects chlorophyll structures from oxidative stress. A deficiency in Zn reduces the synthesis of chlorophyll, causing symptoms like chlorosis, particularly in younger leaves. According to Cakmak (2008), Zn-deficient plants show significantly lower chlorophyll content due to impaired protein synthesis and disrupted structural integrity of chloroplast membranes. Zinc also stabilizes ribosomal activity, necessary for the formation of photosynthetic enzymes that govern chlorophyll synthesis. A number of experimental studies have confirmed the positive effect of Zn application on chlorophyll content in wheat. Ghanem et al. (2025) reported that foliar application of ZnSO₄ at tillering and heading stages significantly enhanced chlorophyll a and b levels, leading to improved photosynthetic activity and grain filling. They attributed this to the enhanced uptake of Zn and its role in maintaining membrane structure and enzyme activity related to chlorophyll metabolism. A study by Narimania and Sharifib (2023) further confirmed that Zn application (both soil and foliar) not only increased chlorophyll content but also improved leaf area index (LAI), light interception, and net photosynthetic rate in wheat under field conditions. The effect of Zn on chlorophyll content depends on the source, application method, and crop stage. Foliar application tends to have a quicker effect on chlorophyll concentration due to direct leaf absorption. Piyawan et al. (2018) found that foliar Zn application at 0.5%

concentration during the booting stage resulted in higher SPAD values than basal soil application. Chelated Zn sources such as Zn-EDTA have also shown better performance in maintaining higher chlorophyll levels than inorganic salts due to improved solubility and bioavailability, especially in calcareous soils (Shivay & Prasad, 2012). Moreover, integrated Zn management combining organic manures (e.g., FYM) and Zn fertilizers enhances nutrient use efficiency, microbial activity, and sustained chlorophyll synthesis throughout the growing season.

Zinc also mitigates chlorophyll degradation under abiotic stress conditions such as drought, salinity, or heat, which are common in wheat-growing areas. Zn reduces the generation of reactive oxygen species (ROS) and lipid peroxidation, thereby protecting chlorophyll molecules from oxidative damage. Fatemeh et al. (2024) demonstrated that wheat plants under drought stress retained 15–20% more chlorophyll when treated with Zn than untreated controls. Zinc application significantly improves chlorophyll content in wheat by enhancing enzymatic functions, stabilizing chloroplast structure, and protecting against oxidative stress. The method, form, and timing of Zn application influence the extent of chlorophyll enhancement. Adequate Zn nutrition, especially in deficient soils, is thus essential for optimizing photosynthesis and improving wheat productivity. Numerous studies have reported significant increases in chlorophyll content following Zn application in wheat, especially under Zn-deficient conditions or environmental stresses such as drought or salinity (Cakmak, 2000). This is attributed to Zn's role in protecting chlorophyll from oxidative damage and enhancing nitrogen metabolism. Foliar and soil Zn applications have been shown to improve SPAD (Soil Plant Analysis Development) readings in wheat leaves, indicating improved photosynthetic efficiency and plant vigor.

Zinc fertilization has a multifaceted influence on soil properties and wheat physiology. It can modulate the availability and uptake of key soil macronutrients such as Ca, Mg, Na, and K, while slightly altering pH and EC depending on the application rate and soil conditions. Furthermore, Zn enhances microbial activity and organic matter dynamics, contributing to improved soil fertility. Importantly, Zn plays a direct role in increasing chlorophyll content, thereby promoting photosynthesis and biomass accumulation in wheat. For sustainable wheat production, integrated Zn management should consider its interactions with soil chemical properties and its physiological effects on the plant.

Zinc is a vital micronutrient required for wheat (*Triticum aestivum* L.) growth, playing a central role in enzyme activation, protein synthesis, antioxidant defense, and hormonal regulation. Zn uptake by wheat plants is strongly influenced by soil moisture conditions, particularly under extremes such as drought stress or optimal (field capacity) moisture levels. These contrasting conditions significantly affect Zn solubility, root activity, and transport mechanisms within the plant.

Under field capacity, Zn availability in the soil solution is generally higher due to adequate water content facilitating nutrient diffusion and mass flow to the root surface. At this optimal moisture level, root growth is vigorous, root hairs are well-developed, and active transport mechanisms operate efficiently, resulting in higher Zn uptake and translocation to shoots and grain. Moreover, soil microbes that assist in Zn solubilization thrive under field capacity, enhancing bioavailability through organic acid secretion and enzymatic activity.

In contrast, drought stress reduces Zn mobility in the soil because of limited water for diffusion and increased Zn adsorption onto soil particles. Root growth is also restricted, and physiological stress impairs nutrient uptake. However, wheat plants under drought often respond to Zn fertilization more positively than under well-watered conditions due to Zn's role in maintaining membrane integrity, reducing oxidative stress, and supporting osmotic adjustment (Cakmak, 2000). Studies have shown that foliar Zn application during drought can partially offset limited root uptake and enhance stress tolerance by boosting antioxidant enzyme activity and chlorophyll content.

Overall, while Zn uptake is naturally higher at field capacity, targeted Zn application during drought stress can mitigate nutrient limitations and improve wheat resilience. Understanding the interaction between Zn availability and moisture conditions is essential for optimizing fertilization strategies, especially in semi-arid and arid wheat-growing regions.

2.4. Salinity Effect

The adverse effects of salinity have been attributed to the increase of Na⁺ and Cl⁻, which are considered to be the most important ions that induce several disorders in the physiological processes of different plants (Tavakkoli et al. 2010). Salinity-sodicity stress is a concern because it generally negatively alters physicochemical properties of soil, reducing soil fertility and hindering plant growth and yield (Syed

et al. 2021). Elevated levels of salinity and sodicity can also lead to nutrient imbalance, particularly influencing phosphorus (P) and zinc (Zn), thereby adversely affecting the yield and quality of important crops such as rice and wheat. Salinity increases ion toxicity levels while reducing water availability to plants and absorption of essential nutrients (Grattan and Grieve 1999). Khalilzadeh et al. 2018 stated that salinity stress decreases chlorophyll content, quantum yield, relative water content (RWC) and grain filling parameters, but increases leaf electrical conductance (EC) and dry matter remobilization in wheat. Salinity affects Zn availability in soil through multiple mechanisms. It alters soil pH, displaces Zn^{2+} from exchange sites, and affects its solubility through the formation of insoluble complexes or interactions with other ions. While chloride (Cl^-) can enhance Zn mobility by forming Zn-Cl complexes, the dominance of sodium (Na^+) often leads to competitive inhibition at root uptake sites, reducing Zn absorption. In saline soils, Zn deficiency is exacerbated due to precipitation reactions and competition with Na^+ and Ca^{2+} . Furthermore, Zn availability decreases at high pH, a condition often induced by salinity through bicarbonate accumulation. The cumulative effect is a reduction in bioavailable Zn and its movement into wheat tissues. Salinity-induced osmotic stress reduces root growth and function, limiting Zn uptake.

2.5. Drought versus Field capacity

Drought reduces nutrient uptake by roots and transport from roots to shoots because of restricted transpiration rates and impaired active transport and membrane permeability, resulting in a reduced root adsorbing power of crop plants. Fahad *et al.* (2017) stated that drought stress affects the cycling, absorption, and availability of nutrients to plants, decreasing uptake of nutrient and disrupting plant functions. The plants grown under drought stress are imposed to several physiological and biochemical alterations, including reduction of leaf water status, CO_2 assimilation, and gas exchange rates (Desoky et al. 2020). In addition, water deficit lowers stomatal conductance and, in turn, raises leaf temperature and leaf wilting (El-Mageed et al. 2021). Generally, drought reduces nutrient uptake by roots and transport from roots to shoots because of restricted transpiration rates and impaired active transport and membrane permeability, resulting in a reduced root absorbing power of crop plants (Kramer and Boyer, 1995). Limited soil moisture is widely reported to accelerate Zn deficiency in wheat (Karim and Rahman 2015), especially

due to dry topsoil at the grain-filling stage (Cakmak and Kutman 2018). At field capacity, the soil retains optimal water without waterlogging, maintaining ideal conditions for root activity and nutrient transport. Zinc, being moderately mobile in the soil, is transported primarily through diffusion and mass flow. Adequate soil moisture facilitates mass flow and root growth, enhancing Zn movement toward the root surface and subsequent uptake (Alloway, 2008). Under field capacity, ZIP family transporters responsible for Zn uptake are more active, and xylem loading processes function efficiently, promoting Zn translocation to shoots. Research shows that wheat grown under field capacity conditions typically exhibits higher Zn concentrations in both shoot and grain compared to drought-stressed plants. Field capacity improved Zn solubility and root uptake efficiency in a pot study. Similarly, studies using chelated Zn forms reported improved Zn translocation to wheat shoots under adequate moisture conditions. Drought stress adversely affects Zn uptake and movement through a combination of physical, chemical, and physiological mechanisms. Reduced soil moisture decreases Zn diffusion rates, hinders mass flow, and causes root desiccation and shrinkage, reducing root surface contact with soil particles. As a result, Zn availability at the root-soil interface drops significantly. Additionally, drought limits transpiration, which is essential for xylem-mediated transport of Zn from root to shoot. Reduced transpiration flow under drought conditions compromises Zn mobilization and partitioning to aerial tissues (Tavakkoli et al., 2020). At the molecular level, drought stress downregulates the expression of ZIP transporters, further impeding Zn uptake. In drought-prone soils, wheat plants often exhibit reduced Zn content in the shoot and grain, contributing to poor nutritional quality. Moreover, drought-induced oxidative stress can impair root metabolism, reducing the energy available for active Zn transport. Wheat grown at 80% field capacity had significantly higher shoot Zn concentrations compared to those at 40% water-holding capacity. Wheat cultivars grown under drought conditions had lower root and shoot Zn, while foliar Zn application partially mitigated the deficit. Interestingly, foliar Zn application has been shown to be more effective under drought conditions, bypassing the impaired soil-root pathway. However, combining soil and foliar Zn treatments under field capacity offers the most robust improvements in shoot Zn content.

2.6. Soil Zinc application

Soil application of Zn fertilizer improves grain Zn concentration by only 29.1%, below the biofortification target of 40 mg kg⁻¹ required for human nutrition (Hui et al, 2025). Soil Zn application increased soil DTPA-Zn in calcareous soils, but there was no significant increase in grain Zn concentration possibly due to the low diffusion coefficients of Zn in these soils (Zhao et al. 2014). According to Rehnem et al 2018, high pH, CaCO₃, low moisture, and organic matter contents hinder Zn solubility and diffusion to roots, even after Zn application to the soil. These conditions restrict the amount of Zn available in the rhizosphere for root uptake by wheat crops, resulting in low grain Zn concentrations. In addition, high soil electrical conductivity and pH, and a higher concentration of Ca, Na, Mg, and HCO₃ are the principal reasons for low Zn availability. Zinc deficiency is most common in arid and semiarid environments in saline soils. Higher Ca together with high pH reduces Zn availability to plants (Alloway 2008). Zinc deficiency is prevalent in soils, which are naturally high or low in organic carbon, waterlogged, or light-textured (Ahmad et al. 2012). In soils with low organic matter content, increasing the amount of soil organic matter can enhance the formation of soluble complexes, which may increase Zn uptake by plants (Ozkutlu et al. 2006). Zinc uptake by roots may be restricted due to reduced root activity resulting the poor root uptake and shoot transport, which ultimately hinders the Zn accumulation in grain (Zou et al. 2012). To achieve a substantial enhancement in grain Zn concentration in wheat production, Zn fertilizers should be applied to soils with low Zn availability and high pH values. For an increase of more than 20% in wheat grain Zn concentration, Zn fertilizer application is recommended to soils with available Zn levels below 0.5 mg kg⁻¹ and pH levels between 6.4 and 8.6 (Hui et al, 2025). Soil Zn application remains a primary agronomic strategy to correct Zn deficiency and enhance crop yield and quality, especially in staple crops like wheat, rice, and maize.

Globally, approximately 50% of arable lands are considered Zn-deficient (Alloway, 2008). Deficiencies are more prevalent in soils with high pH (alkaline), low organic matter, high phosphorus content, and sandy texture. In such soils, Zn becomes immobilized due to precipitation or sorption to carbonates, oxides, or clay particles, rendering it unavailable to plants (Cakmak, 2008). In South Asia and sub-Saharan Africa, widespread Zn deficiency poses a significant constraint to food security and human nutrition due to the low Zn concentration in cereal grains (Shivay

& Prasad, 2012). Soil application is typically done using inorganic Zn fertilizers such as zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), zinc oxide (ZnO), and chelated forms like Zn-EDTA. Among these, ZnSO_4 is the most widely used due to its solubility, cost-effectiveness, and rapid correction of Zn deficiency. Application rates commonly range from 2 to 25 kg Zn/ha, depending on soil properties, crop demand, and severity of deficiency. Soil incorporation before planting ensures better Zn distribution in the root zone and sustained availability during the growing season (Rene et al., 2017). Chelated Zn forms (e.g., Zn-EDTA) are more effective in high pH soils because they remain soluble longer and are less prone to fixation, although they are more expensive. Studies have shown that chelated Zn enhances Zn uptake efficiency by 20–30% compared to ZnSO_4 in calcareous soils (Shivay et al., 2015).

Soil-applied Zn significantly improves plant height, tillering, chlorophyll content, root development, and grain Zn concentration. According to Rehman et al. (2018), Zn application at 10 kg/ha increased wheat grain yield by 22% and Zn content by 35%, compared to untreated control in Zn-deficient soils. Moreover, Zn enhances hormonal regulation, particularly auxin production, which influences root growth and nutrient uptake. Zinc is also involved in antioxidant defense systems, helping plants tolerate abiotic stresses like drought and salinity (Cakmak, 2008). When combined with organic matter, Zn application enhances microbial activity and nutrient cycling. Zn plus farmyard manure (FYM) improved Zn use efficiency and maintained higher soil available Zn throughout the season. Soil-applied Zn undergoes complex interactions. In alkaline and calcareous soils, Zn quickly precipitates as ZnCO_3 or gets adsorbed on clay surfaces, reducing its availability. Regular low-dose application or use of Zn-coated urea or DAP is often recommended to maintain bioavailable Zn without environmental risks. Although Zn is generally safe, excessive application can lead to accumulation in the soil, potentially affecting microbial communities and causing antagonistic interactions with nutrients like phosphorus, iron, and copper.

2.7. Soil versus foliar

Two major agronomic approaches for biofortification are soil application and foliar spraying of Zn fertilizers. Each method has its advantages and limitations, and their effectiveness depends on various factors including soil type, environmental conditions, and wheat growth stage. Zn in soils exists in various chemical forms, and

its bioavailability depends on pH, organic matter, moisture content, and redox potential. Wheat primarily absorbs Zn as Zn^{2+} from the soil solution through root epidermal cells via ZIP (ZRT/IRT-like protein) transporters. After uptake, Zn is transported via the xylem and redistributed through the phloem. However, Zn tends to accumulate in vegetative tissues, and its remobilization to grains is often limited during the reproductive stage. This creates a challenge for achieving effective Zn enrichment in the edible parts of the plant. Soil application involves incorporating Zn fertilizers such as zinc sulfate ($ZnSO_4 \cdot 7H_2O$), zinc oxide (ZnO), or chelated Zn forms (e.g., Zn-EDTA) into the soil before or during planting. It is the most commonly practiced method due to its ease of integration with standard fertilization routines. Soil-applied Zn improves Zn availability in the rhizosphere and supports early root and shoot development. It enhances root Zn uptake throughout the growing season, improves general plant health and yield and it is cost-effective and scalable in large field operations. Moreover, there is low Zn use efficiency (<5–10%) due to Zn fixation in high pH or calcareous soils, limited impact on Zn concentration in the grain due to poor root-to-grain translocation and risk of environmental accumulation or antagonism with other nutrients such as phosphorus. Studies by Alloway (2008) show that while soil application boosts total Zn uptake, its contribution to grain Zn concentration is relatively modest. This is because most of the absorbed Zn remains in vegetative parts, and remobilization to developing grains is insufficient.

Foliar application involves spraying Zn solutions directly on the leaves during critical growth stages, typically booting, flowering, or grain filling stages. This method bypasses soil constraints and delivers Zn directly to the shoot system, increasing the likelihood of Zn translocation to the grains. There is a higher Zn use efficiency due to direct uptake, greater impact on grain Zn concentration, especially when applied at reproductive stages and it is effective even in Zn-fixing or saline soils where root uptake is limited. Moreover, it requires careful timing and repeated applications, it may cause phytotoxicity at high concentrations and it is labor-intensive in large-scale operations if mechanization is not available. Foliar Zn sprays have been shown to significantly increase grain Zn levels. Cakmak et al. (2010) reported up to a 60% increase in grain Zn concentration with foliar Zn application compared to untreated controls. Foliar feeding during grain development stages improves phloem transport of Zn to the developing kernels, where it is stored in the aleurone layer and embryo.

