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MORPHOLOGICAL AND PHYSIOLOGICAL DIVERSITY
IN BREAD (*TRITICUM AESTIVUM* L.),
DURUM (*TRITICUM TURGIDUM* L. VAR. *DURUM*),
EINKORN (*TRITICUM MONOCOCCUM* SPP.
MONOCOCCUM), AND EMMER (*TRITICUM*
DICOCCUM SSP. *DICOCCUM* SCHRANK.)
WHEATS UNDER LEAD STRESS

DOCTOR OF PHILOSOPHY, BIOLOGY

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The thesis entitled “**MORPHOLOGICAL AND PHYSIOLOGICAL DIVERSITY IN BREAD (*TRITICUM AESTIVUM* L.), DURUM (*TRITICUM TURGIDUM* L. VAR. *DURUM*), EINKORN (*TRITICUM MONOCOCCUM* SPP. *MONOCOCCUM*), AND EMMER (*TRITICUM DICOCCUM* SSP. *DICOCCUM* SCHRANK.) WHEATS UNDER LEAD STRESS**” prepared and submitted by **Ashraf Mohammed Salh SOLIMAN** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in Department of **BIOLOGY** with unanimity/majority vote is hereby approved. 6/17/2025

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ABSTRACT

MORPHOLOGICAL AND PHYSIOLOGICAL DIVERSITY IN BREAD (*TRITICUM AESTIVUM* L.), DURUM (*TRITICUM TURGIDUM* L. VAR. *DURUM*), EINKORN (*TRITICUM MONOCOCCUM* SPP. *MONOCOCCUM*), AND EMMER (*TRITICUM DICOCCUM* SSP. *DICOCCUM* SCHRANK.) WHEATS UNDER LEAD STRESS

PHD THESIS

ASHRAF MOHAMMED SALH SOLIMAN
BOLU ABANT IZZET BAYSAL UNIVERSITY
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Wheat is critical to global food security. However, environmental stresses, such as heavy metals, may significantly impact its growth and development. This study evaluated the effect of lead exposure on the growth of seedlings and the accumulation of enzymatic and non-enzymatic antioxidants in various wheat landrace cultivars. The investigation demonstrated the impact of Pb on parametric traits: seed germination, seedling vigor, shoot and root elongation, coleoptile length, root length, shoot fresh and dry weight, and root fresh and dry weight decreased as Pb concentrations increased. On the other hand, low concentrations of lead (50 μ M and 100 μ M) significantly increased these values in the Einkorn, Emmer, and Ekiz species compared to the Durum wheat cultivar. The soluble protein content of Durum at 50 μ M and 100 μ M of Pb (NO_3)₂ was significantly higher than in the other wheat species. However, the Ekiz cultivar exhibited higher protein content than the other species at higher lead concentrations (250 μ M and 500 μ M).

The Durum cultivar lacked certain components (K, Ca, P, Mg, and Fe) at the lead concentration of 500 μ M. Consequently, the amounts of chlorophyll a, chlorophyll b, and proline were lower than in the other wheat landraces. Ancient wheat landrace cultivars: Einkorn and Emmer showed higher antioxidant activity (CAT and SOD), phenolic compounds, carotenoids, and lower H₂O₂ levels compared to modern wheat species Ekiz and Durum when exposed to high concentrations of lead.

The wheat landraces Einkorn and Emmer exhibited more resistance to lead stress in terms of shoot and root toxicity than Ekiz and Durum. Future studies should focus on identifying specific phenolic compounds and the antioxidant enzyme mechanisms underlying lead tolerance in wheat landrace cultivars.

KEYWORDS: Wheat Landraces, Seedling Vigor, Chlorophyll A, B, Carotenoid Antioxidant Enzymes (CAT and SOD), Proline, Phenolics, Protein, Hydrogen Peroxide, Lead Tolerance

ÖZET

**EKMEKLİK (*TRITICUM AESTIVUM* L.), MAKARNALIK (*TRITICUM TURGIDUM* L. VAR. *DURUM*), SİYEZ (*TRITICUM MONOCOCCUM* SPP. *MONOCOCCUM*) VE GERNİK (*TRITICUM DICOCCUM* SSP. *DICOCCUM* SCHRANK.) BUĞDAYLARININ KURŞUN STRESİ ALTINDA MORFOLOJİK VE FİZYOLOJİK FARKLILIKLARI
DOKTORA TEZİ**

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Buğday, dünyanın gıda güvenliği için kritik öneme sahiptir. Ancak ağır metaller gibi çevresel stresler, büyümesini ve gelişimini önemli ölçüde etkileyebilir. Kurşun etkisinin fide büyümesi ve farklı buğday yerel ırklarındaki enzimatik olan ve olmayan antioksidan madde birikimine etkisi değerlendirildi. Araştırma; Pb'nin tohum çimlenmesi, fide canlılığı, sürgün ve kök uzaması, koleoptil uzunluğu, kök uzunluğu, sürgün taze ve kuru ağırlığı ile kök taze ve kuru ağırlığı gibi parametreler üzerinde etkisini göstermiş ve bu parametreler, Pb konsantrasyonu arttıkça azalmıştır. Öte yandan, düşük kurşun konsantrasyonlarında (50 μ M ve 100 μ M), Durum buğday çeşidine göre, Einkorn, Emmer ve Ekiz türlerinde önemli ölçüde artışlar kaydedilmiştir. Durum'un 50 μ M ve 100 μ M Pb (NO₃)₂'deki çözünür protein içeriği, diğer buğdaylara göre önemli ölçüde daha yüksek iken yüksek kurşun konsantrasyonlarında (250 μ M ve 500 μ M) Ekiz çeşidinin diğer türlerden daha yüksek protein içeriğine sahip olduğu saptanmıştır.

Durum çeşidi, 500 μ M kurşun konsantrasyonunda bazı bileşenlerden (K, Ca, P, Mg ve Fe) yoksun olup, bundan dolayı klorofil a, b ve prolin düzeyleri diğer buğday yerel ırklara göre daha düşüktür. Eski buğday yerel ırklarından Einkorn ve Emmer, yüksek kurşun konsantrasyonlarında, modern buğday türlerinden Ekiz ve Duruma göre daha yüksek antioksidan aktivitesine (CAT ve SOD), fenolik bileşiklere, karotenoidlere ve daha düşük H₂O₂ düzeyine sahiptir.

Buğday yerel ırklarından olan Einkorn ve Emmer, kurşun stresine karşı sürgün ve kök toksisitesi açısından Ekiz ve Durum'dan daha dirençlidir. Gelecekteki araştırmalarla, yerel buğday ırklarında kurşun mineraline toleransı sağlayan belirli fenolik bileşikler ile antioksidan enzim mekanizmalarının belirlenmesine odaklanılmalıdır.

ANAHTAR KELİMELEER: Yerel Buğday Irkları, Tohum Gücü, Klorofil A, Klorofil B, Karotenoid, Antioksidan Enzimler (CAT ve SOD), Prolin, Fenolikler, Protein, Hidrojen Peroksit, Kurşun Toleransı

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LIST OF ABBREVIATIONS AND SYMBOLS

μM	: Micro Mole
BSA	: Bovin Serum Albumin
CAT	: Catalase
Durum	: <i>Triticum turgidum</i> L. var. <i>durum</i>
Einkorn	: <i>Triticum monococcum</i> spp. <i>monococcum</i>
Ekiz	: <i>Triticum aestivum</i> L.
Emmer	: <i>Triticum dicoccum</i> ssp. <i>dicoccum</i> Schrank
H₂O₂	: Hydrogen Peroxide
ICP	: Inductively Coupled Plasma Spectroscopy
mm	: Millimeter
NIST	: National Institute of Standards and Technology
PVP	: Polyvinylpyrrolidone
RDW	: Root Dry Weight
RFW	: Root Fresh Weight
S.V.I	: Seedling Vigor Index
SOD	: Superoxide Dismutase
TCA	: Trichloroacetic Acid

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1. INTRODUCTION

1.1 General Information

Wheat (*Triticum* spp.) is one of the most important cereal crops for human civilization. It significantly contributes to world food security (Shiferaw et al., 2013) and supplies humans with the calories and proteins the body requires. It is the essential food ingredient in 40 nations and supports 35% of the world's population (Karagöz & Zencirci, 2005). It was the first crop domesticated between 12,000 and 10,000 years ago in the Fertile Crescent. Early domesticated wheat primarily included species such as Einkorn (*Triticum monococcum* ssp. *monococcum*) and Emmer (*Triticum dicoccum* Schrank), which played a crucial role in the origins of agriculture (Peng et al., 2012; Ulukan, 2009). The hulled wheat has three cultivated species with various levels of ploidy: Einkorn (*Triticum monococcum* ssp. *monococcum* L., $2n = 2x = 14$), Emmer (*Triticum dicoccum* Schrank, $2n = 4x = 28$), and Spelt (*Triticum spelta* L., $2n = 6x = 42$) (Szabó & Hammer, 1996). Einkorn (*Triticum monococcum* ssp. *monococcum*), a diploid ($2n = 2x = 14$, AmAm), was one of the ancient, domesticated wheat lines produced from *T. boeoticum* around 10,000 to 13,000 years ago in the Karacadağ mountain of Southeastern Türkiye (Peng & Gitelson, 2011). Einkorn's whole meal has a low ratio of dietary fiber; however, it is high in proteins, lipids (mainly unsaturated fatty acids), fructose, and trace minerals (especially Zn and Fe) (Hidalgo & Brandolini, 2014). Tetraploid Emmer wheat (*Triticum dicoccum* Schrank) is one of the most cultivated crops in the world. It is a progenitor of hexaploid bread wheat (*Triticum aestivum*) and the direct ancestor of tetraploid durum wheat (*Triticum turgidum* subsp. *turgidum*) (Fuller et al., 2012; Arranz-Otaegui et al., 2016). It is widely cultivated in rural areas in Türkiye by organic farmers, most used for animal consumption and seldom as individual bread (Giuliani et al., 2009). Its flour is used instead of wheat flour for making bread, pasta, sweet-savory biscuits, and cakes (Stallknecht et al., 1996).