Several studies have compared the efficacy of soil and foliar Zn applications on wheat growth, yield, and grain Zn content: Soil application is better for early plant development, improving biomass and yield. Foliar application is more efficient for increasing Zn in grains, which is critical for biofortification. Combined soil and foliar Zn application provided synergistic benefits, enhancing both yield and Zn biofortification. Their results showed that sole soil application increased shoot Zn concentration but had a minimal effect on grain Zn, while foliar application, particularly at grain filling, substantially improved Zn accumulation in wheat grains. Zn use efficiency is generally higher in foliar sprays (20–50%) than soil applications (5–10%). Moreover, foliar Zn is more responsive to environmental and temporal control, allowing farmers to tailor applications to crop needs and growth stages. The combined application of foliar and soil delivery methods resulted in higher Zn uptake in wheat compared to soil application alone. The dual application approach ensures a more comprehensive and efficient uptake of Zn, leading to increased Zn concentrations in wheat tissue (Abbas et al, 2025). Calcareous and alkaline soils reduce Zn availability. Foliar application is preferred in such soils. Drought conditions reduce Zn mobility and uptake from the soil, limiting the effectiveness of soil application. Foliar spraying can mitigate drought-induced Zn deficiency. Zn uptake during grain filling is critical. Soil-applied Zn benefits early growth stages, while foliar application is essential for later stages to enhance grain Zn. Zn Source and Formulation: $ZnSO_4$ is widely used, but chelated forms like Zn-EDTA show better solubility and availability under adverse conditions. Recent trends focus on integrated Zn management combining both soil and foliar applications for synergistic effects. Nano-Zn formulations, slow-release fertilizers, and precision agriculture techniques are also being explored. Zn nanoparticles in foliar sprays enhances uptake efficiency while reducing the total required dose. Furthermore, coupling agronomic biofortification with genetic biofortification (breeding Zn-efficient wheat varieties) and soil health management (e.g., organic amendments, mycorrhizae) can further boost Zn delivery to grains. Both soil and foliar Zn applications have roles to play in wheat biofortification. While soil application supports vegetative growth and overall plant health, foliar application is more effective at enriching wheat grains with Zn. Therefore, a combined strategy—applying Zn to the soil at sowing and supplementing with foliar sprays at reproductive stages—is widely recommended for maximizing yield and nutritional quality.

3. MATERIALS AND METHODS

3.1. Greenhouse setup

The greenhouse experiment was conducted at the Faculty of Agriculture, Ondokuz Mayıs University, Atakum, Turkey, for six months. The crops were sown in November 2024 and were harvested in mid-April 2025. Soil samples were collected from the field close to the greenhouse at a 0-15cm depth, and preliminary tests were conducted (Table 3.1). The soil was crushed and sieved, then potted in a 3 kg pot. Five levels of zinc application (0, 5, 10, 25, 50 kg Zn/ha from ZnSO₄) were applied to the pots after sowing the wheat seeds (twelve seeds, which were later thinned to eight seeds). The irrigation water was prepared with MgCl, NaCl, and CaCl₂ to an electric conductivity of (0.5, 2, 4 dS/m). The plants are irrigated 2-4 times a week at drought level (1335) and field capacity (1440), and during each watering, evapotranspiration data were taken.

Table 3.1: Preliminary test

| Soil Properties | Units | Value |
|----------------------|---------|-------|
| Textural class | - | Clay |
| EC | dS/m | 530.4 |
| pH | - | 7.29 |
| Organic Matter | % | 2.26 |
| Na | Cmol/kg | 1.00 |
| K | Cmol/kg | 1.32 |
| Mg | Cmol/kg | 13.24 |
| Ca | Cmol/kg | 25.16 |
| Available Phosphorus | Ppm | 25.64 |
| Organic Carbon | % | 1.31 |

3.2. Soil sampling and analysis

Soil pH was determined using the 1:2.5 soil-to-water suspension method, following standard procedures described by the Soil Science Society of America (Thomas, 1996). Air-dried soil samples were first sieved through a 2-mm mesh to remove debris and ensure uniformity. For each sample, 20 g of soil was weighed into a clean 100 mL tube, and 20 mL of distilled water was added. The suspension was

stirred thoroughly and allowed to stand for 1 hr with occasional stirring to ensure proper mixing and equilibration. The pH meter was calibrated before use to ensure accuracy. Readings were recorded once the meter stabilized.

Electrical conductivity, which indicates the soluble salt content of the soil, was also measured from the same 1:2.5 soil-water extract used for pH determination. After the 1 hr shaking, the EC of the suspension was measured using a digital conductivity meter (e.g., EC meter with temperature compensation). The EC meter was calibrated using a standard KCl solution of known conductivity before each batch of measurements. The electrode was rinsed with distilled water between readings to prevent cross-contamination. EC readings were expressed in decisiemens per meter (dS/m) at 25°C.

Soil organic carbon (SOC) content was determined using the Walkley-Black wet oxidation method (Walkley & Black, 1934), a widely adopted procedure for estimating the oxidizable organic carbon in soil samples. Air-dried soil samples were first sieved through a 2 mm mesh to remove gravel and plant residues. From each sample, 1.0 g of soil was accurately weighed and placed into a 500 mL Erlenmeyer flask. To this, 10 mL of 1 N potassium dichromate ($K_2Cr_2O_7$) solution was added, followed immediately by 20 mL of concentrated sulfuric acid (H_2SO_4). The mixture was swirled gently and allowed to stand for 15 minutes to ensure complete oxidation of the organic matter. After the oxidation period, 200 mL of distilled water was added to the flask to dilute the solution. Then, 10 mL of phosphoric acid and a few drops of diphenylamine indicator were added. The excess dichromate was titrated with 0.5 N ferrous sulfate ($FeSO_4$) solution until the color changed from violet-blue to green, indicating the endpoint. The amount of dichromate reduced during the reaction was used to calculate the amount of organic carbon in the soil sample. The organic carbon content was expressed as a percentage of the oven-dry soil weight. A correction factor (1.33) accounted for the incomplete oxidation of organic carbon, because only about 75% of the total organic carbon is oxidized under these conditions.

Soil available phosphorus was measured using the Olsen method for neutral to alkaline soils. Air-dried and sieved soil samples (2 mm) were used. 2 g of soil was extracted with 10 mL of 0.5 M sodium bicarbonate ($NaHCO_3$), pH 8.5, in a 100 mL Erlenmeyer flask. The suspension was shaken for 30 minutes on a mechanical shaker and filtered through Whatman No. 42 filter paper. An aliquot of the filtrate was

reacted with ascorbic acid and ammonium molybdate reagent to develop a blue color, which was measured using a spectrophotometer at 882 nm. Available P was calculated using a calibration curve prepared from standard KH_2PO_4 solutions and expressed in mg/kg.

DTPA-extractable Zn was determined following the Lindsay and Norvell (1978) method. 10 g of air-dried, sieved soil was shaken with 20 mL of DTPA extracting solution (composed of 0.005 M DTPA, 0.1 M triethanolamine, and 0.01 M CaCl_2 at pH 7.3) for 2 hours. The suspension was filtered through Whatman No. 42 filter paper. Zinc concentration in the filtrate was measured using an atomic absorption spectrophotometer (AAS) at 213.9 nm wavelength. Results were expressed as mg Zn/kg soil.

Basal respiration was measured to assess microbial activity by quantifying CO_2 evolution from soil. 50 g of fresh field-moist soil was placed in a sealed incubation jar with a vial containing 10 mL of 0.5 M NaOH to trap CO_2 . The jars were incubated at 25°C for 1 day. After incubation, the remaining NaOH was titrated with 0.5 M HCl after carbonate precipitation with BaCl_2 . A blank (jar with NaOH but no soil) was also included. The amount of CO_2 evolved was calculated and expressed as mg $\text{CO}_2\text{-C/kg soil/day}$.

Exchangeable cations were extracted using 1 N ammonium acetate (NH_4OAc) at pH 7.0. 3 g of air-dried, sieved soil was shaken with 25 mL of 1 N NH_4OAc for 5 minutes and then filtered.

- Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) were determined using atomic absorption spectrophotometry (AAS).
- Potassium (K^+) and Sodium (Na^+) were determined using a flame photometer.

The concentrations were calculated using calibration standards and expressed in $\text{cmol}(+)/\text{kg soil}$.

3.3. Plant sampling and analysis

Evapotranspiration was calculated by weighing each pot before and after irrigation and calculating the change in soil moisture.

$$ET = \Delta S \dots\dots\dots(1)$$

Where:

ΔS = Change in soil water storage (initial - final moisture content, mm)

Water Use Efficiency (WUE) is the biomass amount or yield produced per unit of water used.

$$\text{WUE} = \text{Total dry biomass (g)}/\text{Total ET (L or mm)} \dots \dots \dots (2)$$

Relative water content was measured to assess the water status of wheat plants using the method of Barrs and Weatherley (1962).

- Fresh weight (FW) of leaf samples was recorded immediately after harvest.
- Leaves were then floated in distilled water for 4–6 hours at room temperature to reach full turgidity. Afterward, the turgid weight (TW) was measured.
- Leaves were then oven-dried at 70°C for 48 hours to obtain the dry weight (DW).

The Relative Water Content (RWC) was calculated using the formula:

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100 \dots \dots \dots (3)$$

where FW, DW, and TW were the fresh, dry, and turgid weights(g) of the plant samples

Plant chlorophyll was determined by spectrophotometric chlorophyll analysis

- 0.2 g of fresh leaf tissue was homogenized in 10 mL of 80% acetone, then centrifuged.
- The absorbance of the supernatant was read at 645 nm and 663 nm using a spectrophotometer.
- Chlorophyll a, b, and total chlorophyll were calculated using Arnon's equations and expressed in mg/g fresh weight.

Plant Dry Weight was obtained by the Oven drying method by drying in a ventilated oven at 65–70°C for 48–72 hours or until constant weight and weighing using an electronic balance and recording in grams (g).

The concentration of zinc (Zn) in wheat plant tissues was determined using the dry ashing method, followed by analysis using an Atomic Absorption Spectrophotometer (AAS). Plant samples (leaves, stems, and grains) were harvested, thoroughly washed with distilled water to remove surface dust and contaminants, and then oven-dried at 70°C for 48 hours until a constant weight was obtained. The dried samples were ground using a stainless steel mill. 0.5 g of the ground plant sample

was accurately weighed into a porcelain crucible, the crucibles were placed in a muffle furnace and gradually heated to 500°C, the temperature was maintained for 4–6 hours until the sample turned into a light grey or white ash, indicating complete oxidation of organic matter, the crucibles were removed and cooled in a desiccator. The resulting ash was dissolved in 4 mL of 3 N hydrochloric acid (HCl) and gently heated to aid dissolution, the solution was filtered through Whatman No. 42 filter paper into a 50 mL tube and made up to volume with deionized water, zinc concentration in the extract was determined using an Atomic Absorption Spectrophotometer (AAS) at a wavelength of 213.9 nm. Standard zinc solutions were prepared from a stock solution of Zn for calibration. The Zn concentration in plant tissue was expressed as mg Zn per kg of dry weight.

Plant Zn Uptake is the Total amount of Zn absorbed and accumulated in plant tissue.

$$\text{Zn uptake (mg/pot)} = (\text{Zn concentration (mg/kg)} \times \text{Dry weight (g)}) / 1000 \dots \dots \dots (4)$$

3.4. Statistical Analysis

SPSS programme was used to perform the statistical analysis. A three-way Analysis of Variance (ANOVA) was used to evaluate the main and interactive effects of zinc levels, water quality (salinity), and irrigation regime (moisture content) on various soil and plant parameters, including soil pH, electrical conductivity (EC), available nutrients, plant height, chlorophyll content, Zn concentration in shoot, and biomass yield. Significant treatment effects were further explored using Tukey's Honest Significant Difference (HSD) test at a 5% probability level ($p \leq 0.05$) to compare means across treatment combinations. To assess the strength and direction of relationships among key variables such as soil Zn, soil salinity, and shoot Zn, Pearson correlation analysis was performed. This provided insights into the linear associations among physicochemical properties and Zn uptake patterns in wheat. Additionally, linear and multiple regression analyses were conducted to identify significant predictors of plant Zn content and wheat biofortification efficiency. Predictor variables included soil Zn, EC, Zn application rate, irrigation regime, and water quality level. The coefficient of determination (R^2) and significance values (p-values) were used to evaluate model strength and predictive reliability. To identify underlying data structure and multivariate patterns among variables, Principal Component Analysis (PCA) was performed using

Varimax rotation with Kaiser normalization. This helped in identifying groups of correlated variables and reducing data dimensionality. Components with eigenvalues >1 were retained, and variable loadings above ± 0.30 were interpreted to assess contribution to each principal component.

All statistical decisions were made at $p \leq 0.05$, and results were interpreted in conjunction with agronomic relevance. Graphs, scatter plots, and component loading plots were used to visualize treatment effects and associations. These analyses supported the understanding of Zn dynamics in the soil–plant system under varying moisture and salinity conditions.



4. RESULTS AND DISCUSSIONS

4.1. Influence of Zinc application on soil and plant properties

Effects of zinc applications under the different water quality and irrigation levels on some soil and plant properties are given in Table 4.1. According to the statistical analyses, all treatments and their interactions generally had significant effects on the investigated soil and plant properties.

Table 4.1: Mean significance of soil properties and variables

| V. Sources | df | EC | pH. | OM | P | K | Ca |
|-------------------------|----|----------|---------|--------|--------|--------|--------|
| Irrigation | 1 | 38,9** | 0,2 | 3,7* | 12,7** | 5,4* | 14,8** |
| Salt | 2 | 1428,8** | 882,2** | 41,3** | ,020 | 19,7** | 96,0** |
| Znc | 4 | 72,2** | 4,4** | 1,4 | 1,8 | 11,3** | 1,0 |
| Irrigation * Salt | 2 | 13,6** | 5,9** | 15,0** | ,2 | 2,9* | 7,6** |
| Irrigation * Znc | 4 | 5,21** | 1,9 | 2,0 | 3,5* | 2,0 | 2,2* |
| Salt * Znc | 8 | 21,4** | 8,1** | 2,7* | ,4 | 2,4* | 1,9* |
| Irrigation * Salt * Znc | 8 | 1,97* | 3,4** | 3,0** | ,9 | ,8 | 2,8* |

| V. Sources | df | Mg | Na | ET | RWC | SBCO2 | WUE |
|-------------------------|----|--------|---------|---------|------|--------|--------|
| Irrigation | 1 | 13,4** | 13,2** | 141,4** | 3,2* | 0,0 | 59,9** |
| Salt | 2 | 18,1** | 771,6** | 153,1** | 4,0* | 7,5** | 75,7** |
| Znc | 4 | 3,2* | 53,4** | 29,2** | 3,4* | 18,6** | 14,4** |
| Irrigation * Salt | 2 | 0,9 | 6,9** | 1,7 | 0,7 | 17,5** | 0,8 |
| Irrigation * Znc | 4 | 0,9 | 4,2** | 2,4* | 2,7* | 0,9 | 2,3* |
| Salt * Znc | 8 | 1,2 | 17,8** | 8,7** | 2,1* | 0,8 | 5,7** |
| Irrigation * Salt * Znc | 8 | 3,4** | 2,9** | 1,2 | 1,3 | 5,3** | 2,9** |

| V. Sources | df | PdryW | Soil Zn | Pzncont | Zn uptake | K |
|-------------------------|----|--------|---------|---------|-----------|--------|
| Irrigation | 1 | 35,5** | 0,5 | 9,7** | 0,7 | 1,4 |
| Salt | 2 | 21,6** | 4,9* | 55,1** | 38,9** | 19,2** |
| Znc | 4 | 8,4** | 194,5** | 41,2** | 34,9** | 3,3* |
| Irrigation * Salt | 2 | 2,2 | 14,0** | 3,8* | 7,0** | 18,1** |
| Irrigation * Znc | 4 | 2,7* | 16,1** | 5,6** | 3,0* | 7,8** |
| Salt * Znc | 8 | 2,1* | 6,8** | 7,7** | 6,9** | 6,1** |
| Irrigation * Salt * Znc | 8 | 1,9* | 3,1** | 1,5 | 1,8 | 5,2** |

4.1.1. Electrical conductivity (EC)

The soil's electric conductivity (EC) (Fig 4.1) increases with increased salt concentration and soil moisture application. At the same time, it decreases with the increasing ZnSO₄ application at field capacity from 10-50kg/mg Zn. The difference between the treatments is statistically significant at $P < 0.05$ (Fig. 1). The electric conductivity of all samples with ZnSO₄ was higher than that of the control, which signifies that application of ZnSO₄ to soil increases the EC and at drought stress, the soil has low EC compared to a field capacity. The treatment without salt stress and with drought stress recorded the lowest EC, while the maximum EC was observed at 4dS/m salt stress and field capacity. Soil electrical conductivity is related to nutrients in the soil solution and can be affected by soil organic matter, soil texture, temperature, and water content (Kim and Park, 2024). Various studies (Brevik et al. 2006; Mirzakhani-fachi et al, 2022) also reported that soil EC linearly increased with soil moisture content because, as soil moisture increases, more water is available in the soil pores, allowing ions to move freely, which enhances the electrical conductivity of the soil. In saline soil, higher soil moisture can dissolve more salts, increase the concentration of the soil solution, and thus increase the EC. The higher the salinity and moisture content of the soil solution, the higher the EC of the soil solution and vice versa. Upon application of ZnSO₄ to the soil, there is an increase in soluble salt concentration, which raises the soil's EC.

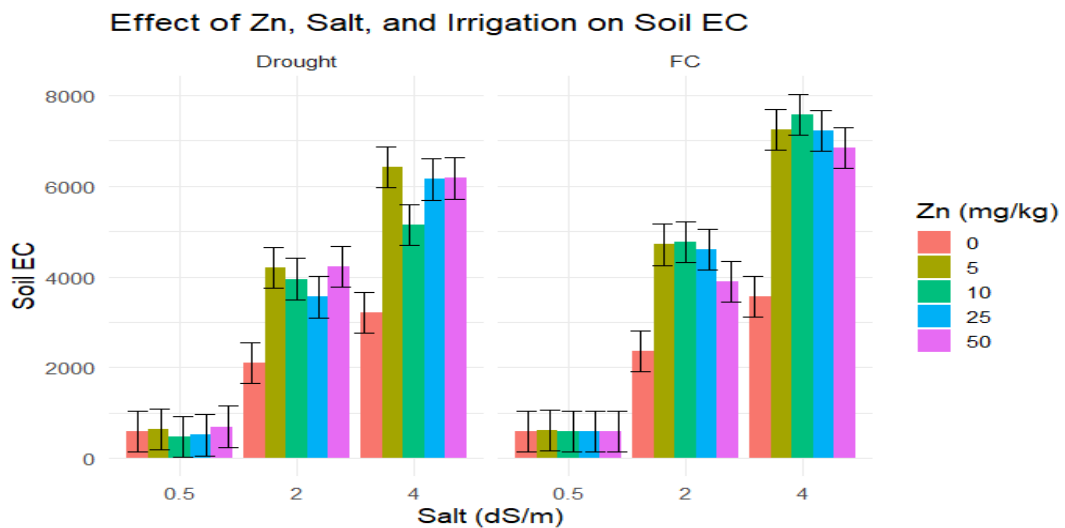


Fig 4.1 Effect of Zn, salt, and irrigation on Soil Electrical conductivity

4.1.2. Soil reaction (pH)

The soil pH, which is the soil's acidity or alkalinity, determines nutrients' availability and solubility. The pH varied between 6.8 and 7.6 (Fig 4.2). At field capacity, the soil becomes slightly alkaline at 0.5dS/m, then neutral and somewhat acidic at 2dS/m and 4dS/m, respectively, indicating a decrease in pH with increasing salinity and an increase in pH with increasing soil moisture. ZnSO₄ application to the soil caused a negligible change to the soil pH. Zinc sulfate (ZnSO₄) can induce slight acidification, particularly in soils with low buffering capacity. ZnSO₄ dissolves in soil moisture, releasing Zn²⁺, SO₄²⁻, and H⁺ ions, where the H⁺ contributes directly to lowering pH. An Indian field study applying ZnSO₄ at 30 kg/ha alongside a base NPK fertilizer showed a drop in soil pH from 7.77 (control) to 7.42 in the Zn-treated plots (Vinothini and Baskar, 2018). Although the magnitude of the change (≈ 0.35 pH units) was moderate, the reduction was consistent with the acidifying effect of acidic salt. This pH decrease is more pronounced in sandy or acid-sensitive soils and becomes negligible in calcareous or clay-rich soils. In most cases, pH drops by 0.1–0.3 units following typical ZnSO₄ applications, and soil buffering systems often neutralize the effect over time. Short-term acidification may be influenced by microbial and root activity, which mobilize additional H⁺ in the rhizosphere.

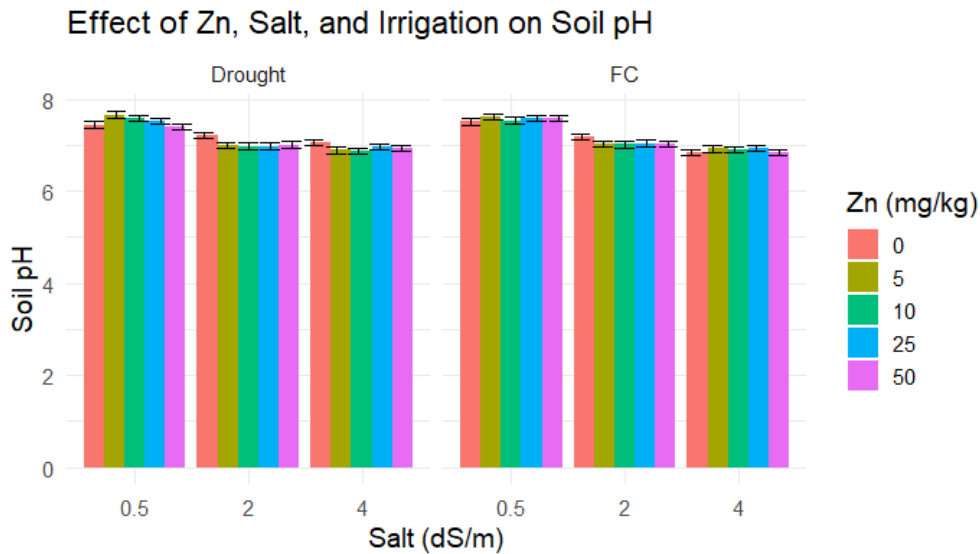


Fig 4.2 Effect of Zn, salt, and irrigation on Soil pH

4.1.3. Organic matter

The results (Fig 4.3) demonstrate that soil organic matter (OM) content was significantly influenced by the interaction of zinc application rates, irrigation regime (drought vs. field capacity), and water salinity levels (0.5, 2, and 4 dS/m). Across all treatments, organic matter content ranged between approximately 1.0% and 3.4%. In the field capacity (FC) regime, soil OM increased with salinity up to 4 dS/m across all Zn application rates. The highest OM content (~3.4%) was observed at 4 dS/m with 5–25 mg/kg Zn, while the lowest (~2.0%) occurred at low Zn levels under 0.5 dS/m. This trend suggests that mild salt stress combined with Zn supplementation may stimulate microbial activity or reduce organic matter mineralization, allowing OM to accumulate under water-limited conditions. Additionally, moderate Zn levels (5–25 mg/kg) were associated with higher OM than no Zn or excessive Zn (50 mg/kg), indicating a potential threshold beyond which Zn toxicity may suppress microbial activity.

Under drought conditions, soil OM declined with increasing salinity from 0.5 to 2 dS/m, but increased again at 4 dS/m. This U-shaped trend may reflect shifts in microbial decomposition rates or root exudate contributions across salinity levels. Notably, at 4 dS/m and FC, the highest OM was observed at 10 mg/kg Zn, again reinforcing the beneficial role of moderate Zn in supporting soil biological activity under saline conditions. Lower Zn rates (0 mg/kg) were generally associated with reduced OM content, particularly at higher salinities.

The results suggest that moderate Zn sulfate application (5–25 mg/kg) improves soil OM, particularly under drought and saline stress conditions, possibly by enhancing plant growth and microbial enzyme activity. However, excessive Zn (50 mg/kg) may suppress OM accumulation, especially under less stressful conditions. The irrigation regime also played a crucial role: drought generally preserved or increased OM compared to field capacity, possibly due to reduced microbial oxidation and lower OM mineralization under limited moisture.

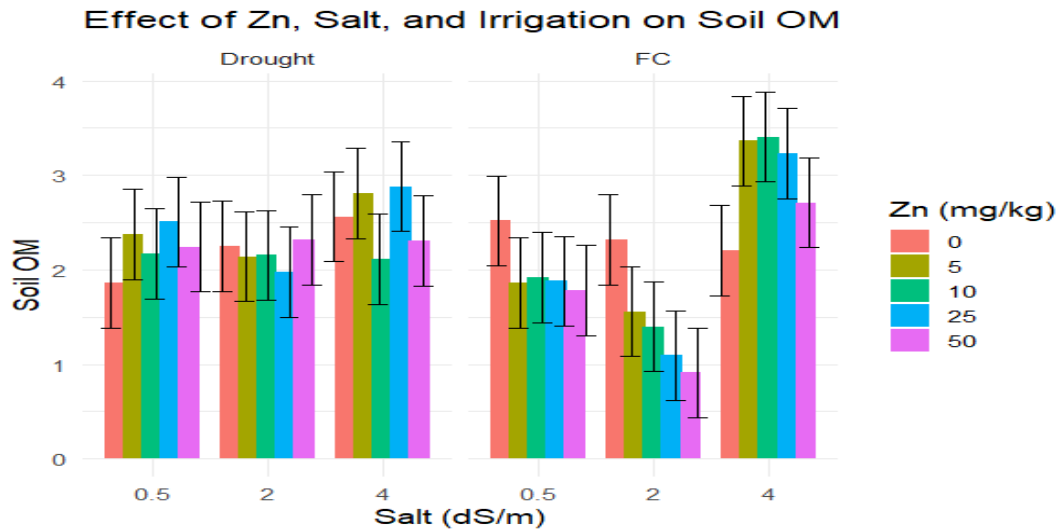


Fig 4.3 Effect of Zn, salt, and irrigation on Soil organic matter

4.1.4. Available Phosphorus

Soil phosphorus (P) levels varied significantly under different zinc application rates, irrigation regimes, and salinity levels (Fig 4.4). Overall, phosphorus concentrations ranged between approximately 60 mg/kg and 117 mg/kg, with notable interactions among the three factors studied. Under drought conditions, soil P increased moderately with salinity from 0.5 to 2 dS/m, but began to decline at 4 dS/m across most zinc treatments. The highest P concentration (~104 mg/kg) was observed at 4 dS/m salinity and 0 mg/kg Zn, while the lowest (~61 mg/kg) occurred at 4 dS/m with 5 mg/kg Zn. This suggests that salinity without Zn application enhanced phosphorus availability under limited moisture, likely due to improved nutrient solubility and microbial mineralization in moderately stressed systems. However, salinity or zinc (50 mg/kg) appeared to suppress P availability, possibly by promoting Zn–P interactions that lead to insoluble Zn-phosphate compounds. Under field capacity (FC) conditions, soil P remained relatively stable across all Zn levels at low salinity (0.5 dS/m), but peaked at 2 dS/m, particularly at 5–25 mg/kg Zn, similar to the drought regime. At 4 dS/m, phosphorus availability declined sharply in low-Zn treatments (0 mg/kg), while moderate to high Zn (5–25 mg/kg) helped maintain elevated soil P levels. This suggests that Zn levels mitigate salinity-induced P immobilization, especially under well-watered conditions. Interestingly, 50 mg/kg Zn did not consistently increase soil P, indicating a potential threshold beyond which Zn addition may lead to competitive or inhibitory effects on phosphorus dynamics. Moderate ZnSO₄ application (5–25 mg/kg) is beneficial for sustaining soil

phosphorus availability under both drought and saline conditions, with 2 dS/m salinity yielding the most favorable P levels. This trend is likely driven by enhanced plant root activity, rhizosphere acidification, and optimal microbial P mineralization supported by zinc-dependent enzymes. In contrast, extreme Zn doses or high salinity may suppress phosphorus availability through chemical precipitation or microbial inhibition.

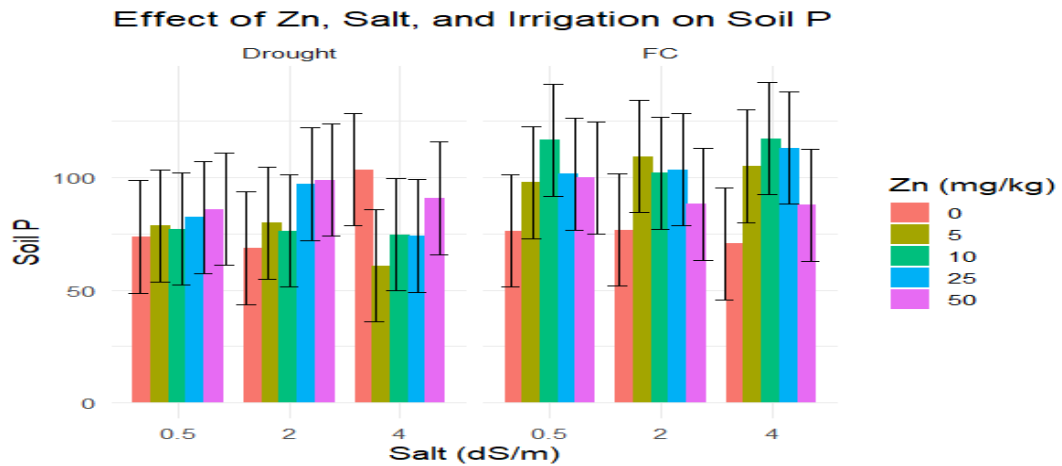


Fig 4.4 Effect of Zn, salt, and irrigation on Soil Phosphorus

4.1.5. Exchangeable Potassium

Soil potassium (K) levels were influenced by the interaction between zinc sulfate ($ZnSO_4$) application rates, salinity (0.5, 2, 4 dS/m), and irrigation regime (drought vs. field capacity) (Fig 4.5). Soil K values ranged from approximately 0.4 to 0.80 cmol/kg, showing variations across treatments. Under drought conditions, the application of $ZnSO_4$ generally increased soil K, particularly at low salinity (0.5 dS/m). The highest soil K (~ 0.80 cmol/kg) was observed at 25 mg/kg Zn, suggesting that moderate Zn levels may enhance K retention or reduce K leaching under limited water availability. As salinity increased to 2 and 4 dS/m, soil K content tended to decline, especially in low Zn treatments (0–5 mg/kg), indicating potential antagonistic effects of sodium (Na^+) on K^+ uptake and retention. Notably, higher Zn levels (25–50 mg/kg) mitigated some of this decline, suggesting a protective role of Zn in maintaining K availability under salt stress. In contrast, under field capacity (FC) conditions, soil K was highest at 0.5 dS/m salinity, particularly in the 25 mg/kg Zn treatment (~ 0.79 cmol/kg). As salinity increased to 2 and 4 dS/m, soil K declined across all Zn levels, with the lowest values found at high salinity and low Zn

application. This pattern suggests that high moisture levels promote leaching losses of K^+ , especially under saline conditions, and that Zn application may partially compensate for such losses by enhancing root health and K uptake efficiency. Moderate to high Zn application (10–50 mg/kg) improves soil K retention, particularly under drought and low-salinity conditions. The decline in soil K under high salinity and FC conditions may be attributed to cation competition (e.g., Na^+ vs. K^+), leaching, or salt-induced K fixation in clay minerals. Zinc alleviates some of these stresses by promoting better root functioning and nutrient balance.