Modern wheat cultivars are hexaploid bread wheat (*Triticum aestivum* L., ($2n = 6x = 42$, AABBDD) and Durum wheat (*Triticum turgidum* durum ssp., ($2n = 4x = 28$, AABB) (Ling et al., 2018). Bread wheat is a nutritional source that provides energy as food for humans and feeds animals, about 95% of the world's wheat production (FAOSTAT, n.d.). However, Durum wheat accounts for 5% of it.

On the other hand, macaroni wheat uses more than bread wheat (Kilian et al., 2009; Alexandratos & Bruinsma, 2012).

1.2 Problem Statement

The sufficient production of wheat for human consumption is becoming challenging due to the growing global population (Alexandratos & Bruinsma, 2012). Additionally, environmental factors including biotic organisms, various pathogens, such as fungi, and abiotic stressors, play a significant role. Factors such as low or high temperatures, drought, salinity, pH, and heavy materials like lead (Pb) affect bread, durum, einkorn, and emmer species (Zencirci et al., 2019), particularly the content of nutrients (K, Ca, P, Mg, and Fe), storage protein amount composition in wheat caryopsis, phenotypic traits, and crop quality (Alkan & Birsin, 2023).

1.3 Research Purpose

To assess the impact of Pb stress on the following morphological parameters in soil and petri dishes: shoot and root dry weight, coleoptile length, root length, and germination percentage (%).

To evaluate the effect of Pb on shoot toxicity, root toxicity, and tolerance index in wheat cultivars.

To determine how Pb stress affects physiological parameters such as hydrogen peroxide levels, antioxidant enzyme activities (CAT and SOD), carotenoids, phenolic content, proline, protein content, photosynthetic pigments (Chlorophyll a (Chl a) and Chlorophyll b (Chl b)), lead accumulation, and the level of essential elements (Pb, Ca, Mg, P, Fe, and K).

1.4 Hypothesis

H₀₁: There is no effect of Pb toxicity on agro morphological features in wheat species.

H₁₁: Pb toxicity affects agro morphological features in wheat varieties.

H₀₂: The physiological traits of different wheat varieties are unaffected by Pb toxicity.

H₁₂: The physiological traits of different wheat species are affected by Pb toxicity.

H₀₃: The physiological and morphological attributes of wheat cultivars are not associated with each other under Pb stress.

H₁₃: The physiological and morphological traits of wheat cultivars are associated with each other under Pb stress.

1.5 Wheat Landraces in Türkiye

Türkiye has a long history of growing wheat, and several researchers from across the globe have gathered, assessed, and documented its landraces due to their varied characteristics (Özkan et al., 2011). In addition to increasing production and fostering stability, landraces have evolved under various harsh conditions, including biotic and abiotic stresses (Dempewolf et al., 2017; Witcombe et al., 1996). Landraces have historically outperformed modern farming in low-input agricultural systems due to their remarkable adaptive capabilities (Dwivedi et al., 2016). Establishing prospective gene pools is the foundation for producing resistant crops to biotic and abiotic stress. Identifying and picking new plant varieties that offer high yields and varying degrees of resistance to environmental conditions is a dynamic, long-term endeavor for research teams. A wheat landrace is not always a homogeneous, unique, or stable entity in genetics and phenotype. It is related to any area and the regular exchange of seeds over both short and long distances among local farmers. (Jaradat, 2006).

According to Zeven (1999) and Lodhi et al. (2020), a wheat landrace is a conventional wheat variety that exhibits stress tolerance, high yield stability, and a moderate crop output level under low conditions. These landraces result from grain types that farmers harvest regularly and are resistant to the same conditions that affect the typical wheat variety (Zeven, 1999). Similarly, due to their ability to cope with biotic and abiotic stressors, they are becoming a diversified and heterogeneous population (Masood et al., 2005). Therefore, novel resistant cultivars are developed using the genes found in landraces (Reynolds et al., 2007). Genetic diversity among crop species has gained significant importance in the context of contemporary rapid agricultural advancements. Therefore, the identification of novel resistance genes greatly depends on landrace research. It is crucial to distinguish between beneficial features related to yield and resistance to abiotic and biotic stresses, especially since many landraces have recently disappeared and been replaced by new wheat

varieties. Furthermore, the collection of landraces has emphasized the need to characterize the gathered germplasm (Lodhi et al., 2018).

Consequently, wheat landraces often represent collections from various geographic locations and serve as a significant source of novel rust-resistant genes (Sthapit et al., 2014). These landraces improve the use of plant genetic resources for the commercially important features in wheat breeding programs (Karagöz & Zencirci, 2005; Ulukan, 2009)

1.6 Wheat Landraces Distribution

Türkiye is one of the gene centers and the source of many plant species worldwide. Numerically, it has 8800 species, of which more than 30% are indigenous and endemic. Additionally, it is the origin and source of genetic diversity for scientifically and internationally significant plants initially domesticated from the wild within the country (Kan et al., 2016). For example, *Triticum monococcum* ssp. *monococcum*, the wild Einkorn wheat, descended from *T. boeoticum* and can survive in challenging environmental conditions (Ünlü et al., 2018). Another important hulled wheat is Emmer (*T. dicoccon*) (Aslan et al., 2016). Since the twentieth century, common wheat (*Triticum aestivum* L.) has replaced the hexaploid spelt (*Triticum spelta* L.), the most common grain crop grown in Europe, starting in the fifth century. Due to the husks' 30% loss and the additional step needed to remove them during milling, spelt yields are much lower (Su & Sun, 2016). Einkorn wheat (*Triticum boeoticum*), the wild ancestor of modern commercial wheat, was first sown between 10,000 and 12,000 years ago by ancient humans in the southeastern Turkish province of Şanlıurfa (Mountains), according to archaeological findings (Kan et al., 2016). Furthermore, Einkorn varieties are distributed across Southeast European nations, including Germany, Türkiye, Switzerland, Spain, and Italy (Wieser et al., 2009). Emmer is cultivated in India, Italy, Ethiopia, and Türkiye (Gioia et al., 2015), while spelt is grown in Germany, the Czech Republic, Switzerland, and other European countries (Trocchi & Codianni, 2005).

Farmers cultivate Emmer and Spelt wheats since these plants are rich in nutritious components and highly resilient to environmental stress (Suchowilska et al., 2010), but their yield levels are rather low. Historically, wheat and barley plants

were also domesticated in western Iran, Jordan, Iraq, Palestine, Syria, Lebanon, and southeast Türkiye (Feldman, 2001; Russell et al., 2011; Dwivedi et al., 2016).

Cultivating and using landraces is affected by climate, physical factors, socio-economic conditions, pricing systems, and market facilities. They are crucial to Türkiye's small-scale farmers' livelihood. Landraces has 800,000 ha in Türkiye. The most effective methods for conserving traditional knowledge about landrace sustainability include their use in organic farming systems, growing landrace mixes, modifying the seed registration framework, setting up a unique landrace register system, and providing breeding facilities for farmers (Karagöz, 2014).

1.7 Genetic Variation in Wheat Landraces

Wheat is the most revolutionary grain in the world. It enabled our hunter-gatherer ancestors to transition to a settled, agricultural society about 10,000 years ago. The domestication process produced the brittle rachis of wild Emmer (*Triticum turgidum* ssp. *dicoccoides* Körnicke) and *Triticum monococcum* ssp. *aegilopoides* evolved to the non-brittle rachis of domesticated Emmer wheat (*Triticum turgidum* ssp. *dicoccon* Schrank Thell) ($2n = 4X = 28$, AABB) and Einkorn wheat [*Triticum monococcum* ssp. *monococcum* ($2n = 2x = 14$, AmAm), respectively, was the other revolution in wheat history. The most widely grown crops up to the early Bronze Age were *Triticum monococcum* and *Triticum dicoccon*. They were then gradually supplanted by free-threshing and high-yielding wheat types. *Triticum durum* $2n = 4X = 28$, AABB, and *Triticum aestivum* $2n = 6X = 42$, AABBDD do the same. Traditional farmers have grown domesticated wheat types after domestication for thousands of years. Farmers select wheat varieties based on their ability to withstand biotic and abiotic stressors, yield quantity, and yield stability in low-input farming settings, despite the absence of organized breeding programs. The process of natural selection within the environment, combined with farmers' preferences for specific types of wheat, contributes to the development and persistence of certain wheat varieties (Zeven, 1999).

1.8 Wheat Landrace Varieties

1.8.1 Ekiz

As is known, bread wheat (*Triticum aestivum* L.) cultivars are the source of essential nutrients for both humans and animals. Moreover, its flour and bran have a unique nutritional composition, rich in protein, B vitamins, minerals, and dietary fiber (Köse et al., 2023). Yiğit & Ereku (2023) reported that "Selimiye", "Kutluk", "İzgi", "Tosunbey", and "Müfitbey" wheat cultivars had superior parameters, including higher protein, total phenol, and total antioxidant activity. They found a high positive correlation between total antioxidant activity, phenolic content, and amino acids. It has a sufficient genetic variation to support the production of over 25,000 types and is suitable for temperate climates, although it has a comparatively recent origin (Feldman et al., 1995).

The highest diversity of bread wheat occurred in regions like Manisa, Konya, and Diyarbakır (Morgounov et al., 2016). Karaman et al. (2022) found significant genotypes for grain yield (GY) (G6, G8, G10, "Müfitbey", "Hanlı", and "Metin"), qualitative attributes (Bezostaja-I, Kenanbey, and Cemre), and physiological traits (Ekiz, G8, and G9). Thousand-grain weight and plant height were positively correlated with the Normalized Difference Vegetation Index (NDVI) and grain yield (GY). In contrast, GY was negatively related to canopy temperature (CT) and heading time (HT). Biplot analysis indicated that genotypes such as "Hanlı", "Metin," G10, G8, and G6 were high-yielding and stable, showing less sensitivity to environmental conditions.