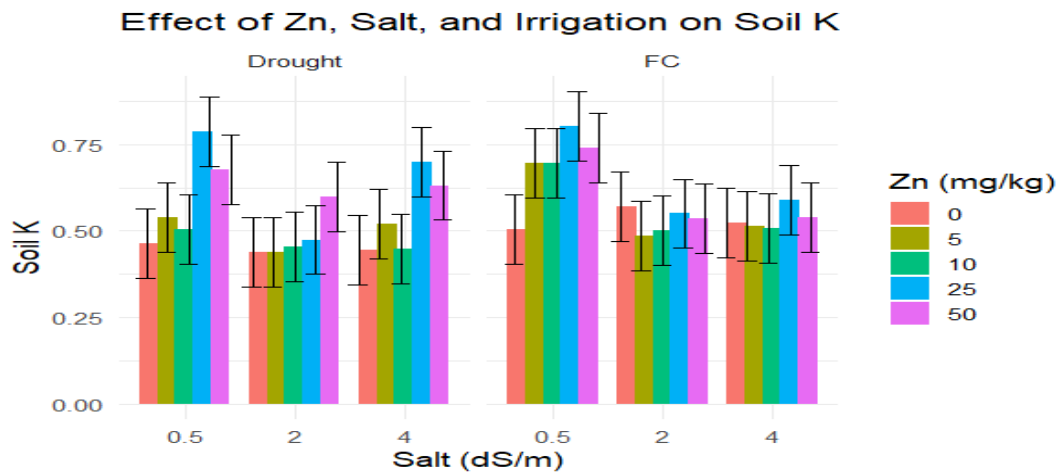


Fig 4.5 Effect of Zn, salt, and irrigation on Soil Potassium

4.1.6. Exchangeable Calcium

Soil calcium (Ca) concentrations showed notable variation across different zinc sulfate ($ZnSO_4$) application rates, salinity levels (0.5, 2, and 4 dS/m), and irrigation regimes (drought vs. field capacity) (Fig 4.6). Soil Ca ranged from approximately 23 mg/kg to 40 mg/kg, reflecting the influence of moisture availability and salinity on calcium dynamics in the soil. Under drought conditions, soil Ca generally increased with salinity, reaching its highest values at 4 dS/m. The most pronounced increase was observed at 5–25 mg/kg Zn, suggesting that moderate Zn application can enhance Ca availability or retention under saline–dry conditions. In low-salinity treatments (0.5 dS/m), Ca levels were relatively lower across all Zn rates, likely due to reduced dissolution of Ca-bearing minerals and minimal competitive displacement from other cations. Interestingly, the highest Zn rate (50 mg/kg) did not consistently cause higher Ca values, suggesting a possible antagonistic interaction or Zn-induced microbial suppression at excessive doses. In

the field capacity (FC) regime, a different trend emerged. Soil Ca was relatively stable or slightly declined at lower salinity levels but increased significantly at 4 dS/m, especially at Zn rates between 10 and 25 mg/kg. This increase under high salinity and adequate moisture could be due to the displacement of Ca^{2+} by Na^+ ions on soil exchange sites, a process enhanced under saline irrigation. Zn application appears to mitigate this, possibly by improving root uptake efficiency or stabilizing the soil's cation exchange complex. Overall, the results suggest that moderate ZnSO_4 applications (10–25 mg/kg) under higher salinity conditions (4 dS/m) are associated with increased soil calcium availability. These findings indicate that Zn may help preserve or enhance soil Ca under salinity stress, although the effect varies with moisture regime. Excessive Zn (50 mg/kg), however, does not further enhance Ca levels and may even reduce them in some cases, possibly due to negative effects on microbial activity or root function.

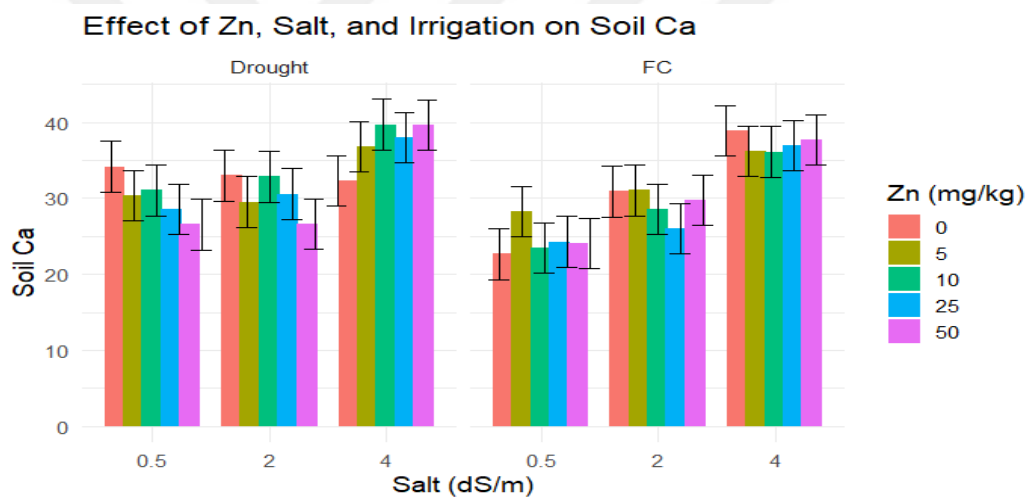


Fig 4.6 Effect of Zn, salt, and irrigation on Soil Calcium

4.1.7. Exchangeable Magnesium

Soil magnesium (Mg) levels showed varied responses to the interaction of zinc sulfate (ZnSO_4) application rates, salinity levels (0.5, 2, and 4 dS/m), and irrigation regimes (drought vs. field capacity) (Fig 4.7). The measured soil Mg ranged from approximately 11 to 24 mg/kg, with distinct trends emerging under different treatment conditions. Under drought conditions, soil Mg was highest at 0.5 dS/m across all Zn application rates, with the maximum concentration (~22 mg/kg) observed at 50 mg/kg Zn. As salinity increased to 2 and 4 dS/m, Mg levels declined steadily, especially in treatments with lower Zn rates (0–10 mg/kg). Under saline-dry

conditions, Mg availability may be reduced due to cation competition, particularly with Na^+ , or precipitation as insoluble salts. However, moderate to high Zn rates (25–50 mg/kg) appeared to buffer this decline, likely by enhancing root function and microbial nutrient cycling even under stress. In Field capacity (FC), a more balanced trend was observed. Soil Mg levels were relatively stable across salinity treatments, though slightly higher at 0.5 and 2 dS/m. Here, Zn application between 5 and 25 mg/kg supported higher Mg availability, while the highest Zn rate (50 mg/kg) showed inconsistent effects. The best Mg levels under FC were recorded at 25 mg/kg Zn and 2 dS/m, indicating an optimal range for maintaining magnesium under saline-irrigated conditions. Excessive Zn appeared to offer no further benefit and may have led to antagonistic interactions or micronutrient imbalance.

Moderate ZnSO_4 application (10–25 mg/kg) enhances soil Mg availability under both drought and irrigated conditions, particularly when salinity is low to moderate. High salinity (4 dS/m) tends to suppress Mg levels, especially in the absence of adequate Zn, likely due to competitive interactions with other cations and reduced plant uptake. The positive role of Zn may stem from its ability to support root health, enzyme activity, and microbial processes that contribute to the cycling of Mg in soil systems.

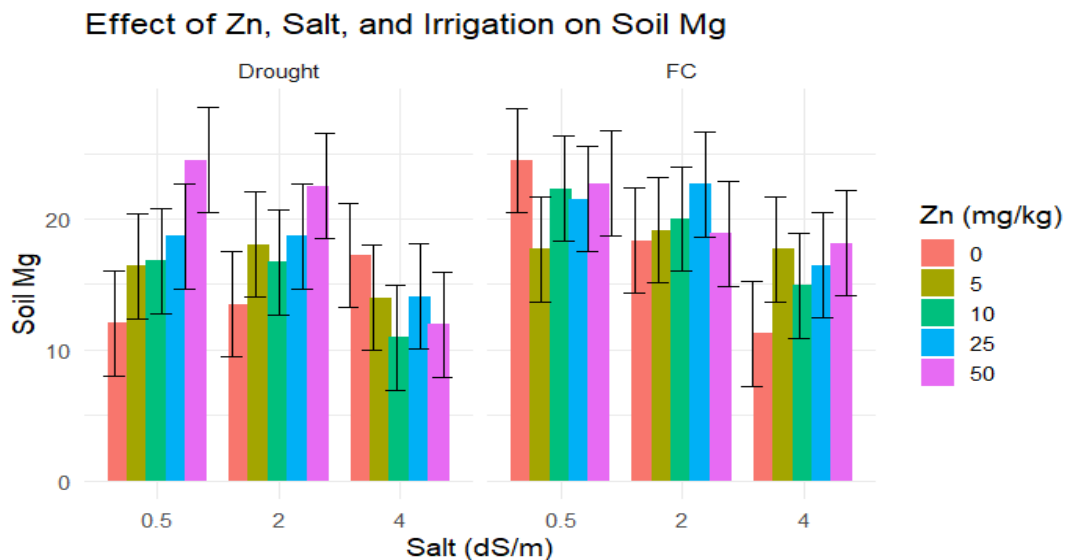


Fig 4.7 Effect of Zn, salt, and irrigation on Soil Magnesium

4.1.8. Sodium

Soil sodium (Na) content was significantly affected by the interaction of zinc sulfate (ZnSO_4) application, salinity levels, and irrigation regimes. Na concentrations ranged from approximately 0.4 to 5.0 cmol/kg, with a marked influence of salinity and moisture availability (Fig 4.8). Under drought conditions, soil Na remained relatively low at 0.5 and 2 dS/m, across all Zn levels, with values below 2.0 cmol/kg. However, at 4 dS/m, a substantial increase in Na was observed, particularly in the 0 and 5 mg/kg Zn treatments, where levels reached up to 5.0 cmol/kg. This sharp rise under high salinity suggests the accumulation of Na^+ due to limited leaching and concentration of salts under low-moisture conditions. Notably, Zn application at 25–50 mg/kg slightly reduced Na accumulation at 4 dS/m, indicating a potential ameliorative role of Zn in moderating Na toxicity, possibly by enhancing plant uptake of K^+ or Ca^{2+} , which competes with Na^+ at exchange sites. Under field capacity (FC) conditions, soil Na increased progressively with salinity, reaching a peak at 4 dS/m. Compared to drought, the Na accumulation was more gradual but still significant. Interestingly, at both 2 and 4 dS/m, higher Zn treatments (25–50 mg/kg) were associated with higher Na levels, unlike in the drought regime. This may be due to greater Na mobility under high moisture conditions, with Zn-induced alterations in cation exchange dynamics or root exudation patterns. At lower salinity (0.5 dS/m), soil Na remained uniformly low, with minimal variation across Zn rates. Overall, the findings suggest that salinity is the primary driver of soil Na accumulation, with Zn application modulating its effect depending on the irrigation regime. Under drought, moderate to high Zn may help reduce Na accumulation or toxicity by improving nutrient balance and root function. However, under field capacity, Zn application appears to have less of a mitigating effect, even coinciding with higher Na content, possibly due to Zn-induced solubilization of native salts or interactions at the rhizosphere. Zinc uptake decreases under saline soils due to strong competition between Zn and salt cations at the root interface (Tinker and Lauchli 1984). For example, in saline–sodic soils, the exchange sites are occupied by Na^+ , resulting in leaching of Zn, especially if the irrigated water has high Na^+ (Alloway 2008). Under saline conditions, Na in the growth medium might compete with other cations such as K, Ca, and Mg, among others, resulting in low absorption by the roots. It is generally recognized that K and Ca uptake by the plant

and their deposition in both expanding and expanded tissues are reduced under saline conditions (Lazof and Bernstein, 1999).

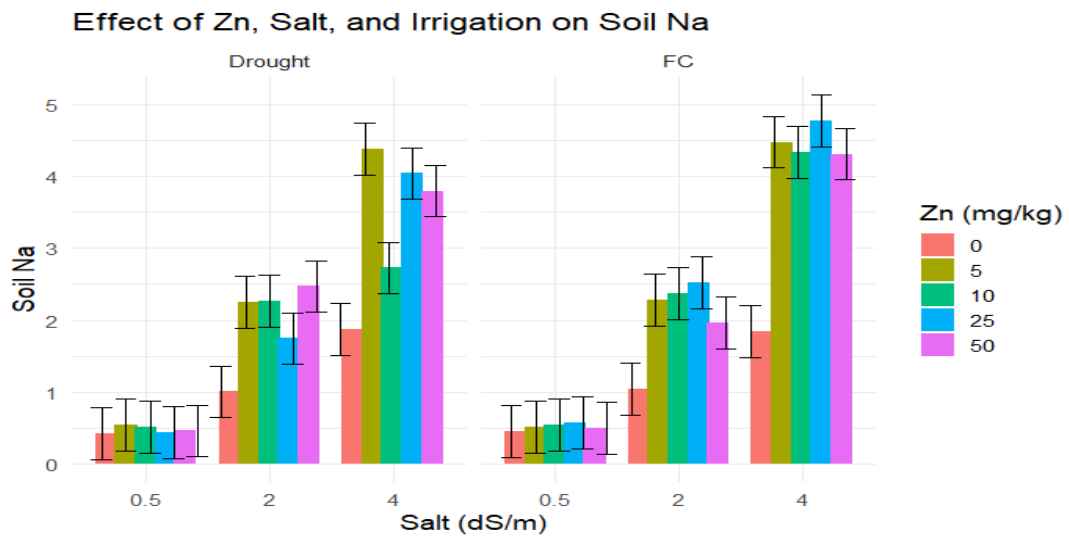


Fig 4.8 Effect of Zn, salt, and irrigation on Soil Sodium

4.1.9. Soil basal respiration

Soil basal respiration ($SBCO_2$), an indicator of microbial activity and organic matter decomposition, was significantly influenced by interactions between zinc sulfate ($ZnSO_4$) application, salinity, and irrigation regimes (Fig 4.9). The measured $SBCO_2$ values ranged from approximately 0.03 to 0.22 mg CO_2 /g soil/day, with noticeable variation across treatments. Under field capacity, $SBCO_2$ displayed high variability. At 0.5 dS/m, respiration was relatively high (~0.13–0.20), especially at low Zn levels (0–5 mg/kg). However, as salinity increased to 2 and 4 dS/m, $SBCO_2$ tended to decrease, reaching minimum values below 0.07 mg/g/day, particularly at high Zn doses (25–50 mg/kg). This decline reflects microbial stress due to increased salinity and Zn toxicity, which can suppress enzyme activity and microbial proliferation. Notably, spikes in $SBCO_2$ were still observed at intermediate Zn levels under mild salt stress, suggesting a stimulatory effect of moderate Zn on microbial respiration under favorable moisture conditions. Under drought conditions, $SBCO_2$ fluctuated across treatments but showed a slightly higher overall baseline than at FC. Maximum values approached 0.20 mg/g/day, particularly at 0.5 and 2 dS/m with 10–25 mg/kg Zn. This indicates that moderate Zn doses supported microbial activity even under moisture-limited conditions, possibly by enhancing microbial enzyme function or stabilizing microbial membranes. However, at 4 dS/m and high Zn rates,

SBCO₂ declined again, showing the compounded stress effect of drought and high salt/Zn levels.

Overall, the results suggest that:

- Moderate Zn application (10–25 mg/kg) supports soil microbial respiration under both irrigation regimes.
- Excess Zn (50 mg/kg) and high salinity (4 dS/m) suppress SBCO₂, especially in well-watered soils.
- SBCO₂ under drought was relatively more resilient, likely due to slower mineralization rates and reduced microbial turnover, which conserve energy.

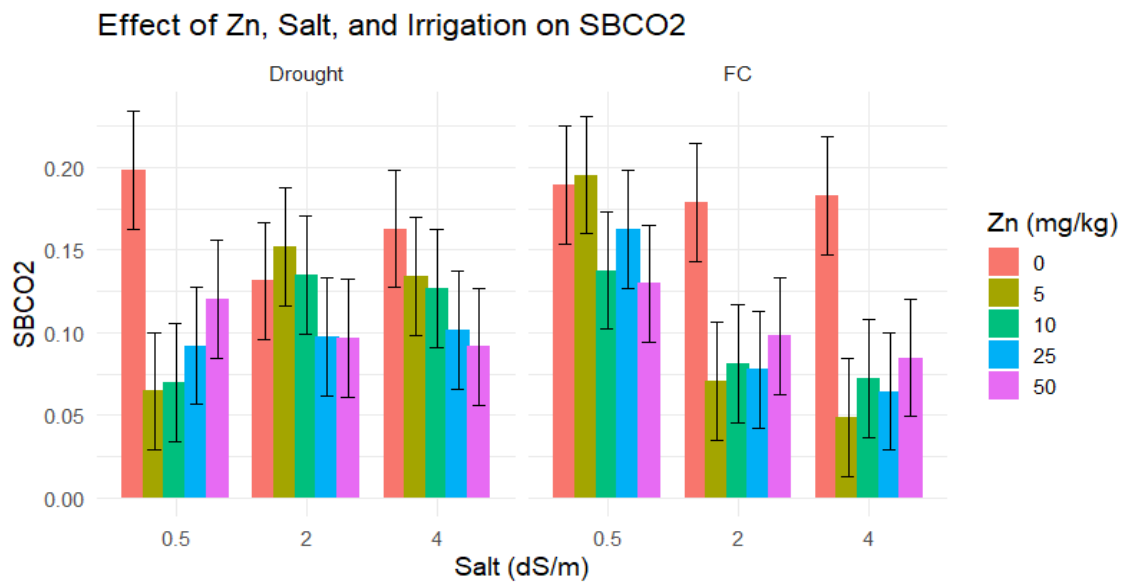


Fig 4.9 Effect of Zn, salt, and irrigation on Soil basal respiration

4.1.10. Plant dry weight

Plant dry weight (PdryW) is a critical indicator of crop growth performance and biomass accumulation under varying environmental and nutrient conditions. In this study, PdryW was measured across different zinc sulfate (ZnSO₄) application rates (0–50 mg/kg), salinity levels (0.5, 2, and 4 dS/m) (Fig 4.10), and two irrigation regimes (field capacity and drought). The data reveal that PdryW remained relatively stable, ranging from 9 – 12 g per pot across all treatments. There were slight fluctuations due to Zn, salt, and irrigation changes, indicating that biomass production was resilient to the tested stress levels and treatments. The narrow variation suggests that either:

- The plants adjusted physiologically to the imposed salinity and Zn conditions, or
- The combined duration and stress intensity did not significantly hinder biomass accumulation.

Under field capacity, PdryW tended to be slightly higher overall, especially at lower salinity (0.5 dS/m), but the effect was modest. Under drought, plant dry weight remained fairly consistent, although slightly reduced at 4 dS/m across all Zn levels. This suggests that increasing salinity may have a minor negative effect on biomass under moisture-limited conditions. Zinc application had no clear linear effect on dry weight. While Zn is essential for growth, the applied rates (0–50 mg/kg) did not dramatically increase biomass in this experiment. This implies that Zn’s main benefits may have been nutrient use efficiency, stress resistance, or physiological traits (such as RWC and WUE) rather than boosting bulk biomass.

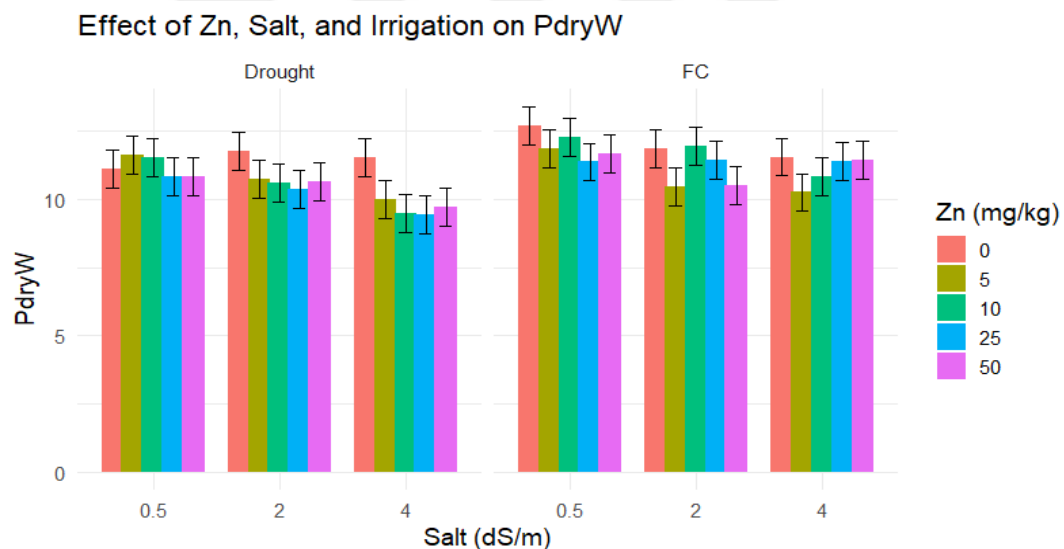


Fig 4.10 Effect of Zn, salt, and irrigation on Plant dry weight

4.1.11. Relative water content

Relative water content (RWC), a physiological indicator of plant hydration and cell turgor, responded sensitively to variations in zinc sulfate ($ZnSO_4$) application, salinity levels, and irrigation regimes (Fig 4.11). Relative water content (RWC) implies the water status in plant leaves (Kabiri et al. 2014). RWC values ranged from approximately 62% to 90%, revealing the influence of salt stress and

nutrient interactions on plant water status. Under drought conditions, RWC was generally higher at moderate salinity levels (2 dS/m) than at 0.5 or 4 dS/m. The highest RWC (~90%) occurred at 2 dS/m with 0 or 5 mg/kg Zn, indicating improved water conservation or osmotic adjustment in mildly stressed plants. However, RWC declined notably at 4 dS/m, particularly under high Zn levels (25–50 mg/kg), likely due to the combined effect of osmotic stress from salinity and Zn toxicity. This suggests that while low to moderate Zn may support cell hydration under moderate salt stress, excessive Zn application can impair water retention in plant tissues. Under field capacity (FC), RWC values were more uniform across treatments, ranging from 70% to 85%. There was no consistent increase in RWC with Zn application, though moderate Zn (10–25 mg/kg) appeared to maintain slightly higher RWC across all salinity levels. At 4 dS/m, the lowest RWC values were observed, particularly under 0 and 50 mg/kg Zn, indicating that extreme Zn doses (too little or too much) can reduce cell water content, especially under saline irrigation. Moderate Zn application (5–25 mg/kg) supports plant water retention, especially under moderate salinity. Excess Zn (50 mg/kg), particularly in combination with high salinity (4 dS/m), is detrimental to RWC, possibly due to ionic toxicity or disruption of root water uptake. The buffering effect of field capacity reduced the magnitude of RWC decline compared to drought, emphasizing the importance of soil moisture in mitigating salinity and Zn stress. The decrease in RWC with increased salinity may be attributed to the accumulation of toxic ions such as Na⁺ and Cl⁻, reducing leaf expansion, LAI, and stomata conductance, and decreasing stomata conductance or stomata closure, leading to a reduction in intracellular CO₂ partial pressure (Hasegawa et al., 2000).

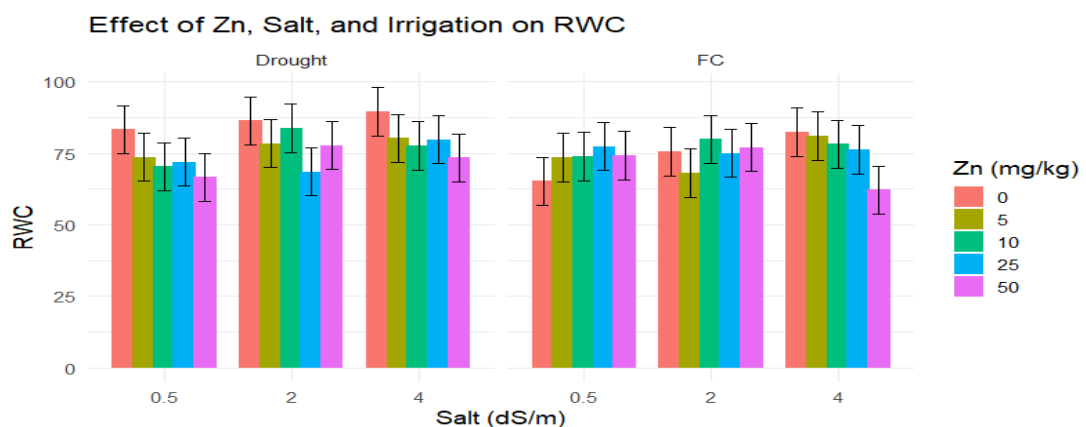


Fig 4.11 Effect of Zn, salt, and irrigation on Soil Relative water content

4.1.12. Evapotranspiration

Soil evapotranspiration (ET), a measure of water loss through both soil evaporation and plant transpiration, was notably influenced by salinity, irrigation regime, and zinc sulfate (ZnSO_4) application rate (Fig 4.12). The measured ET values ranged from approximately 156 to 363 mm, and varied trends under drought and field capacity (FC) conditions. Under drought conditions, ET generally decreased with increasing salinity across all Zn application rates. The highest ET values (~300–310 mm) were recorded at 0.5 dS/m salinity, particularly with 0 and 10 mg/kg Zn, while the lowest ET (~156 mm) occurred at 4 dS/m, especially under higher Zn rates (25–50 mg/kg). This decline is likely due to a combination of osmotic stress from salinity and reduced plant water uptake, especially when high Zn levels may have added to the stress burden. The lower ET at higher Zn rates under high salinity suggests a possible suppressive effect on plant transpiration, potentially due to stomatal closure or reduced root conductivity under combined salt and Zn stress. Under field capacity (FC), ET decreased as salinity increased. At 0.5 dS/m, ET values were highest (~320–363 mm), especially with 0–10 mg/kg Zn, while values declined sharply at 2 and 4 dS/m. However, compared to drought, the reduction in ET was less drastic under FC, reflecting the buffering effect of moisture in mitigating salinity stress. Zn application at low to moderate levels (5–10 mg/kg) maintained slightly higher ET at 2 dS/m than other rates, possibly due to improved root activity and water uptake. Excess Zn (25–50 mg/kg) again coincided with the lowest ET values under high salinity. Overall, the results indicate that salinity is the dominant factor reducing ET, with moisture availability modifying its impact. While moderate Zn application may support ET under mild salinity, high Zn doses under salt stress appear to compound water-use limitations. This underscores the importance of optimizing Zn application—not exceeding 10–25 mg/kg—especially under saline conditions to avoid impairing water uptake and use efficiency.

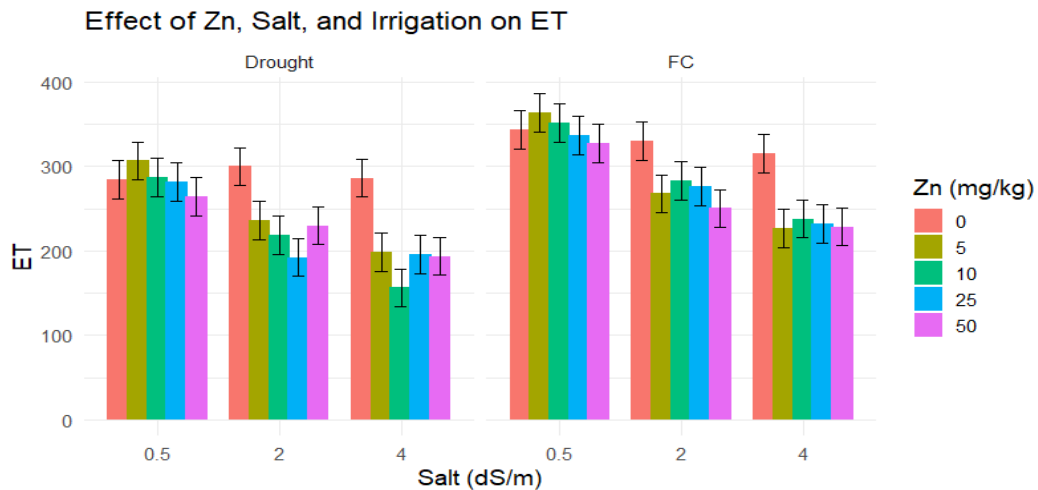


Fig 4.12 Effect of Zn, salt, and irrigation on Soil Evapotranspiration

4.1.13. Water use efficiency

Water use efficiency (WUE), which measures biomass or yield produced per unit of water used, responded strongly to zinc sulfate (ZnSO_4) application rates, salinity levels, and irrigation regime (Fig 4.13). The WUE values ranged approximately from 33 to 61, revealing patterns of optimization and stress response under different environmental and nutrient conditions. Under drought conditions, WUE increased with salinity up to 2 dS/m, particularly at moderate Zn levels (10–25 mg/kg). The highest WUE (~62) was recorded at 4 dS/m with 25 mg/kg Zn, suggesting that zinc at optimal levels can enhance physiological efficiency and stress tolerance in moderately saline-dry conditions. At 4 dS/m, however, WUE declined across all Zn treatments, particularly with no Zn (0 mg/kg) and excess Zn (50 mg/kg), indicating the limits of plant adaptation to combined high salinity and zinc stress. The lowest WUE was seen at 4 dS/m under 0 mg/kg Zn, confirming the critical role of Zn in maintaining water efficiency under harsh conditions. Under field capacity (FC), WUE values were generally lower than under drought, and increased progressively with salinity, but the rate of increase was milder. The highest WUE at FC was still observed at 4 dS/m and 50 mg/kg Zn, values remained in the 40–50 range, suggesting that high moisture may reduce WUE due to increased evapotranspiration and reduced plant stress-induced efficiency. Zn application at 10–25 mg/kg again supported better WUE outcomes, while 0 and 50 mg/kg were associated with poorer performance. Moderate Zn application (especially 25 mg/kg) significantly enhances WUE, especially under moderate salinity and drought. This

may be attributed to improved root function, enzyme activity, and stomatal regulation promoted by zinc. Conversely, excess Zn (50 mg/kg) or no Zn impaired WUE under both irrigation regimes, highlighting the narrow threshold within which Zn is beneficial.

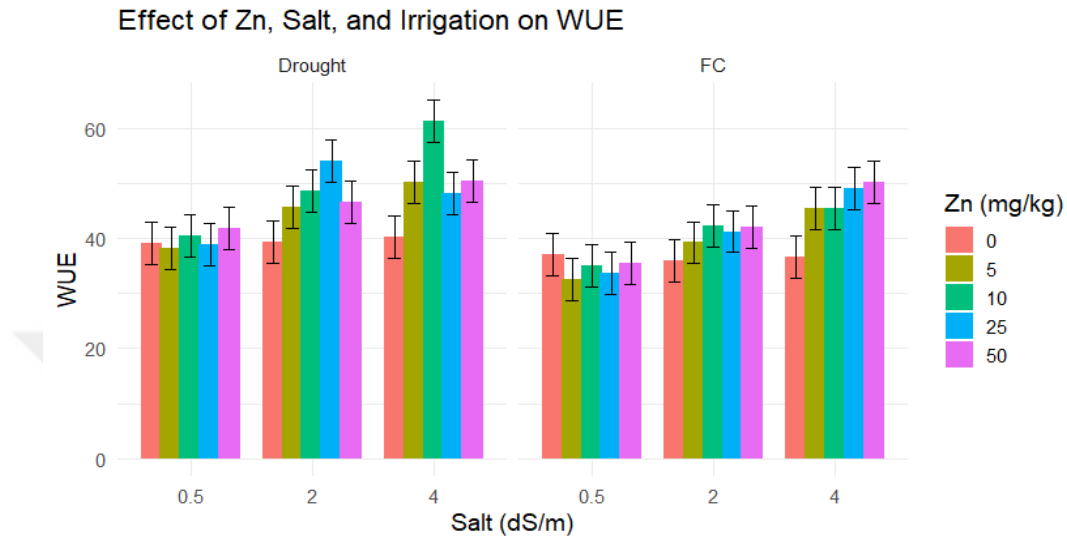


Fig 4.13 Effect of Zn, salt, and irrigation on Soil Water use efficiency

4.1.14. Total chlorophyll

Total chlorophyll content (TChlr) is a critical physiological indicator of photosynthetic capacity and plant health. In this study, TChlr was influenced by the combined effects of zinc sulfate ($ZnSO_4$) application, salinity (0.5, 2, 4 dS/m), and irrigation regime (field capacity and drought)(Fig 4.14). Chlorophyll values ranged from approximately 1.2 to 5.6 units, with distinct trends observed under varying conditions. Under field capacity, TChlr remained relatively stable across all Zn levels and salinity treatments, mostly between 1.9 and 4.0. There was no significant spike in chlorophyll content, although moderate Zn levels (10–25 mg/kg) slightly enhanced TChlr under 0.5–2 dS/m salinity. This suggests that under well-watered conditions, Zn may play a role in maintaining photosynthetic pigment synthesis without causing drastic fluctuations. Under drought conditions, TChlr exhibited a marked peak at 2 dS/m salinity and 5-10 mg/kg Zn, reaching values above 5.0, the highest recorded in the experiment. This indicates that moderate Zn application under moderate salinity stress enhances chlorophyll synthesis, possibly due to Zn's role as a cofactor in chlorophyll biosynthetic enzymes and its protective effect against oxidative damage.

However, beyond 25 mg/kg Zn, TChlr sharply declined, especially at 4 dS/m, showing that excess Zn under drought-saline stress may impair pigment synthesis or lead to chlorosis due to nutrient imbalances or toxicity.

Overall, the results show that:

- Moderate Zn (10–25 mg/kg) enhances chlorophyll content, particularly under mild to moderate salinity and drought stress.
- High Zn rates (50 mg/kg) may negatively affect chlorophyll levels under saline conditions.
- Plants under drought with 2 dS/m salinity are more responsive to Zn for chlorophyll production, likely due to induced stress adaptation mechanisms that require Zn-dependent enzymatic support. El-Desouky et al. (2021) elucidated that the application of zinc considerably enhanced chlorophyll content in sunflower, wheat, rapeseed, and tomato under drought stress.