Kizilgeçi et al. (2019) reported that Australian wheat cultivars such as LPB 08-1799, Eagle Rock, Magenta, Emu Rock, Wyalkatchem, Young, Calingiri, Yitpi, Mace, Envoy, and Corack outperformed Turkish bread wheat varieties (Kaşifbey 95, Basribey 95, and Alibey) in terms of GY and quality features when cultivated under the ecological conditions of Diyarbakır in Türkiye.

1.8.2 Einkorn

Einkorn (*Triticum monococcum* L. ssp. *monococcum*), known locally as siyez, is one of the Neolithic founder crops, cultivated for over eight thousand years in the Near East. The current limited production of einkorn, primarily in isolated mountainous communities in the Mediterranean region, emphasizes its semi-wild nature and unique characteristics as a hulled wheat variety. Einkorn has a higher protein content (19–21%) and higher concentrations of microelements and antioxidant compounds than modern wheat (Hidalgo & Brandolini, 2014). Because of its high nutritional content, it's an excellent choice for sustainable farming methods and a better diet (Gazza et al., 2023).

Turkish-cultivated Einkorn wheat landrace populations exhibit high genetic diversity, and seed endosperm proteins (gliadins and glutenins) were found to have a genetic diversity value ($H_e = 0.65$). The gliadin proteins were superior to the high molecular weight-glutenin proteins in terms of allele combinations and genetic diversity (Keskin et al., 2015).

Einkorn wheat has health benefits, including higher protein levels, minerals (especially Zn and Fe), and phenolic compounds than common wheat (Hidalgo & Brandolini, 2014). Its flour could be mixed with wheat flour in varying proportions, which allows for the production of lighter and brighter bread. Key parameters affecting bread structure, such as modified Zeleny sedimentation and modified sodium dodecyl sulfate (SDS) sedimentation values, showed significant decreases as the proportion of einkorn and wholemeal flour increased (Keçeli et al., 2021).

1.8.3 Emmer

Triticum dicoccum Schrank ex Schübl. ($2n = 4x = 28$, AABB), It is a hulled tetraploid species first domesticated in the Near East and still grown in Turkey under the local name “çatalsiyez.” The specific epithet dicoccum (“two-grained”) reflects its typical pair of kernels per spikelet. Historical synonyms include *Triticum turgidum* L. subsp. dicoccum (Schübl.) Thell. and *T. amyleum* Seringe, though modern taxonomy consolidates these under *T. dicoccum* Schrank ex Schübl. Emmer’s tightly enclosed, hard-husked grains and dense ears distinguish it from other wheat types, and its adaptability to marginal environments, along with a distinctive nutrient profile, has been well documented (Nevo, 2002; Bavec & Bavec, 2006).

The production of Emmer wheat has reduced significantly during the past century due to the substitution of high-yielding wheat types. However, the cultivation area has increased to more than 2000 hectares due to the growing demand for nutritious foods and their agronomic and nutritional benefits in recent years. According to the agro-morphological and genetic investigation, Italian cultivars of *Triticum turgidum* L. spp. *dicoccum* Schrank ex Schübler showed a great deal of variability both across and within the varieties (Pagnotta et al., 2005).

Emmer wheat was one of the essential cereal crops in Neolithic agriculture, and its domestication was crucial to the onset of farming. Its wild ancestor, *Triticum dicoccoides* (Körn. Ex Asch. et Graebn.) Schweinf was domesticated around 10,000 years ago. *Triticum dicoccum* (emmer) retains a wider array of gene variants than *Triticum monococcum* (einkorn) or *T. spelta* (spelt), a diversity that underlies its stronger resilience against pathogens, pests, drought, salinity, and temperature extremes (Salamini et al., 2002; Dempewolf et al., 2017).

Emmer is the world's most significant cereal crop due to its high energy, vitamins, minerals, protein, and dietary fiber. Micronutrients, including minerals and antioxidants, are more abundant in ancient grains like emmer than in modern wheat varieties. Emmer flour was for cakes, pasta, bread, and sweet-savory biscuits. On the other hand, it forced cattle feeding in the highlands of Italy, Spain, Germany, Switzerland, Türkiye, Russia, and the United States (Zommita et al., 2022).

1.8.4 Durum

Durum wheat represents about 5% of the world's wheat production and only 8% of the total area cultivated for wheat. The main durum producers are the European Union, the United States, Algeria, Türkiye, Canada, Mexico, and Kazakhstan, whereas Australia, Tunisia, Syria, India, Chile, Argentina, and Morocco are minor producers. The EU's leading producers are Greece, France, Italy, and Spain. Durum wheat is the primary crop in many parts of the Mediterranean basin as the raw material for a variety of finished products consumed globally, including couscous and pasta, while being a relatively minor crop globally (Kabbaj et al., 2017). *Triticum turgidum* ssp. *dicoccum*, the cultivated tetraploid wheat, originated from wild wheat in the Karacadağ Mountain in southeast Anatolia, Türkiye. Many edible agricultural plants, including tetraploid wheats, are grown in a small area of provinces (Özbek, 2014). The importance of Türkiye and the Mediterranean region has grown recently on a global scale. During the Ottoman Empire in the 20th century, 65-70% of Türkiye's wheat land was covered by durum wheat (*Triticum durum* Desf.) (Martínez-Moreno et al., 2022). (RAPD) markers used for observable heterogeneity and diversity of genes demonstrate that durum wheat landraces have a larger in exhibit higher levels of genetic diversity due to a paucity of high-quality raw materials for the pasta industry, its rising relevance at international commerce and the food scarcity that numerous developing countries confront, (Özbek, 2021; Aslan et al., 2017). Additionally, to understand the genetic mechanisms that underlie the domestication and geographic spread of wheat, as well as to formulate basic techniques for the future application of the unique traits of performance, ability to adapt, tolerance to stress, and end-utilization qualities displayed by landrace cultivars (Zencirci & Karagoz, 2005).

Durum wheat cultivars are less resistant to cold because they are susceptible to Zn deficiency, which is prevalent in many Turkish soils (Martínez-Moreno et al., 2022). A better understanding of adaptation mechanisms and (Environment x Genotype) interactions, increased grain yield stability, responsiveness to inputs and efficient use of resources in different farming systems, resilience against such as pests and diseases, as well as environmental challenges like drought, salinity are some other important cultivation/limitation factors that make cultivation appropriate (Xynias et al., 2020). Additionally, with slight adjustments, similar

methods can be applied to increase output and reduce the length of the breeding program (Xynias et al., 2020; Martínez-Moreno et al., 2022).

1.9 Impact of Heavy Metals on Plants

The growing global population is one of the most significant challenges humanity will face in the future. A critical aspect of coping with this challenge is the development of crops capable of producing sufficient yields under stress conditions, such as salinity, drought, cold, diseases, and micro/macro element toxicity. This remains a demanding task in modern agriculture (Padulosi et al., 1996).

Heavy metals are classified into two types according to their physicochemical properties: redox-active metals (Cr, Fe, Cu, and Mn) and non-redox-active metals (Pb, Ni, Cd, Hg, Al, and Zn) (Schutzendubel & Polle, 2002; Yadav, 2010). Redox-active metals can trigger oxidative stress directly through mechanisms such as the Haber-Weiss and Fenton reactions, which are known to generate free radicals in plant systems (Schutzendubel & Polle, 2002; Halliwell, 2006). In contrast, metals that are non-redox active contribute to oxidative damage indirectly. Reducing the availability of glutathione (GSH) stimulates pro-oxidative enzymes such as Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, forming bonds with protein sulfhydryl groups, or inhibits the function of antioxidative enzymes (Solanki & Dhankhar, 2011).

1.10 Lead Stress and Mechanism of Tolerance in Plants

It is an amphoteric trace metal found as free or complex ions, and it is the second most hazardous heavy metal in plants after arsenic. Moreover, it has no recognized physiological role or importance (Kumar & Prasad, 2018). Pb and Cd are the most common heavy metal contaminants in the environment, with both natural and anthropogenic sources, such as mining, smelting, and other industrial processes. These pollutants often act as co-pollutants, resulting in simultaneous exposure to living organisms and synergistic adverse health effects (Patra et al., 2011).

Studies have shown that Pb and Cd concentrations in cultivated barley (*Hordeum vulgare*) and wheat seedlings inhibited seed germination, as well as root and shoot development, with Cd being more toxic (Titov et al., 1996). Similarly,

Turk et al. (2018) revealed that Pb toxicity significantly reduced the development of early wheat seedlings. Antioxidant enzyme activity increased compared to the control group, while the ratio of ascorbate and glutathione shifted in favor of oxidized forms, indicating oxidative stress. Additionally, Pb poisoning significantly reduced amylase activity by decreasing the amount of Ca^{2+} in endosperm supplements and increasing amylase activity by increasing the amount of Ca^{2+} when exposed to Pb and alpha-lipoic acid (LA). In a study by Kaur et al. (2012), it was found that root and shoot length significantly decreased with increasing lead concentrations $\text{Pb}(\text{NO}_3)_2$ at 500, 1000, and 2500 μM compared to an unstressed, water-treated control variant. Moreover, lead exposure also affects photosynthetic activity by inducing oxidative stress.

Rahman et al. (2023) found that tetracycline (TC), Cd, and Pb significantly decreased the growth and development of *Triticum aestivum* by 11%, 9%, and 5%, respectively. Pb and Cd exhibited the most severe negative effects on *T. aestivum* yield, while TC had no significant impact. Exposure to these contaminants reduced the photosynthetic rate due to a reduction in chlorophyll content. Cd had the most substantial effect (58%), followed by Pb (37%) and TC (8%).