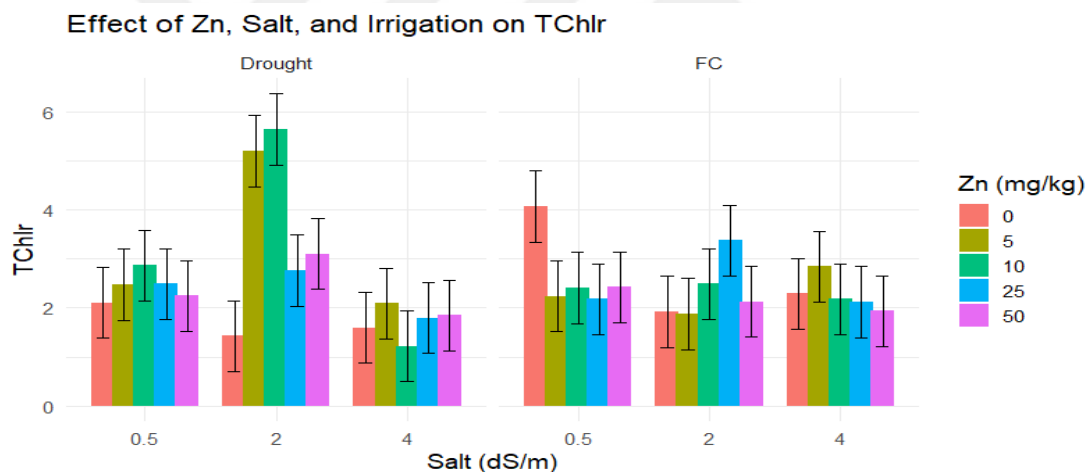


Fig 4.14 Effect of Zn, salt, and irrigation on Total chlorophyll

4.1.15. Soil Zinc

Soil zinc (Zn) availability is a key factor in micronutrient management and was notably influenced by zinc sulfate ($ZnSO_4$) application rate, salinity levels (0.5, 2, and 4 dS/m), and irrigation regime (drought vs. field capacity) (Fig 4.15). The measured soil Zn values ranged from approximately 5 to 30 mg/kg, showing clear trends aligned with Zn treatment levels and environmental conditions. Under drought conditions, soil Zn concentrations increased consistently with Zn application rate. The highest soil Zn (~30 mg/kg) was recorded at 50 mg/kg Zn application, regardless

of salinity level, demonstrating the retention of applied Zn in the soil under low moisture conditions. Notably, at 2 dS/m, moderate Zn rates (10–25 mg/kg) also maintained elevated Zn availability (~15–25 mg/kg), while control and low Zn treatments remained below 10 mg/kg. The drought conditions likely limited Zn leaching, allowing higher retention and slower mobility of Zn ions. Under field capacity (FC) conditions, soil Zn values were generally lower at higher salinity (4 dS/m), especially for the 50 mg/kg Zn treatment. This suggests that increased leaching and ionic competition under wet and saline conditions reduced Zn retention in the soil. Saline soils limit Zn uptake due to a high concentration of Na on exchangeable sites together with other ions, Ca^{2+} , Cd^{2+} , and Cl^- (Rehnam et al, 2018). Nonetheless, the trend of increasing soil Zn with increasing ZnSO_4 application was still evident across all salinity levels. The highest Zn concentration (~28 mg/kg) occurred at 50 mg/kg Zn and 0.5 dS/m, while values at 4 dS/m declined to around 20 mg/kg or less, even for the same Zn rate.

Overall, the results confirm that:

- Soil Zn increases proportionally with ZnSO_4 application, regardless of salinity or moisture.
- Salinity and irrigation regime influence Zn mobility and retention, with more Zn retained under drought and lower salinity, and greater Zn losses under field capacity at higher salinity.
- Moderate Zn doses (10–25 mg/kg) provided a good balance of availability and efficiency, especially under stress conditions.

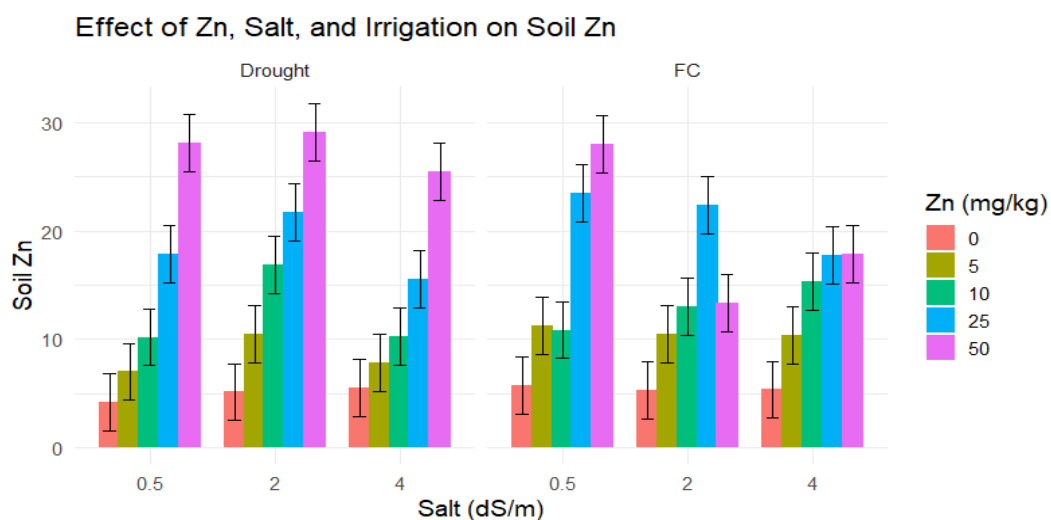


Fig 4.15 Effect of Zn, salt, and irrigation on Soil Zinc

4.1.16. Plant Zinc Concentration

The zinc (Zn) content in plant tissues indicates zinc uptake efficiency and biofortification potential. The plant Zn content was significantly influenced by the interaction of zinc sulfate (ZnSO_4) application rate, irrigation regime (drought vs. field capacity), and salinity (0.5, 2, and 4 dS/m) (Fig 4.16). Plant Zn concentrations ranged from approximately 6 to 27 mg/kg, depending on the treatment combinations. Under drought conditions, plant Zn content increased steadily with ZnSO_4 application rate across all salinity levels. The highest plant Zn (~27 mg/kg) was observed at 10 mg/kg Zn application and 4 dS/m salinity, suggesting enhanced Zn uptake under moderate salinity stress. At 0.5 and 4 dS/m, plant Zn was also elevated with increasing Zn application, but slightly lower than at 2 dS/m. This pattern indicates that moderate salinity under drought may stimulate Zn uptake by affecting root permeability or enhancing Zn solubility. Under field capacity, a similar trend was observed, but with slightly lower overall Zn uptake compared to drought. Plant Zn content increased with Zn application rate, though the effect was more gradual. At higher salinity (4 dS/m), Zn content in plants peaked with 50 mg/kg Zn application, indicating that even under wet and saline conditions, plants can accumulate Zn when sufficiently supplied. However, the uptake efficiency appeared somewhat reduced at 0 and 5 mg/kg Zn levels, especially under low salinity.

Overall, the results show that:

- Plant Zn uptake increases with Zn application, and is enhanced under moderate salinity (2 dS/m).
- Drought conditions favor greater Zn accumulation in plant tissues, likely due to reduced leaching and more concentrated Zn in the root zone.
- The most effective Zn enrichment occurred with 25–50 mg/kg Zn application, highlighting the potential for soil Zn application to improve Zn biofortification in wheat, particularly under mild salt and moisture stress.

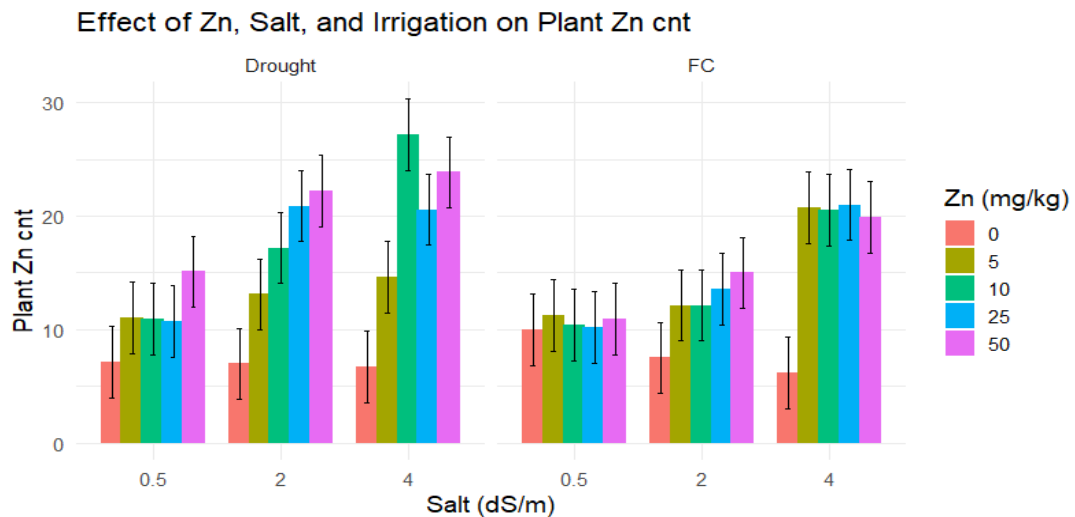


Fig 4.16 Effect of Zn, salt, and irrigation on Plant Zn Concentration

4.1.17. Plant Zinc Uptake

Plant zinc (Zn) uptake reflects the Zn uptake efficiency and its translocation to aerial plant tissues. It was influenced significantly by ZnSO₄ application rate, salinity levels, and irrigation regimes (Fig 4.17). Plant Zn uptake ranged approximately from 0.07 to 0.25 units (likely mg/g or %), varying across treatments. Under drought conditions, plant Zn concentration increased with Zn application, peaking at 2 dS/m salinity and 25–50 mg/kg Zn. The highest concentration (~0.25) was recorded under these conditions, suggesting enhanced Zn bioavailability and uptake due to increased root-to-soil contact and possible salt-induced Zn mobilization. Conversely, the lowest plant Zn concentrations (~0.07–0.10) were observed in control and 5 mg/kg Zn treatments, particularly at 0.5 dS/m salinity. This trend confirms that Zn application rate and moderate salinity promote higher tissue Zn accumulation under water-limited conditions. Plants become more sensitive to Zn deficiency under low water availability (Bageci et al. 2007). Under a limited water supply, Zn movement in the soil is limited, restricting plants' Zn uptake (Marschner 2012). Under field capacity (FC), plant Zn concentrations also increased with Zn application, but with a more gradual trend. The highest values (~0.20–0.23) were seen at 4 dS/m with 25–50 mg/kg Zn. This indicates that even under well-watered conditions, Zn bioavailability and uptake improve at higher Zn application rates and elevated salinity. However, at 0.5 dS/m and low Zn rates, Zn concentrations remained low (~0.08–0.12), consistent with limited Zn availability in

low-salt, low-Zn environments. In drought-stressed plants, the effect of irrigation on grain yield is maximized with adequate Zn fertilization (Amirazad 2010)

Key Takeaways

- Zn application is the primary driver of Zn concentration in plant tissues.
- Moderate salinity (2 dS/m) enhances Zn uptake, especially under drought, possibly due to salt-induced Zn desorption from soil particles.
- 25–50 mg/kg Zn yielded the highest Zn concentrations in plants, under both irrigation regimes.
- Field capacity supports Zn uptake, but Zn accumulation was more efficient under drought, likely due to reduced leaching and higher root-zone Zn concentration.

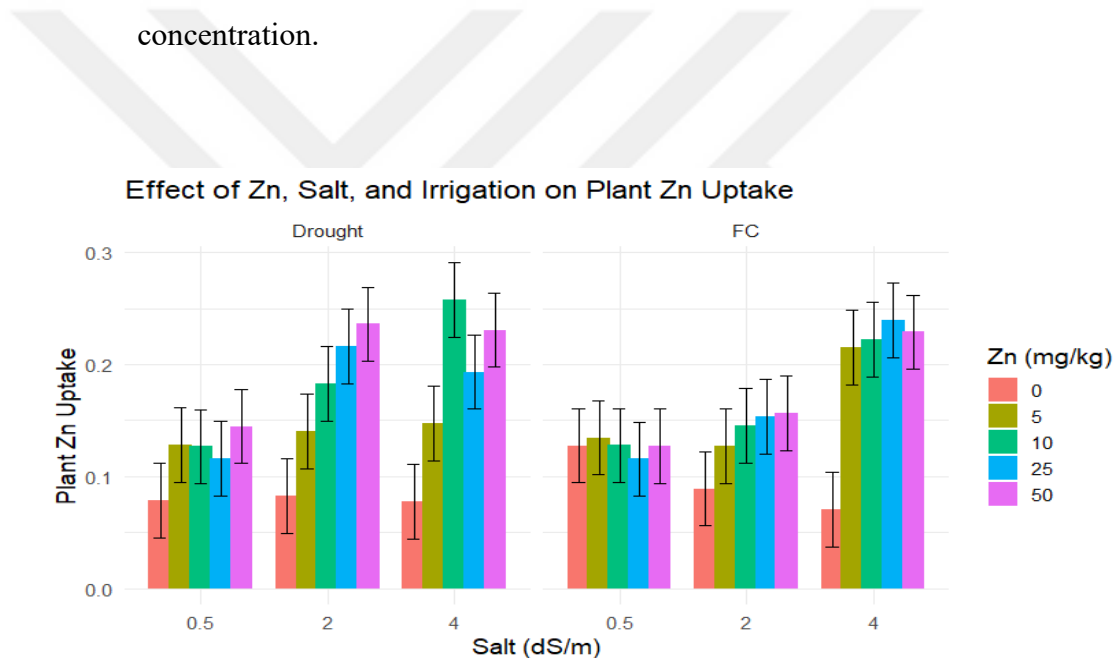


Fig 4.17 Effect of Zn, salt, and irrigation on Plant Zinc uptake

4.2. Correlation of soil properties, variables, and management practices

The Pearson correlation matrix offers crucial insights into the relationships between plant zinc concentration (PZNcont) and various soil, plant, and environmental parameters (Table 4.2). Among the most influential variables were electrical conductivity (EC), sodium (Na), evapotranspiration (ET), water use efficiency (WUE), and soil Zn. PZNcont exhibited a strong positive correlation with EC ($r = 0.613$, $p < 0.01$) and Na ($r = 0.615$, $p < 0.01$), indicating that increased soil salinity enhances Zn uptake. This is likely due to ionic competition in saline soils, which promotes Zn desorption and mobilization into the soil solution (Ali et al., 2020). Similarly, the positive association with WUE ($r = 0.741$, $p < 0.01$) supports findings that efficient water use enhances nutrient uptake efficiency, particularly under moderate salinity stress (Waraich et al., 2011). Conversely, pH negatively correlated with PZNcont ($r = -0.510$, $p < 0.01$), aligning with known mechanisms where higher pH reduces Zn solubility, thereby limiting plant availability (Faezeh et al., 2024). ET also showed a strong negative correlation ($r = -0.748$, $p < 0.01$), likely due to excessive water loss and reduced translocation efficiency of Zn under high evapotranspiration rates (Zhao et al., 2022). Soil Zn demonstrated a significant positive correlation with PZNcont ($r = 0.440$, $p < 0.01$), supporting its direct role in enhancing shoot Zn content. These findings are consistent with past research highlighting soil Zn availability as a primary determinant of plant Zn status (Alloway, 2009). In addition, calcium (Ca) also had a moderate positive correlation ($r = 0.388$, $p < 0.01$), which may indicate interactions that support root membrane stability and cation uptake (Nataly et al., 2021). Negative correlations with plant dry weight (PdryW, $r = -0.561$) and subsurface CO₂ (SBCO₂, $r = -0.457$) suggest possible Zn dilution effects in higher biomass conditions or inhibitory interactions with soil microbial respiration (Liu et al., 2020). Zn uptake is significantly modulated by soil salinity, pH, water efficiency, and total Zn reserves. These interactions highlight the importance of balanced soil-water management in Zn biofortification efforts under saline or semi-arid conditions.