Gillani et al. (2021) investigated the impact of salicylic acid (SA) with different concentrations (0, 0.1, and 0.5 mM) in combination with varying Pb levels (0, 0.5, 1, 1.5 mM) on wheat cultivars ("Millat-2011", "Aas-2011", and "FSD-2008"). Increasing Pb concentration caused significant reductions in soluble sugar, protein, carotenoids, and chlorophyll contents in the wheat varieties. Conversely, the amount of proline increased with higher Pb levels, indicating stress. The application of SA notably mitigated the adverse effects of Pb, enhancing the biochemical attributes of *T. aestivum* L. cultivars.

Chen et al. (2017) declared that lead (Pb) and cadmium (Cd) significantly harmed wheat seed germination and growth. Exposure to Pb leads to an increase in malondialdehyde (MDA), superoxide anion radical (O_2^-) levels, and electrolyte leakage (CEL). However, treatments with magnetic fields (MF) or laser radiation (LR) improved germination rates by increasing the activity of antioxidant enzymes such as glutathione reductase (GR), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). These treatments alleviate the detrimental physiological effects of Pb and Cd.

Selim et al. (2024) reported that an increase in lead (Pb) concentrations from PbSO₄ and Pb(NO₃)₂ significantly affected the vegetative growth of pepper plants (*Capsicum annuum*). Pb(NO₃)₂ exhibited a more substantial inhibitory effect compared to PbSO₄. The Pb salts negatively affect photosynthetic pigments, leaf water deficit, osmotic pressure, membrane integrity, total water content, relative water content (RWC), transpiration rate, and leaf succulence.

Moringa oleifera demonstrated significant uptake at concentrations of 500 mg kg⁻¹ in the roots and 224 mg kg⁻¹ in the shoots when exposed to 5 mM Pb for 30 days. The levels of metallothionein protein (93.55 µmol g⁻¹ × 10⁻³), proline (0.49 µmol g⁻¹ FW), and polyphenols (75.83 mg g⁻¹ FW) were increased in Pb-treated plants, suggesting enhanced stress response mechanisms. The activity of antioxidant enzymes, including CAT, GR, and ascorbate peroxidase (APX), was significantly higher in Pb-treated plants than in the control plant, indicating a protective response to oxidative damage (Mathur et al., 2023).

According to Tan et al. (2022), lead (Pb) stress increased the amount of Pb in pakchoi leaves depending on the concentrations. Malondialdehyde (MDA) levels were enhanced with exposure to lead. On the other hand, the activity of GSH-Px, SOD, and total antioxidant capacity (T-AOC) decreased. Pb stress adversely affected photosynthesis, as indicated by reduced values of photosynthetic parameters such as vapor pressure deficit (VPD), transpiration rate (Tr), intercellular CO₂ concentration (Ci), net photosynthetic rate (Pn), stomatal conductance (Gs), Chlorophyll and carotenoid contents also significantly decreased in the high-dose group.

Bouziani et al. (2023) found a significant decrease in chlorophyll pigment content, relative water content (RWC), and sodium (Na) levels in both the roots and aerial parts of the plants. Additionally, calcium (Ca) and potassium (K) levels decreased in the roots, indicating that lead interferes with nutrient uptake and transport in broad bean (*Vicia faba* L.) plants under lead stress. Moreover, the dry weight of the aerial and root parts significantly decreased with increasing lead concentrations. For example, reductions exceeded 76% in roots and over 66% in aerial parts at the highest lead dose (2000 ppm).

Urooj et al. (2022) discovered that growth parameters such as root, shoot, seedling length, and biomass production in *Sporobolus coromandelianus* (Retz.) Kunth (True Grass) and *Euphorbia hirta* Forsk (Weed) were adversely affected by

the toxic Pb concentrations (40, 60, 80, and 100 ppm). More resistance was observed in *S. coromandelianus* than in *E. hirta*.

Bloem & Ciaranek (2021) declared that soil contamination with lead, specifically $\text{Pb}(\text{NO}_3)_2$, negatively affected the germination of maize (*Zea mays* L.) grains. Higher lead concentrations harmed all measured parameters: elongation growth and the fresh and dry mass decreased significantly due to lead exposure. Photosynthetic activity was affected by the rate of transpiration, which varied with the concentration of lead solutions used.

Lestari & Rosyidah (2022) reported that Pb exposure caused morphological alterations in spinach (*Spinacia oleracea* L.) and kale (*Brassica oleracea* var. *Acephala*), although it did not affect the basal leaves. The tops of spinach leaves remained unchanged, whereas the tips of kale leaves grew blunt. Root development was better in the control group, with longer roots and more root hairs compared to the Pb-treated plants. Total chlorophyll content was higher in spinach control plants than in kale, but it decreased with an increase in Pb concentration in treated plants. Spinach was more sensitive to Pb exposure, showing more visible morphological damage by the end of the study.

Lead (Pb) treatment significantly reduced shoot heights and root lengths in *Leucaena leucocephala* seedlings, particularly at $\text{Pb}(\text{NO}_3)_2$ concentrations of 300, 500, and 700 μm . The PN, Gs, and Tr decreased at Pb concentrations. Plant leaves showed a significant increase in protein and carbohydrate content at these Pb concentrations. However, lower Pb concentrations (25, 50, and 100 μm) did not harm the plants (Alkhatib et al., 2019).

Herlina et al. (2021) reported that the growth parameters, such as photosynthetic pigments, stem dry biomass, leaf, root biomass, the leaf area, and the number of leaves decreased in the *Cordyline fruticosa* as lead concentrations in the soil increased. Additionally, the activity of antioxidant enzymes, specifically ascorbate peroxidase (APX) and catalase (CAT), increased in response to higher lead concentrations, indicating that the plant may be activating its defense mechanisms against lead toxicity. In line with (Khan et al., 2021), high lead concentrations (1.2 mM) dramatically decreased plant height, the number of tillers, panicles per plant, and spikelets per panicle in lead-sensitive rice cultivars. Biomass dry weight decreased when exposed to lead. CAT and POD, proline accumulation, and sucrose content increase with higher lead concentrations.

However, total protein and chlorophyll contents significantly decreased. The rice cultivars Tunnae and Mashkab had a high degree of tolerance to lead stress. This is consistent with the findings of Nandikonda and Sadhu (2020), who reported that mustard (*Brassica juncea* L.) varieties, including NRCHB-101MAYA, NRCDR-02, RH0406, and DRMRIJ-31, exhibited reduced seed germination and slower seedling growth under Pb exposure. Additionally, levels of antioxidative enzymes such as SOD, APX, and POD, along with proline and MDA content, increased in response to lead stress. In contrast, CAT activity and soluble protein levels declined. Similarly, Hamim et al. (2019) observed that increasing concentrations of lead significantly inhibited growth measurements, such as shoot and root dry weights, number of leaves, and plant height, in castor bean, jatropha, bead-tree, and Philippine-tung decreased with higher concentrations of Pb. MDA content significantly increased, indicating the development of free radicals and oxidative stress, whereas chlorophyll content decreased in all species under lead treatment. The thickness of leaves, spongy mesophyll tissue, lower epidermis, and upper epidermis was dramatically reduced due to increasing Pb (NO₃)₂ concentration. Philippine-tung was the most resistant to Pb toxicity compared to the other cultivars.

Mohamed et al. (2020) declared that the roots of *Jatropha curcas* seedlings contained more lead (Pb) than the stems and leaves, indicating a pattern of translocation and absorption. Rising Pb concentration caused a significant decrease in the dry weight of the roots, stems, and leaves. The total protein concentration decreased by 29% compared to the control at the highest lead dosage (40 mg/L). The total phenol and flavonoid components rose in response to enhancing Pb levels. Specifically, the total phenolic content doubled, and the flavonoid content increased sixfold at the highest Pb dose. The plant responded to heavy metal exposure by upregulation of the phenylalanine ammonia-lyase (PAL) gene in *Jatropha curcas* (JcPAL) in response to Pb stress. The tolerance index (TI) was higher in the seedlings.

Ravikumar & Thamizhiniyazn (2014) revealed that the development of the roots and shoots of the black gram (*Vigna mungo*) was dramatically reduced compared to the control group. The dry weight of the plants and the total chlorophyll content significantly decreased with exposure to different Pb concentrations. However, the leaves of plants treated with lead showed a significant

increase in proline content and the activity of enzymes, particularly catalase and peroxidase, suggesting that these enzymes may be involved in the plant's defensive system against lead-induced oxidative stress.

According to Rossato et al. (2012), *Pluchea sagittal* roots accumulate substantially more lead (Pb) than shoots. Furthermore, the concentration and quantity of Pb in the roots, stems, and leaves increased due to rising external Pb levels. Pb exposure caused a reduction in the transpiration ratio, leaf fresh weight, leaf area, shoot length, and nutritional solution intake. Nonetheless, there were no significant changes in the dry weights of the root and shoot. The roots were resistant to Pb than the shoots, indicating a differential response based on plant organs. The lipid peroxidation and H₂O₂ levels increased in both roots and leaves. Moreover, the activity of antioxidative enzymes such as APX and CAT increased, indicating a defense mechanism against oxidative stress.

2. MATERIALS and METHOD

2.1 Seed Samples

The experiment was conducted at Bolu Abant İzzet Baysal University's Plant Genetic and Pathology Lab, part of the Department of Biology. Wheat landrace varieties, including Emmer wheat, Einkorn wheat, Bread wheat (Ekiz), and Durum wheat, were collected from various regions in Türkiye, as shown in Table 2.1.

Table 2.1. Seeds collected from different regions in Türkiye

Common name	Region	Registration date	Scientific name	Location by GPS
Ekiz	Bahri Dağdaş Uluslararası Tar. Araş. Ens. Çumra Konya	01-04- 2004 Hasadi 2016	<i>Triticum aestivum</i> L.	37°34'15.0"N 32°43'38.7"E
Einkorn	Seben Güneyce Köyü	Hasadi 2021	<i>Triticum monococcum</i> spp. <i>monococcum</i>	40°21'46"N 31°31'6"E
Emmer	Sinop Durağan Gölgerişi Köyü	Hasadi 2020	<i>Triticum dicoccum</i> ssp. <i>dicoccum</i> Schrank	41°26'15"N 35°22'54"E
Durum	Bahri Dağdaş Uluslararası Tar. Araş. Enst. Karapınar Konya	06-05-1997 Hasadi 2016	<i>Triticum turgidum</i> L. var. <i>durum</i>	37°43'57.5"N 33°31'36.0"E

2.2 Effect of Lead Nitrate (Pb (NO₃)₂) on Morphological Traits of Wheat Landrace Cultivars in Petri Plate

The experiment could be summarized as follows: The 150 seed husks from each cultivar were cleaned and peeled. The surfaces of them were sterilized for ten minutes with 0.1% sodium hypochlorite, washed under running tap water, and rinsed five or six times with distilled water (Kumar et al., 2018). The seeds were then placed into petri dishes with three replicates of ten seeds each, exposed to various Pb nitrate concentrations (50, 150, 250, and 500µM). A 5 ml solution of the respective lead nitrate concentration was added one day after placement, along with 5 ml of dH₂O₂ as a control every two days for seven days (Kumar et al., 2018). After eight days, morphological traits such as fresh and dry weight, root length, coleoptile length, germination percentage (%), and seedling vigor (S.I.V.) (mm)

were evaluated for the wheat landrace cultivars (Kim et al., 1994). The fresh roots were then dried in an oven at 65 °C for 48 hours to calculate the dry weight (Weatherley, 1950).



Photo 2.1. Effect of Pb (NO₃)₂ concentrations on germination

2.3 Effect of Lead Nitrate (Pb (NO₃)₂) on Morphological and Physiological Features of Wheat Landrace Cultivars in Soil

Twenty seeds from each wheat cultivar were sterilized and planted in a plastic soil container. The control groups of cultivar seedlings received 150 ml of dH₂O on the first day of the growth stage. However, after eight days of plant growth, when two leaves appeared, the seedlings were treated with lead nitrate in different concentrations (50, 150, 250, and 500 µM), receiving 150 ml of the solution every 2 days for 28 days. The plant was stored in a growth room at 25 °C. The germination percentage, root elongation, shoot elongation, shoot, root toxicity tolerance index, and fresh and dry weight were measured based on the method outlined by Idrees et al. (2015) and calculated using the following formula:

$$\text{Germination (\%)} = \frac{\text{Total seed germination}}{\text{Total seed of plant}} \times 100$$

$$S.V.I. = \text{Seedling length (mm)} \times \text{Germination percentage (\%)}$$

$$\text{Shoot toxicity (\%)} = \frac{\text{Shoot length of cotrol} - \text{Shoot lenght of treatment}}{\text{Shoot leght of control}} \times 100$$

$$\text{Root toxicity (\%)} = \frac{\text{Root length of cotrol} - \text{Root lenght of treatment}}{\text{Root leght of control}} \times 100$$

$$\text{Tolerance toxicity (\%)} = \frac{\text{Mean root length in stress}}{\text{Mean root leght in control}} \times 100$$



Photo 2.2. Effect of Pb (NO₃)₂ concentrations on germination

2.4 Determination of Photosynthetic Pigments Contents

The photosynthetic pigments carotenoids, chlorophyll a (Chl a), and chlorophyll b (Chl b) were isolated and examined. Cut into short pieces, 0.5 g of control and lead nitrate-treated leaves were crushed with 10 ml of methanol. Plant extract methanolic solutions at the required concentrations (1.0 to 4.0 mg/ml) were measured using He\ios UV/VIS spectrophotometer at 470, 653, and 666 nm. The total of chlorophylls a and b, and carotenoids was evaluated according to Lichtenthaler & Wellburn (1985) and calculated by the following formula:

$$\text{Chlorophyll } \alpha \text{ (mg/L)} = (15.65 \text{ Abs}_{666} - 7.340 \text{ Abs}_{653})$$

$$\text{Chlorophyll } b \text{ (mg/L)} = (27.05 \text{ Abs}_{666} - 11.21 \text{ Abs}_{666})$$

$$\text{Total carotenoids (mg/L)} = (1000 \text{ Abs}_{470} - 2.860 \text{ Ca} - 129.2 \text{ Cb})/24$$

2.5 Determination of Total Phenolic Content (mg GA/g)

The phenolics from the leaves of wheat species were extracted by macerating 0.5 g of leaf tissue in a pestle and mortar with a 10 mL methanol/water (1:1, v/v) mixture. The suspended macerate was ultrasonicated at an ambient temperature for 15 minutes and then centrifuged at 1500×g for 15 minutes. After collecting the transparent, greenish supernatant, the remaining cell debris underwent a second 4-hour extraction process. The extraction procedure follows Waterhouse's (2002) methodology, which is given in the first step below. The extracted sample or blank (20 µl) was combined with 1.58 ml of water and Folin-Ciocalteu reagent (100 µl) for five min. After that, 300 µl of 20% NaCO₃ was added to the sample test tube and kept at 40°C for 20 minutes. A spectrophotometer was then used to measure the absorbance at 765 nm. The results were plotted versus concentration, with methanol acting as a blank.

The phenolic plant extraction content was measured using the following equation, expressed in gallic acid equivalents (GAE) / dry extract (dE):

$$C = cV/m$$

C = total phenolic components (mg GAE/g) (dry extract (dE)); c = gallic acid concentration (mg/ml) derived from the calibration curve; V = volume of extract; m = weight of phenolic extract from wheat landraces.

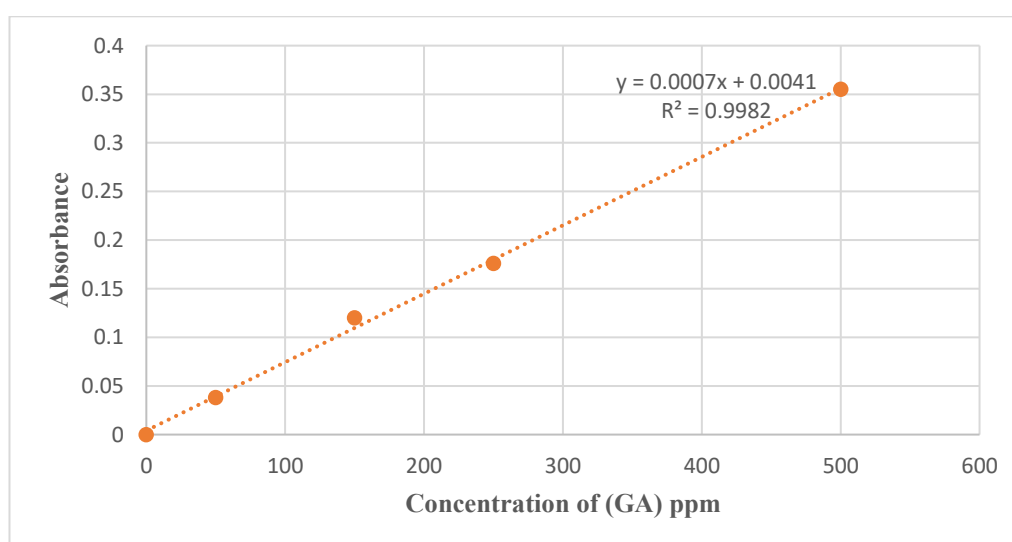


Figure 2.1. Gallic acid (ppm) standard curve

2.6 Determination of Proline Content ($\mu\text{mol/g}$)

The proline content was determined using the Bates et al. (1973) method, which involved grinding 0.5 g of seedlings in 5 mL of sulfosalicylic acid 3% (w/v) and centrifuging the mixture for 20 minutes at $5000 \text{ rpm min}^{-1}$. A 2 mL sample of the supernatant was then reacted with 2 mL of gallic acid and 2 mL of ninhydrin in a test tube for an hour at 100°C . The reaction stopped in an ice bath. The mixture was extracted using 4 mL of toluene and vigorously mixed with a test tube stirrer for 15-20 seconds. The proline content was measured using spectrophotometry at 520 nm against the standard proline.

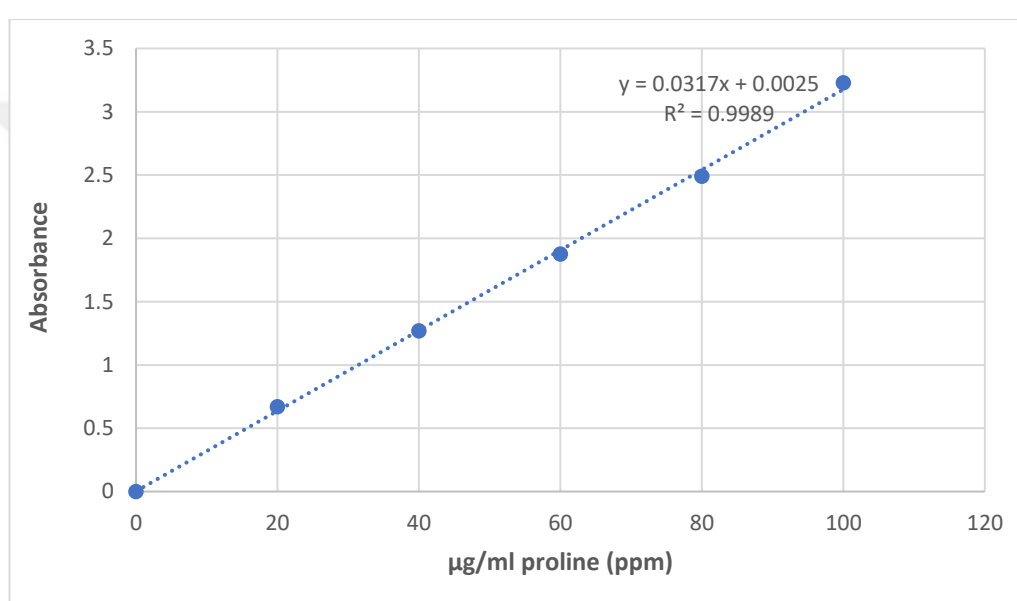


Figure 2.2. Proline (ppm) standard curve

2.7 Determination of Soluble Protein Content (mg/g)

The soluble protein content was determined using the method Lowry et al. (1951). A 0.2 mL sample of each extract was placed in a test tube, and 2 mL of alkaline copper sulfate (CuSO_4) reagent was added. After ten minutes of incubation at room temperature, the absorbance at 660nm was measured using a spectrophotometer, with the blank and standard protein as references. Standard protein concentrations of 0, 50, 100, 200, 600, and 800 mg/l of Bovine serum albumin (BSA) were used for calibration.