Table 4.2. Correlation matrix

| | PZNcont | EC | pH | OM | P | K | Ca | Mg | Na | ET | RWC | SBCO2 | WUE | PdryW | soilZn |
|----------|---------|---------|---------|--------|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Znuptake | ,973** | ,599** | -,471** | ,224* | ,084 | -,084 | ,331** | -,107 | ,610** | -,671** | -,121 | -,469** | ,715** | -,393** | ,432** |
| PZNcont | 1 | ,613** | -,510** | ,218* | ,040 | -,085 | ,388** | -,162 | ,615** | -,748** | -,073 | -,457** | ,741** | -,561** | ,440** |
| EC | ,613** | 1 | -,888** | ,358** | ,156 | -,269* | ,626** | -,294** | ,974** | -,694** | ,122 | -,421** | ,623** | -,475** | ,095 |
| pH | -,510** | -,888** | 1 | -,169 | -,046 | ,432** | -,596** | ,306** | -,811** | ,712** | -,154 | ,276** | -,666** | ,461** | -,017 |
| OM | ,218* | ,358** | -,169 | 1 | -,022 | ,140 | ,420** | -,238* | ,446** | -,194 | ,117 | -,048 | ,185 | -,121 | -,094 |
| P | ,040 | ,156 | -,046 | -,022 | 1 | ,267* | -,173 | ,255* | ,157 | ,118 | -,191 | -,183 | -,126 | ,125 | ,252* |
| K | -,085 | -,269* | ,432** | ,140 | ,267* | 1 | -,304** | ,280** | -,169 | ,322** | -,120 | ,091 | -,347** | ,085 | ,423** |
| Ca | ,388** | ,626** | -,596** | ,420** | -,173 | -,304** | 1 | -,836** | ,624** | -,547** | ,191 | -,168 | ,502** | -,435** | -,219* |
| Mg | -,162 | -,294** | ,306** | -,238* | ,255* | ,280** | -,836** | 1 | -,284** | ,295** | -,286** | ,033 | -,276** | ,275** | ,355** |
| Na | ,615** | ,974** | -,811** | ,446** | ,157 | -,169 | ,624** | -,284** | 1 | -,672** | ,102 | -,426** | ,599** | -,457** | ,118 |
| ET | -,748** | -,694** | ,712** | -,194 | ,118 | ,322** | -,547** | ,295** | -,672** | 1 | -,032 | ,400** | -,923** | ,765** | -,233* |
| RWC | -,073 | ,122 | -,154 | ,117 | -,191 | -,120 | ,191 | -,286** | ,102 | -,032 | 1 | ,186 | -,057 | -,126 | -,249* |
| SBCO2 | -,457** | -,421** | ,276** | -,048 | -,183 | ,091 | -,168 | ,033 | -,426** | ,400** | ,186 | 1 | -,319** | ,273** | -,283** |
| WUE | ,741** | ,623** | -,666** | ,185 | -,126 | -,347** | ,502** | -,276** | ,599** | -,923** | -,057 | -,319** | 1 | -,513** | ,184 |
| PdryW | -,561** | -,475** | ,461** | -,121 | ,125 | ,085 | -,435** | ,275** | -,457** | ,765** | -,126 | ,273** | -,513** | 1 | -,238* |
| soilZn | ,440** | ,095 | -,017 | -,094 | ,252* | ,423** | -,219* | ,355** | ,118 | -,233* | -,249* | -,283** | ,184 | -,238* | 1 |
| TChlr | -,003 | -,070 | ,010 | -,071 | -,043 | -,179 | -,204 | ,162 | -,076 | -,005 | -,087 | ,033 | ,014 | ,111 | ,074 |

4.3. Model Development

To evaluate the predictive strength, two models were generated (Table 4.3 to Table 4.8), comparing the measured plant Zn content (ppm) with the estimated values. Each model incorporated a combination of soil, salinity, irrigation, and nutrient input variables relevant to Zn biofortification.

Table 4.3: Model 1 summary

| Model | R | R Square | Adjusted Square | R Std. Error of the Estimate |
|-------|--------------------|----------|-----------------|------------------------------|
| 1 | 0,676 ^a | 0,457 | 0,438 | 4.57728 |

a. Predictors: (Constant), ZnL, SL, IRL

Table 4.4: ANOVA^b

| Model | | Sum of Squares | Df | Mean Square | F | Sig. |
|-------|------------|----------------|----|-------------|--------|--------------------|
| 1 | Regression | 1514,322 | 3 | 504,774 | 24,093 | 0,000 ^a |
| | Residual | 1801,829 | 86 | 20,951 | | |
| | Total | 3316,151 | 89 | | | |

a. Predictors: (Constant), ZnL, SL, IRL b. Dependent Variable: PZNcont

Table 4.5: Coefficients^a

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|-------|------------|-----------------------------|------------|---------------------------|--------|-------|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 8,995 | 1,415 | | 6,358 | 0,000 |
| | IRL | -0,027 | 0,015 | -0,147 | -1,849 | 0,068 |
| | SL | 2,097 | 0,337 | 0,495 | 6,231 | 0,000 |
| | ZnL | 0,147 | 0,027 | 0,436 | 5,480 | 0,000 |

a. Dependent Variable: PZNcont

$$\text{Zncont} = 8,995 - 0,027 * \text{IRL} + 2,097 * \text{SL} + 0,147 * \text{ZnL}$$

4.3.1. Model 1: Effect of Zn Level, Salinity, and Irrigation Regime on PZNcont

The first regression model (Table 4.3) examined the effect of Zn application level (ZnL), salinity level (SL), and irrigation regime level (IRL) on plant zinc content (PZNcont). The model exhibited a moderate-to-strong correlation ($R = 0.676$) and explained approximately 45.7% of the variance in PZNcont ($R^2 = 0.457$, Adjusted $R^2 = 0.438$).

From the coefficients (Table 4.5):

- ZnL significantly and positively influenced Zn content ($\beta = 0.147$, $p < 0.001$), suggesting that increased Zn fertilization enhances Zn uptake by wheat.
- SL also had a strong positive effect ($\beta = 2.097$, $p < 0.001$), indicating that moderate salinity may promote Zn desorption from soil particles and enhance mobility.

- IRL showed a negative and marginally significant effect ($\beta = -0.027$, $p = 0.068$), suggesting irrigating excessively may reduce Zn availability due to leaching or dilution effects.

These findings align with previous studies, which report that salinity can enhance Zn availability by displacing Zn from soil colloids and increasing its presence in soil solution (Ali et al., 2020). However, excessive irrigation may suppress uptake by altering the rhizosphere redox environment or triggering leaching losses (Guochun et al., 2024).

Table 4.6: Model 2 Summary

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|-------|--------------------|----------|-------------------|----------------------------|
| 1 | 0,613 ^a | 0,375 | 0,368 | 4,85145 |
| 2 | 0,723 ^b | 0,522 | 0,511 | 4,26768 |
| 3 | 0,750 ^c | 0,563 | 0,547 | 4,10696 |

a. Predictors: (Constant), EC b. Predictors: (Constant), EC, soilZn c. Predictors: (Constant), EC, soilZn, IRL

Table 4.7: ANOVA^d

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|-------|------------|----------------|----|-------------|--------|--------------------|
| 1 | Regression | 1244,935 | 1 | 1244,935 | 52,894 | 0,000 ^a |
| | Residual | 2071,216 | 88 | 23,537 | | |
| | Total | 3316,151 | 89 | | | |
| 2 | Regression | 1731,611 | 2 | 865,805 | 47,537 | 0,000 ^b |
| | Residual | 1584,540 | 87 | 18,213 | | |
| | Total | 3316,151 | 89 | | | |
| 3 | Regression | 1865,581 | 3 | 621,860 | 36,868 | 0,000 ^c |
| | Residual | 1450,570 | 86 | 16,867 | | |
| | Total | 3316,151 | 89 | | | |

a. Predictors: (Constant), EC b. Predictors: (Constant), EC, soilZn

c. Predictors: (Constant), EC, soilZn, IRL d. Dependent Variable: PZNcont

Table 4.8: Coefficients^a

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|-------|------------|-----------------------------|------------|---------------------------|--------|-------|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 9,039 | 0,889 | | 10,168 | 0,000 |
| | EC | 0,002 | 0,000 | 0,613 | 7,273 | 0,000 |
| 2 | (Constant) | 5,050 | 1,099 | | 4,597 | 0,000 |
| | EC | 0,001 | 00,000 | 0,576 | 7,737 | 0,000 |
| | soilZn | 0,303 | 0,059 | 0,385 | 5,169 | 0,000 |
| 3 | (Constant) | 7,481 | 1,364 | | 5,483 | 0,000 |
| | EC | 0,001 | 0,000 | 0,598 | 8,298 | 0,000 |
| | soilZn | 0,298 | 0,057 | 0,378 | 5,280 | 0,000 |
| | IRL | -0,038 | 0,013 | -0,202 | -2,818 | 0,006 |

a. Dependent Variable: PZNcont

$$\text{Zn_cont} = 7,481 + 0,0017 * \text{EC} + 0,298 - 0,038 * \text{IRL}$$

4.3.2. Model 2: EC, Soil Zn, and Irrigation Regime as Predictors of PZNcont

The second regression set included a stepwise model incorporating EC, soil Zn, and IRL. The models were (Table 4.6):

- Model 1 (EC only): $R^2 = 0.375$
- Model 2 (EC + soil Zn): $R^2 = 0.522$
- Model 3 (EC + soil Zn + IRL): $R^2 = 0.563$

The final model had the highest explanatory power (Adjusted $R^2 = 0.547$) with strong statistical significance ($F = 36.868$, $p < 0.001$). The regression equation was:

$$\text{PZNcont} = 7.481 + 0.0017 \times \text{EC} + 0.298 \times \text{SoilZn} - 0.038 \times \text{IRL}$$

EC emerged as the strongest predictor (Table 4.8) ($\beta = 0.598$, $p < 0.001$), supporting its critical role in solubilizing Zn ions. Soil Zn also showed a significant positive effect ($\beta = 0.378$, $p < 0.001$), indicating that total Zn reserves still contribute meaningfully to shoot Zn levels. IRL again displayed a significant negative effect ($\beta = -0.202$, $p = 0.006$), reinforcing the need for optimized irrigation.

These results are supported by research demonstrating that higher EC enhances Zn availability through ionic competition and increased diffusion rates

(Qian et al., 2025). Similarly, maintaining optimal soil Zn is crucial for ensuring sufficient translocation to edible plant parts (Alloway, 2009; Cakmak, 2008).

4.3.3. Interpretation Across Models

Across both models:

- The strongest positive influences on PZNcont were EC, SL, ZnL, and soil Zn.
- The negative effect of IRL across models suggests over-irrigation may hinder Zn uptake, possibly due to reduced root oxygenation or Zn dilution.
- Salinity and EC, often considered stress factors, enhance Zn uptake within moderate ranges, highlighting the importance of controlled saline environments in biofortification (Yamshi et al., 2020).

This evidence supports a multi-factorial approach to Zn biofortification, considering soil chemistry and water management strategies. Soil testing and salinity monitoring should be integral to Zn management practices in wheat production, particularly in semi-arid regions.

4.3.4. The rotated Principal Component Analysis (PCA) plot

It visually summarizes the relationships among soil properties, treatment levels, and Zn content in wheat (PZNcont) (Fig 4.18). Two main components were extracted, accounting for most of the variance in the dataset. Variables that cluster closely together are positively correlated, while those on opposite sides of the plot are inversely related.

Component 1 is heavily influenced by salinity level (SL), Ca, EC, and organic matter (OM). These variables cluster toward the positive side of Component 1, indicating that salinity and soil chemical enrichment play a dominant role in shaping the plant's physiological environment. This aligns with findings that high EC and Ca can increase Zn bioavailability by displacing Zn from soil particles (Faezeh et al 2024)

Component 2 is influenced strongly by soil Zn, Zn level (ZnL), and PZNcont, all located in the upper-right quadrant, suggesting a strong mutual relationship. Their close grouping suggests that soil Zn concentration and external Zn application directly enhance Zn accumulation in wheat shoots. This supports previous reports where Zn fertilization significantly boosted tissue Zn levels (Alloway, 2009).

pH and PdryW (plant dry weight) are on the opposite end of this axis, implying a negative relationship with Zn uptake. Higher pH values reduce Zn solubility, while increased biomass may dilute Zn concentration in plant tissue (Natasha et al., 2022). IRL (irrigation regime) is near the origin, indicating a relatively weaker but still relevant influence across both components. The moderate proximity to Zn-related variables suggests that water availability may mediate Zn uptake indirectly through soil solution dynamics (Zhao et al., 2022). Interestingly, Mg and K are moderately loaded along Component 2, suggesting they are more associated with plant nutrient balance than direct Zn uptake. This agrees with results showing antagonistic or synergistic interactions between Zn and other cations (Rengel, 2015). OM is slightly negatively associated with PZNcont. This could be due to the high binding capacity of organic matter, which might immobilize Zn in certain soil types.

The Principal Component Analysis (PCA) with Varimax rotation identified two principal components that summarize the interrelationships among soil properties, treatment factors, and wheat zinc concentration (PZNcont). These components capture underlying patterns in the dataset and help reduce dimensionality while retaining meaningful variance.

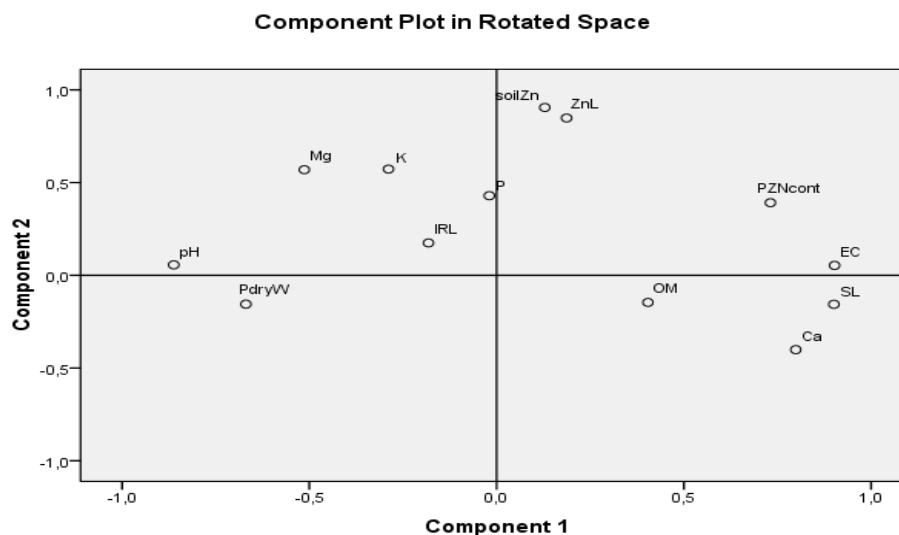


Fig 4.18: Principal component analysis plot

4.3.4.1. Component 1: Soil Salinity and Physicochemical Influence

Variables with strong positive loadings on Component 1 (Table 4.9) include electrical conductivity (EC, 0.200), Ca (0.165), Salt Level (0.194), and PZNcont (0.172). This component reflects the influence of salinity-related soil properties on Zn uptake. High EC and salt levels are typically associated with increased Zn desorption due to ionic competition, which enhances Zn availability in the soil solution (Ali et al., 2020). The moderate loading of Zn level (ZnL, 0.065) further suggests that applied Zn interacts synergistically with salinity to enhance PZNcont. Conversely, pH (-0.188) and plant dry weight (PdryW, -0.152) had negative loadings, indicating that alkaline conditions and high biomass may reduce Zn concentration in tissues due to lower solubility or dilution effects (Faezeh et al., 2024).

4.3.4.2. Component 2: Nutrient Composition and Fertilization Influence

Component 2 (Table 4.9) is characterized by high loadings for soil Zn (0.330), ZnL (0.312), Mg (0.191), K (0.199), and P (0.154). This suggests that nutrient-rich soils and Zn fertilization directly affect the availability and uptake of Zn by wheat. The relatively high loading of PZNcont (0.162) in this component supports this interpretation, emphasizing that nutrient availability, especially Zn and synergistic cations, governs Zn accumulation in plant tissues (Cakmak, 2008). Interestingly, organic matter (OM) and pH had negligible contributions to Component 2, suggesting their effects are aligned with the ionic dynamics captured in Component 1. Irrigation regime level (IRL) also showed weak loadings in both components, indicating a less direct but potentially interacting influence on Zn uptake.