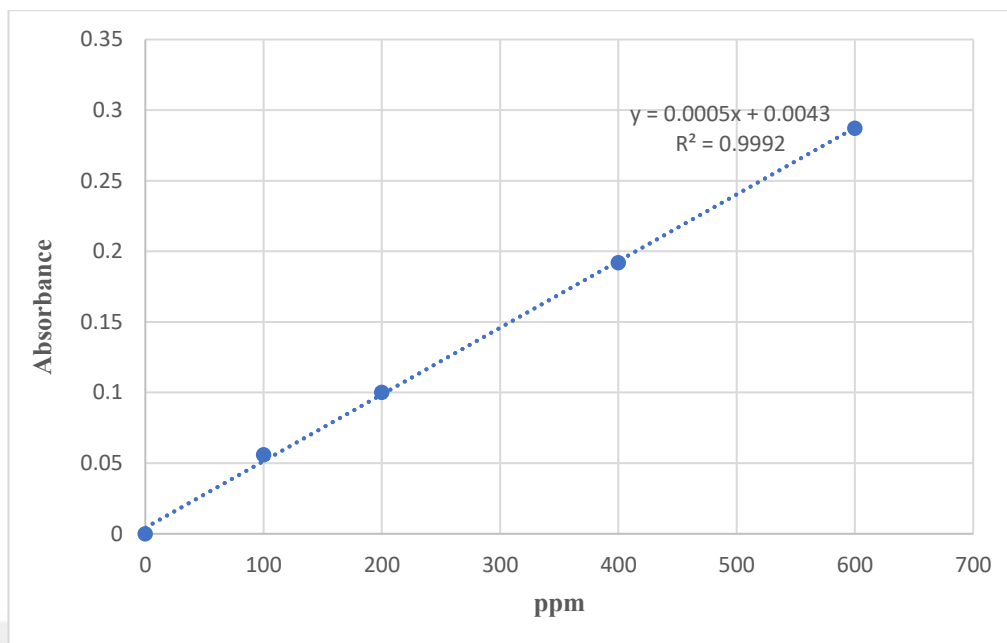


Figure 2.3. BSA standard curve

2.8 Extraction of Wheat Cultivar Leaves for Enzyme Analysis

The levels of the enzymes superoxide dismutase (SOD) and catalase (CAT) were determined. To prepare the leaf extract for enzyme analysis, 0.5 grams of fresh leaf material was homogenized in 2 mL of a solution containing 50 mM potassium phosphate buffer, 2 mM sodium EDTA, and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was then centrifuged at 12,000 rpm for 10 minutes at 4°C. The resulting supernatant was collected and stored at -20°C for subsequent catalase (CAT) and (SOD) assays (Şahin, 2019)

2.8.1 Determination of CAT Activity

CAT activity was measured using the method described by Lartillot et al. (1988). In each test tube, 3 ml of the reaction mixture, 50 mM of phosphate buffer (pH 7.0), 10 mM of H₂O₂, and 20 µl of the isolated enzyme were combined. The reaction was terminated by adding 0.5 ml of 1 M HCL. The absorbance of the solution containing 2.5 ml of the reaction mixture and 0.5 ml of 1M HCL was measured spectrophotometrically at 240 nm to detect the initial absorbance of H₂O₂ (AS 0.467). To detect protein absorbance (AT), the absorbance of a solution containing 20 µl enzyme extract, 2.5 ml buffer, and 0.5 ml 1 M HCl was measured spectrophotometrically. The following formula

was used to determine the enzymatic activity based on changes in absorbance:

$$A = \text{Seedling length } (A_s + A_t) - A_r$$

The enzyme activity solution in U/ml-1/ mg protein was calculated.

$$\text{Activity} = \text{Seedling length } (A \times VT) / (E \times t \times V_e) \times (\text{mg protein})$$

V_t = The total volume of reaction (ml)

V_e = Volume of enzyme extraction (ml)

ϵ = extinction coefficient of H₂O₂

t = Reaction time (min)

2.8.2 Determination SOD Activity

The activity of SOD was determined according to the method described by Sun et al. (1988). The inhibition of nitroblue tetrazolium reduction (NBT) was used, with xanthine oxidase as a SOD generator (EC 1.15.1.1). The following reagents were prepared: 20 ml of 0.6 mM EDTA solution, 20 ml of 150 mM nitroblue tetrazolium solution, 12 ml of 400 ml of Na₂CO₃ solution, and 6 ml of BSA (1 g/l). These were combined in a 200 ml beaker. For each test tube, 1.425 mL of SOD assay reagent was mixed with 0.05 mL of the extracted enzyme and incubated in a water bath at 25 °C. The formation of formazan was measured using a spectrophotometer at 560 nm. Inhibition values were determined using the equation below:

$$\% \text{ Inhibition} = (\text{Absorbance blank} - \text{Absorbance sample})$$

$$\text{Activity (U/ml)} = (\% \text{ Inhibition}) / (50 \times 0.1)$$

$$\text{Specific} = (\text{Activity (U/ml)}) / (\text{mg/ml protein})$$

2.8.3 Determination of Hydrogen Peroxide

The hydrogen content was measured using the method by Velikova et al. (2000) with the following steps: 0.5g of fresh wheat leaf tissue was homogenized separately in 5 mL of 0.1 % trichloroacetic acid (TCA). The homogenate was then centrifuged at 10000xg for 15 min at 4°C. 1 ml of the supernatant, 1 ml of 10 mM phosphate buffer (pH 7.0), and 2 ml of 1M KI were combined to obtain a total of 4 ml of the assay mixture. The extinction coefficient (ϵ) of 0.28 $\mu\text{mole}^{-1} \text{ cm}^{-1}$ was used to identify the H₂O₂ content, expressed as $\mu\text{mol g}^{-1}$ fresh weight (fwt). The absorbance was determined

spectrophotometrically at 390 nm. The level of H_2O_2 was measured using a standard curve.

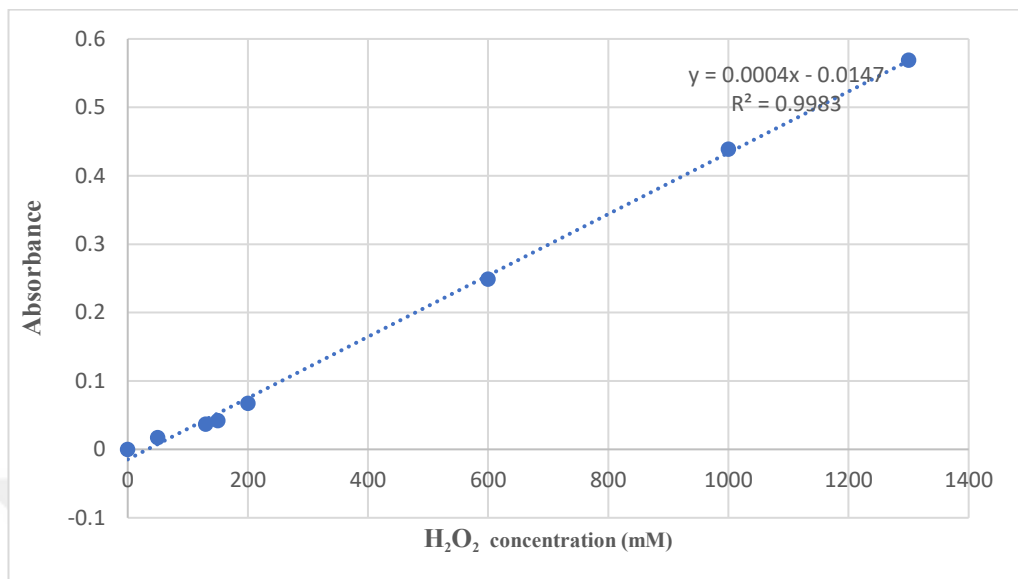


Figure 2.4. Standard curve of H_2O_2

2.9 Determination of Elemental Composition and Pb Toxicity in Wheat Landraces

The elemental content in wheat landrace cultivars was measured according to the method described by Alhagri & Albeshry (2023) and Albeshry (2023). A 0.5 g sample of dry leaf wheat was subjected to lead nitrate concentrations of 100 μM and 500 μM , excluding the control group for comparison purposes. The samples underwent digestion using Milestone microwave equipment. The process was conducted under a pressure of 100 bar at a temperature of 300°C for 54 minutes. The digestion process used 3 mL of reagent-grade H_2O_2 (33% (w/v)) and 9 mL of 8 mol/L nitric acid (65%) (Sigma-Aldrich, Germany). Wheat flour from the National Institute of Standards and Technology (NIST, USA) was used as the standard reference material (SRM 1567b). Calibration solutions were prepared by serial dilution of an ICP multielement standard solution stock solution containing Pb, Mg, K, Ca, P, Fe, and K, with an average concentration of 10 mg/L.

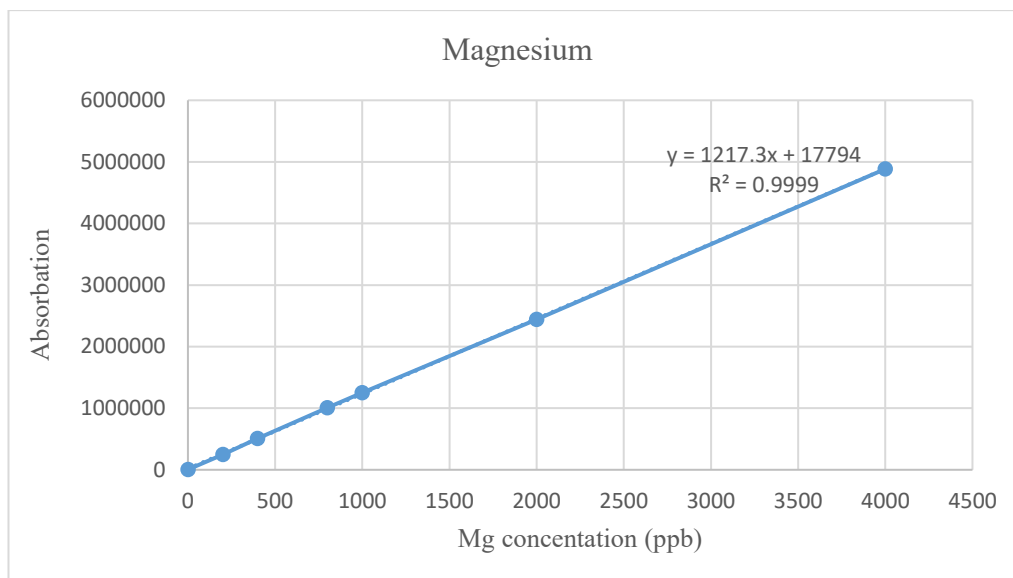


Figure 2.5. Magnesium standard curve in wheat varieties leaves

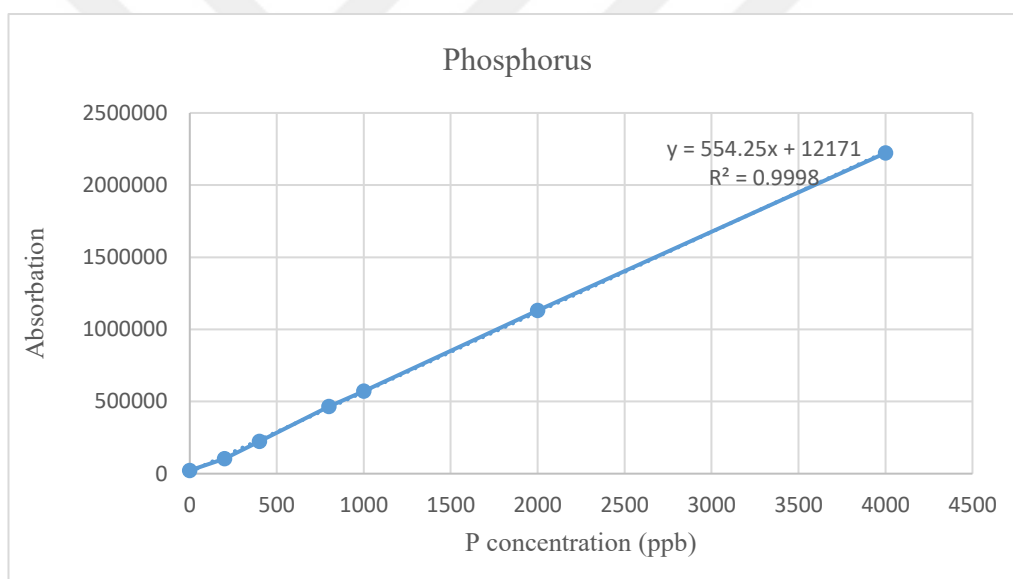


Figure 2.6. Phosphorus standard curve in wheat varieties leaves

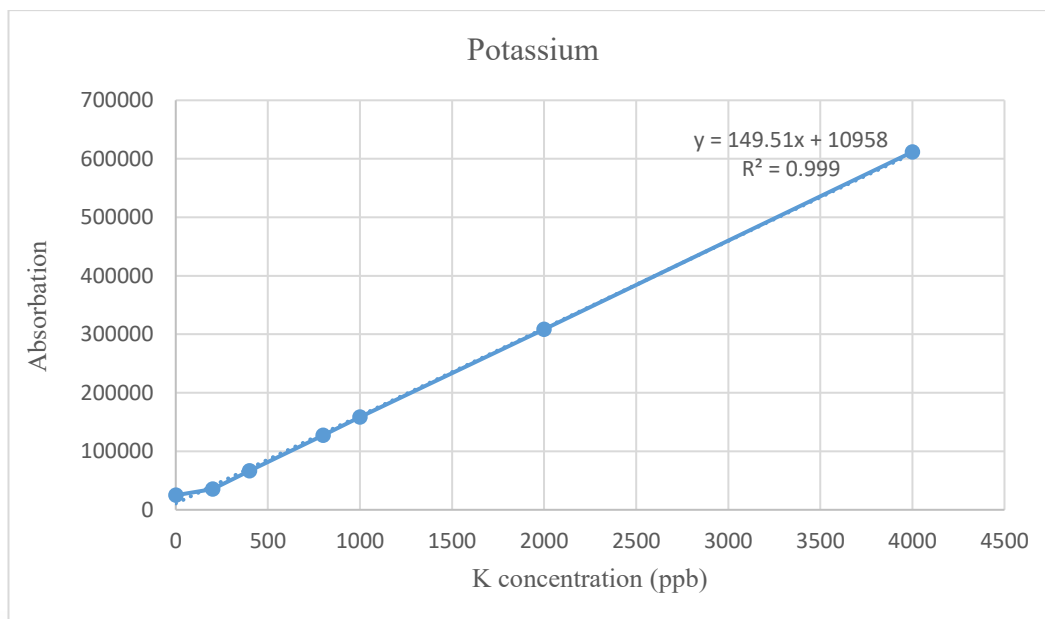


Figure 2.7. Potassium standard curve in wheat varieties leaves

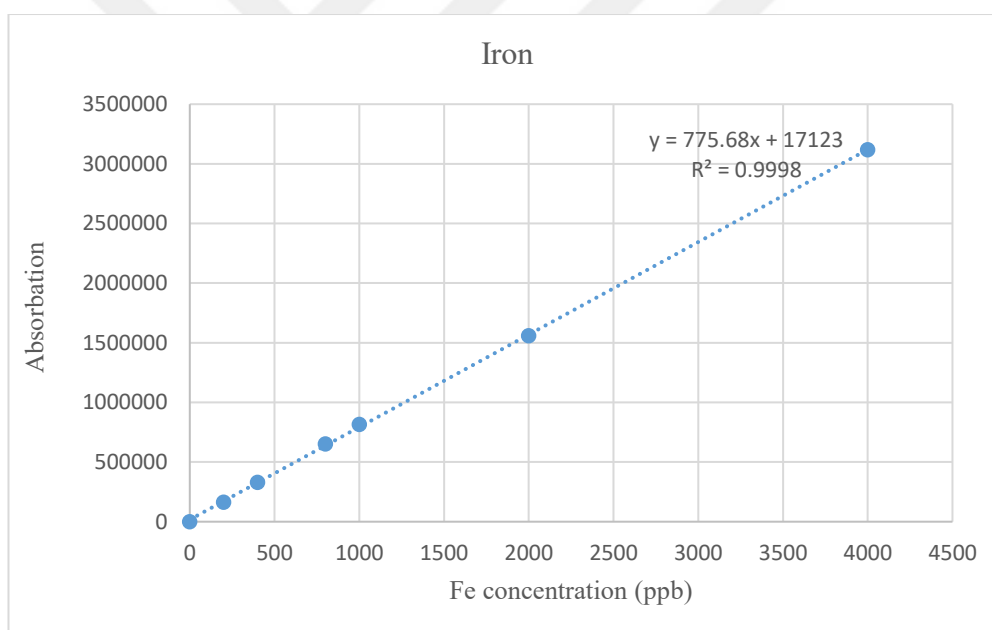


Figure 2.8. Iron standard curve in wheat varieties leaves

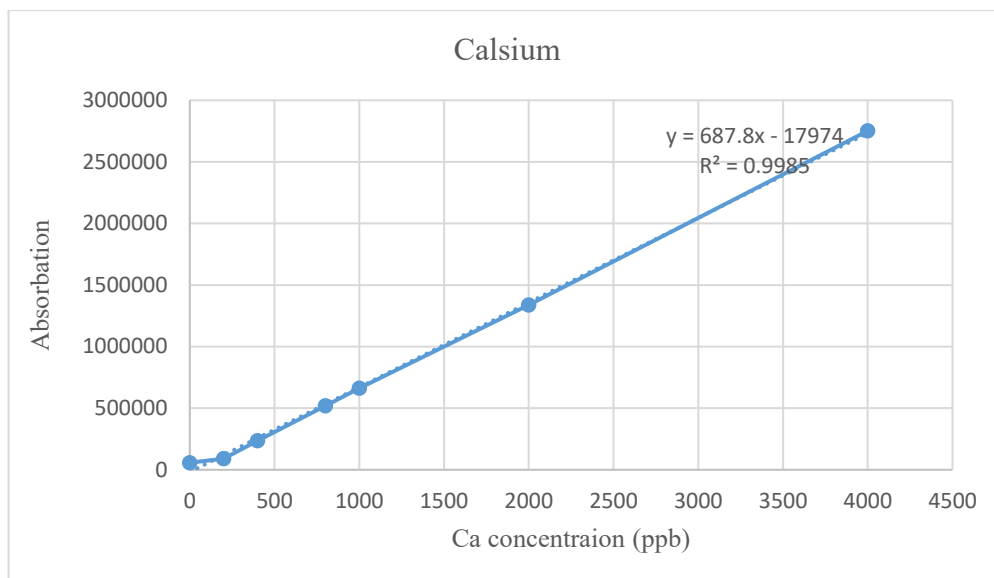


Figure 2.9. Calcium standard curve in wheat varieties leaves

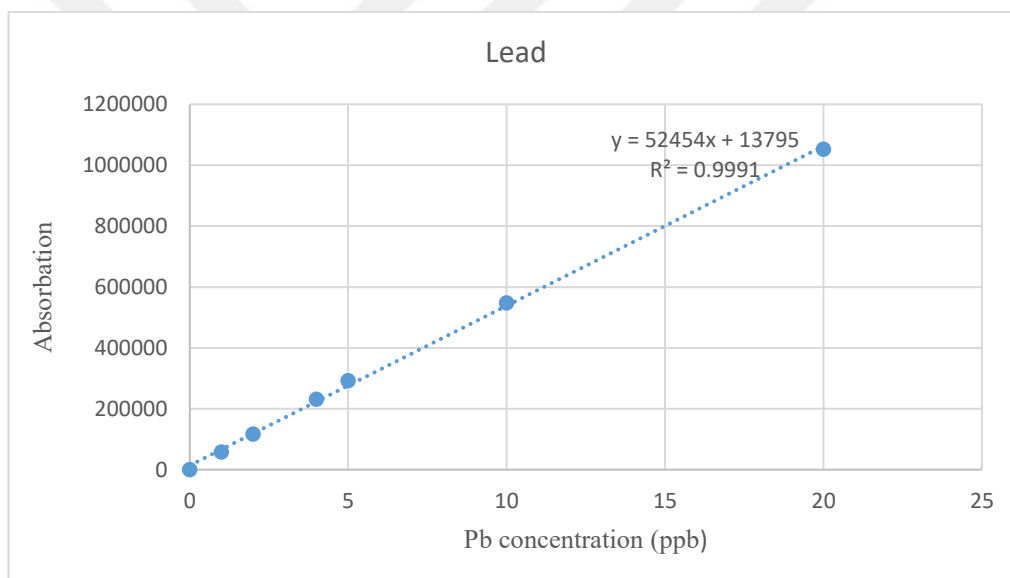


Figure 2.10. Lead standard curve in wheat varieties leaves

2.10 Measurement of the Water Holding Capacity (WHC) of the Soil

The water holding capacity (WHC) of the soil sample was determined using the funnel method described by Bernard (1963) with the following procedure: the soil samples were placed in the funnel with a 100 mL volume, supported by filter paper mounted on the top of a graduated cylinder (500 mL volume). Each sample was then saturated with 100 ml of water and left to drain for 72 hours. The volume of water collected in the graduated cylinder was recorded. The WHC is calculated using the following equation:

$$WHC = (A - B)$$
$$WHC (\%) = \frac{\text{Volume of water retained by soil (V1 - V2)}}{\text{Weight of the sample soil}} \times 100$$

2.11 Statistical Analysis

The data were evaluated by an ANOVA test mean comparison (Duncan's Multiple Range Test) in IBM SPSS Version: 26.0. Statistical significance was set at $P \leq 0.05$ for the lead treatments. Growth parameters were measured in three replicates using Petri dishes. Additionally, physiological traits, including photosynthetic pigments (Chlorophyll a, Chlorophyll b, and Carotenoids), proline, protein content, H_2O_2 levels, and antioxidant enzyme activities (CAT and SOD), were analyzed in three replicates using soil samples.

3. RESULTS

3.1 Effect of $\text{Pb}(\text{NO}_3)_2$ on Germination Parameters of Wheat

Landraces in Petri Dishes

The effect of different concentrations of $\text{Pb}(\text{NO}_3)_2$ on the germination parameters of wheat landraces after 8 days in a petri dish is shown in Table 3.1. These parameters include germination percentage (%), coleoptile length (mm), and root length (mm).