Table 4.9: Component Score Coefficient Matrix

| | Component | |
|---------|-------------|-------------|
| | 1 | 2 |
| EC | ,200 | ,044 |
| pH | -,188 | -,004 |
| OM | ,085 | -,042 |
| P | ,008 | ,154 |
| K | -,048 | ,199 |
| Ca | ,165 | -,123 |
| Mg | -,097 | ,191 |
| PdryW | -,152 | -,075 |
| soilZn | ,054 | ,330 |
| IRL | -,035 | ,058 |
| SaltL | ,194 | -,031 |
| ZnL | ,065 | ,312 |
| PZNcont | ,172 | ,162 |

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

4.3.5. Model Visualization and Comparison

The scatter plot (Fig 4.19) illustrates the relationship between measured plant Zn content and estimated Zn content using Model 1, which integrates predictors such as Zn level (ZnL), salinity level (SL), and irrigation regime level (IRL). The regression line in the plot is given by:

$$y = 0.4659x + 7.7525 \text{ with } r = 0.676^{**}$$

This equation reflects a moderately strong positive correlation ($r = 0.676$, $p < 0.01$), indicating that the model performs well in predicting plant Zn content from the chosen environmental and management variables. The positive slope signifies that an increment in measured Zn content leads to a corresponding increment in model estimates, although the model slightly underestimates Zn values at higher concentrations.

The coefficient of determination $r^2 = 0.457$ (from previous regression results) indicates that approximately 46% of the variability in measured Zn content is explained. This level of prediction accuracy is acceptable in agronomic modeling,

especially when dealing with complex field interactions like salinity, water dynamics, and nutrient uptake (Alloway, 2009; Cakmak, 2008).

The intercept (7.7525) suggests a baseline Zn content likely driven by native soil Zn levels, even without additional Zn input. The result aligns with various wheat genotypes, particularly those grown in Zn-moderate soils

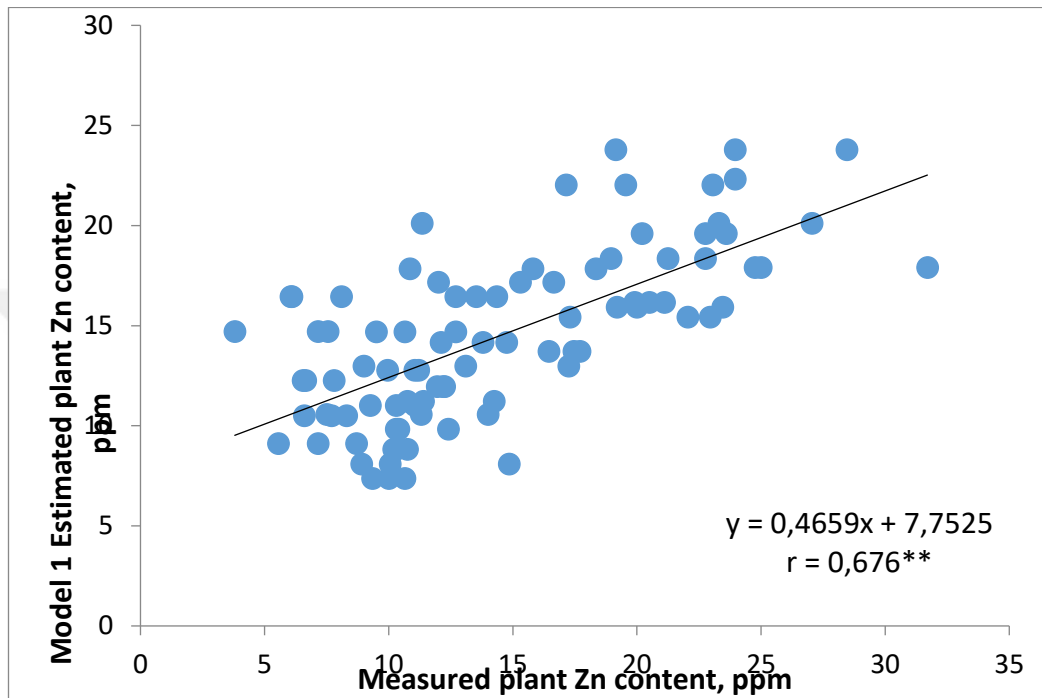


Figure 4.19: Model 1 visualisation

Despite the model's performance, the spread around the regression line suggests some unexplained variability, likely due to additional factors not included in the model, such as:

- Soil organic matter-Zn binding,
- pH-induced Zn immobilization, or
- plant genotype-specific Zn efficiency

Furthermore, data points clustering at lower measured Zn levels suggest possible Zn deficiency in a plot portion, underscoring the need for improved Zn fertilization practices. The scatter plot also serves as a validation tool, showing how modeled results align with observed data. The moderate correlation observed here is comparable with other plant modeling studies where multivariate regression has been used to estimate micronutrient uptake (Shabani, 2016).

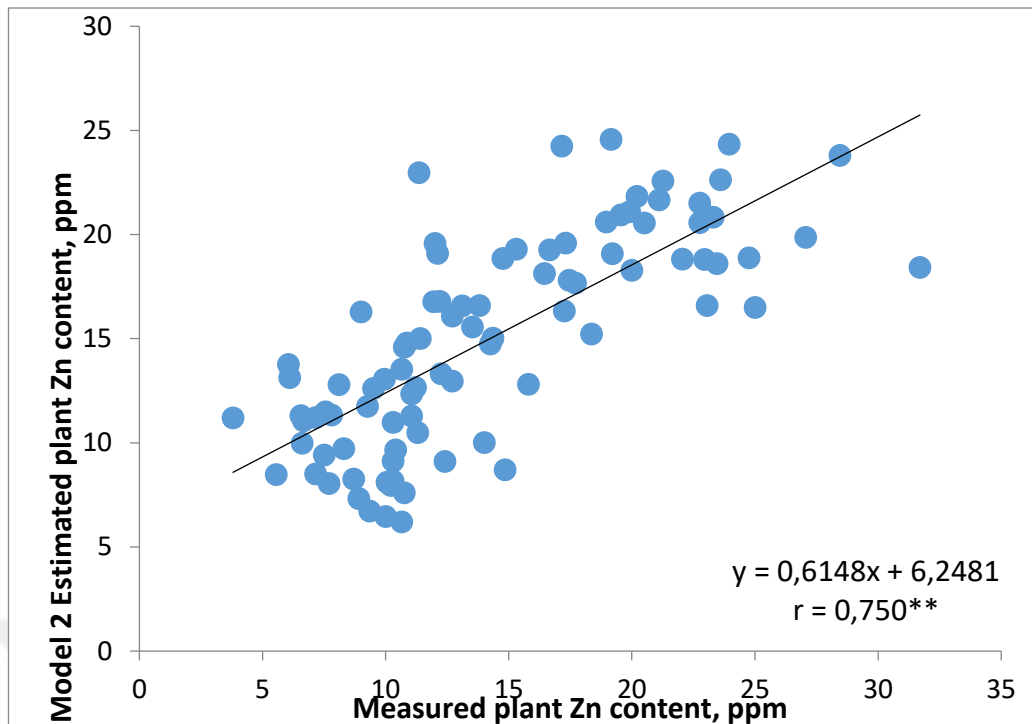


Fig 4.20: Model 2 visualisation

The scatter plot (Fig 4.20) presents the relationship between measured plant Zn content (ppm) and Model 2 estimated Zn content. The regression equation derived from the graph is:

$$y = 0.6148x + 6.2481 \text{ with } r = 0.750^{**}$$

This equation demonstrates a strong positive linear correlation ($r = 0.750$, $p < 0.01$) between measured and predicted values, indicating that Model 2 has improved prediction accuracy over Model 1 ($r = 0.676$). The coefficient of determination ($r^2 = 0.563$) suggests that approximately 56.3% of the variability in plant Zn content can be explained by this model. This enhancement in predictive performance is likely due to the inclusion of EC (Electrical Conductivity), Soil Zn, and Irrigation Regime Level (IRL)—variables that influence Zn availability and uptake. EC and soil Zn, in particular, play great roles in controlling Zn solubility and root accessibility.

The slope of the regression line (0.6148) indicates that for every 1 ppm increase in measured Zn content, the model predicts a 0.61 ppm increase, suggesting a slight underestimation by the model at higher values. However, the relatively small intercept (6.2481) confirms a consistent baseline prediction likely due to residual soil Zn levels and background availability (Alloway, 2009). Compared to Model 1, the tighter clustering of data points around the regression line and higher correlation coefficient indicate better model robustness and reliability. This suggests that

integrating EC and soil Zn into predictive models enhances Zn bioavailability forecasting, especially under varied irrigation conditions.

Moreover, high EC may promote Zn uptake by reducing Zn adsorption to soil particles, enhancing Zn presence in the soil solution (Qian et al., 2024). Similarly, a moderate reduction in IRL contributes positively by preventing Zn leaching and promoting better Zn root zone concentration (Rehman et al., 2012).

Overall, Model 2 reflects an improved empirical framework for estimating plant Zn uptake, emphasizing the importance of including environmental interactions in biofortification modeling strategies.

4.3.6. Comparison of Model 1 and Model 2 Performance in Predicting Plant Zn Content

Model 1 Performance

The regression equation from Model 1 was:

$$\text{Estimated Zn (ppm)} = 0.4659 \times \text{Measured Zn} + 7.7525, \quad r = 0.676^{**}$$

Model 1 included Zn level (ZnL), salinity level (SL), and irrigation regime level (IRL) as predictors. The correlation coefficient ($r = 0.676$) indicates a moderate-to-strong relationship between measured and predicted Zn values. However, the spread of points around the regression line and a slightly lower slope reflect a model that underestimates high Zn values and captures only ~45.7% of the total variation ($R^2 = 0.457$). This result suggests that while Zn application and salinity positively influence Zn uptake, excluding variables like soil Zn or EC limits the model's ability to account for Zn mobility and plant availability across variable soil environments (Alloway, 2009).

Model 2 Performance

The second regression model expanded the predictor set to include electrical conductivity (EC), soil Zn, and IRL, yielding the regression equation:

$$\text{Estimated Zn (ppm)} = 0.6148 \times \text{Measured Zn} + 6.2481, \quad r = 0.750^{**}$$

Model 2 showed a stronger linear relationship with a higher correlation ($r = 0.750$) and improved explanatory power ($R^2 = 0.563$). 56.3% of the measured Zn content variability was predicted. The improved performance is evident in the tighter clustering of data points around the regression line and a slope closer to 1, suggesting better accuracy across all concentration levels. Including EC and soil Zn significantly

improved model accuracy, as both are critical factors influencing Zn desorption, solubility, and uptake efficiency under different salinity and moisture conditions.

Table 4.10: Comparative interpretation

| Metric | Model 1 | Model 2 |
|-----------------------------------|-------------------------------------|----------------------------|
| Predictors | ZnL, SL, IRL | EC, Soil Zn, IRL |
| Regression Slope | 0.4659 | 0.6148 |
| Intercept | 7.7525 | 6.2481 |
| Correlation (r) | 0.676** | 0.750** |
| Coefficient of Determination (R2) | 0.457 | 0.563 |
| Visual Fit | More scattered, underpredicts highs | Tighter, more accurate fit |

Model 2 outperformed Model 1 in all statistical metrics (Table 4.10), making it a more robust and reliable tool for estimating Zn uptake in winter wheat. The results affirm that including soil chemical properties (like EC and total soil Zn) significantly improves prediction accuracy by capturing key mechanisms of Zn bioavailability. This model comparison also reinforces the importance of integrating environmental and management factors into Zn biofortification modeling, particularly in salinity-affected or nutrient-depleted soils typical of semi-arid wheat-growing regions (Rehman et al., 2012)

5. CONCLUSION

Soil Zn application is an effective strategy for correcting Zn deficiency and improving crop productivity and nutritional quality. The choice of Zn source, application method, and integration with organic inputs must be tailored to soil type and crop demand. With rising concerns about micronutrient malnutrition and soil degradation, agronomic Zn management will continue to play a vital role in sustainable agriculture and human health. Zn application modifies the dynamics of both Na and K in soil by influencing ion exchange processes, plant physiological responses, and microbial-mediated nutrient cycling. These interactions are critical in managing saline soils and enhancing plant nutrient efficiency in Zn-deficient regions. Zinc fertilization influences soil pH and EC depending on soil properties, fertilizer form, and environmental conditions. While minor acidification can enhance micronutrient availability, increases in EC should be carefully managed to prevent salt accumulation, particularly in sensitive or poorly drained soils. These findings highlight the need for site-specific Zn management strategies while considering the interactive effects on soil chemical properties. The effect of Zn application on SOM is context-dependent. In low-Zn/degraded soils, moderate Zn application may enhance microbial activity and carbon cycling. However, excessive or repeated Zn inputs, especially in contaminated areas or through industrial sources, can reduce SOM decomposition and biological functions. Hence, Zn application should be managed based on soil type, initial organic matter levels, and environmental risks. Integrating Zn fertilization with organic inputs and monitoring microbial indicators (e.g., basal respiration, enzymatic activity) can optimize SOM preservation and improve soil health. Zinc application significantly improves chlorophyll content in wheat by enhancing enzymatic functions, stabilizing chloroplast structure, and protecting against oxidative stress. The method, form, and timing of Zn application influence the extent of chlorophyll enhancement. Adequate Zn nutrition, especially in deficient soils, is thus essential for optimizing photosynthesis and improving wheat productivity.

Zn uptake is significantly modulated by soil salinity, pH, water efficiency, and total Zn reserves. These interactions highlight the importance of balanced soil-water management in Zn biofortification efforts under saline or semi-arid conditions. This study reveals that Zn application rate, salinity, electrical conductivity, and soil Zn

levels significantly influence Zn accumulation in wheat. Conversely, over-irrigation appears detrimental. Optimizing these factors can improve Zn biofortification strategies and enhance the nutritional quality of wheat grains. The PCA differentiates two major dimensions influencing Zn uptake in wheat: one related to soil chemical and salinity conditions (Component 1), and the other to Zn-specific variables such as soil Zn and Zn fertilization (Component 2). These insights support the need for integrated soil management practices that optimize Zn availability, especially under varying salinity and irrigation conditions. Model 1 offers a reliable estimation of wheat Zn content, accounting for nearly half of the variation in measured values. The significant correlation ($r = 0.676^{**}$) suggests that Zn fertilization, salinity, and irrigation are key levers in managing Zn uptake. However, further model refinement with variables like soil pH, organic matter, and genotype may enhance predictive accuracy. Model 2 provides a strong predictive capacity for wheat Zn content, with over 56% of the variance explained. The high correlation and improved accuracy underscore the importance of incorporating EC, soil Zn, and irrigation practices into predictive models to optimize Zn nutrition under field conditions. The comparative analysis reveals that Model 2, with EC and soil Zn, provides superior prediction of plant Zn content compared to the simpler Model 1. These findings underscore the necessity of multi-variable models in biofortification research and provide a foundation for precision Zn management in winter wheat cultivation.

Zn biofortification through agronomic methods is a practical, immediate, and scalable solution to increase wheat grain Zn concentration and reduce hidden hunger. Context-specific Zn management strategies adoption, considering soil properties, crop stage, and genotype, can significantly improve crop productivity and human health outcomes. Continued research on cost-effective and farmer-friendly Zn delivery systems will be key to its global success.

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Publications :

1. Chukwu, E. C., and Coskun, G. (2025). Morphological, physiological, and anatomical effects of heavy metals on soil and plant health and possible remediation technologies. *Soil Security*, 100178.

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2. Chukwu, E. C., and Coskun, G. (2024). Effects of Zn Application to Wheat under Drought Stress Condition- A Review. International Symposium on Soil Science and Plant Nutrition. International Symposium on Soil Science and Plant Nutrition, 10th International Scientific Meeting, pp 60-67.

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3. Chukwu, E. C., Okorie, B. O., and Azuka, C. V. (2024). Effects of different levels of spent engine oil on soil physicochemical properties using different texturally contrasting soils. *Journal of Agricultural Science and Practice*, 9(5), 147-156. <http://dx.doi.org/10.21203/rs.3.rs-2231711/v1>

4. Okorie, B. O., Chukwu, E., Ezeifeke, O. A., Ukwuaba, K. U., Asadu, C. L. A., Umeugokwe, C. P., Ayogu, C. J. and Ezeaku, V. I. (2022). Topsoil's nutrient dynamics of compound farms in upper and lower slopes of University of Nigeria, Nsukka. International Symposium on Soil Science and Plant Nutrition, 7th International Scientific Meeting, 16-23.

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5. Chukwu E. C., and Azuka C. V (2022). Phytoremediation Potentials of Sorghum (*Sorghum Bicolor*), Sunflower (*Helianthus Amarus*) and Fluted Pumpkin (*Telfaria Occidentallis*) On Spent Engine Oil Polluted Texturally Contrasting Soils. *Medicon Agriculture & Environmental Sciences* 3(2) 27-33.

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Won Awards, Incentives, and Scholarships

- | | |
|-----------------------------------|-------------|
| 1. Erasmus Mundus Scholarship | 2023 |
| 2. Second-best graduating student | 2019 |