At 50 μM of lead, the highest increase in coleoptile length was observed in Einkorn, which increased by 10.68%, and Emmer, which increased by 5.12%, compared to Ekiz and Durum, which showed decreases of 0.99% and 4.54%, respectively. The Einkorn cultivar exhibited a 10.69% increase. In contrast, under 100 μM of lead, Ekiz, Emmer, and Durum showed decreases of 0.50%, 2.84%, and 5.4%, respectively.

At 250 μM of $\text{Pb}(\text{NO}_3)_2$, the wheat cultivars Ekiz and Emmer showed reductions of 1.98% and 2.54%, respectively, compared to Durum (7.52%) and Einkorn (8.84%). At 500 μM $\text{Pb}(\text{NO}_3)_2$, Emmer and Durum exhibited significant decreases in coleoptile length by 26.99% and 25.30%, respectively, while Ekiz and Einkorn showed decreases of 12.35% and 25.30%, respectively (Figure 3.1).

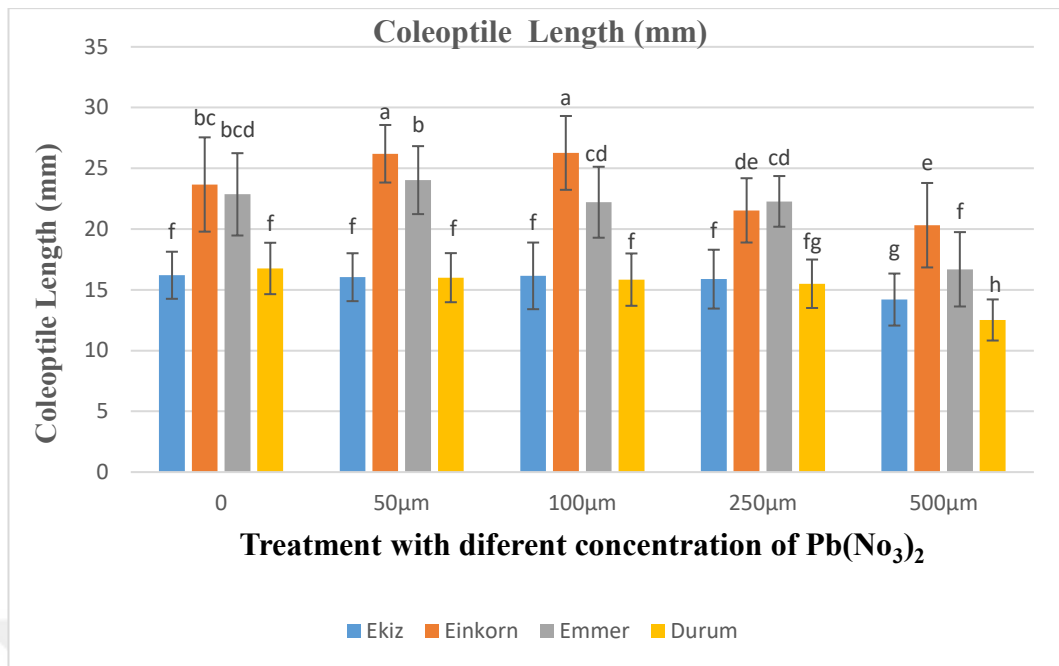


Figure 3.1. Impact of lead on the coleoptile length of wheat landraces in petri dishes

Table 3.1. Effect of Pb(NO₃)₂ on germination parameters of wheat species after 8 days of cultivation in petri dishes

Wheat Landraces	Pb concentration	Coleoptile length (mm)	Root length (mm)	Germination Percentage %
<i>Triticum aestivum</i> L. (Ekiz)	Control	16.2 ^f ±1.94	32.77 ^e ±4.63	100 ^a ± 0.00
	50µm	16.04 ^f ±1.97	28.75 ^f ±5.73	93.33 ^{abc} ±5.77
	100µm	16.15 ^f ±2.74	29.19 ^f ±6.70	90 ^{bcd} ±0.00
	250µm	15.88 ^f ±2.42	26.42 ^f ±5.53	86.67 ^{cd} ±5.77
	500µm	14.2 ^g ±2.14	19.36 ^g ±4.59	83.33 ^d ±5.77
<i>Triticum monococcum</i> ssp. <i>monococcum</i> L. (Einkorn)	Control	23.67 ^{bc} ±3.88	44.37 ^c ±6.61	100 ^a ±0.00
	50µm	26.2 ^a ±2.37	54.9 ^{ab} ±6.98	100 ^a ±0.00
	100µm	26.27 ^a ±3.04	57.8 ^a ±6.99	100 ^a ±0.00
	250 µm	21.54 ^{de} ±2.65	32.5 ^e ±5.05	93.33 ^{abc} ±5.77
	500µm	20.32 ^e ±3.48	20.39 ^g ±3.47	93.33 ^{abc} ±5.77
<i>Triticum dicoccum</i> Schrank (Emmer)	Control	22.86 ^{bcd} ±3.39	47 ^c ±4.58	96.66 ^{ab} ±5.77
	50µm	24.03 ^b ±2.80	57.34 ^{ab} ±9.03	96.67 ^{ab} ±5.77
	100µm	22.21 ^{cd} ±2.92	53.93 ^b ±7.55	96.67 ^{ab} ±5.77
	250µm	22.28 ^{cd} ±2.09	53.79 ^b ±5.68	96.67 ^{ab} ±5.77
	500µm	16.69 ^f ±3.06	33.31 ^e ±4.24	96.66 ^{ab} ±5.77
<i>Triticum turgidum</i> ssp. <i>durum</i> (Durum)	Control	16.76 ^f ±2.12	55.21 ^{ab} ±7.70	96.67 ^{ab} ±5.77
	50µm	16 ^f .00±2.02	43.92 ^c ±5.40	86.67 ^{cd} ±5.77
	100µm	15.84 ^f ±2.15	43.76 ^c ±8.38	83.33 ^d ±5.77
	250µm	15.5 ^{fg} ±1.99	39.36 ^d ±4.75	73.33 ^e ±5.77
	500µm	12.52 ^h ±1.69	28.67 ^f ±4.39	70 ^e ±0.00

Values indicate means ± SE from three replicates. According to Duncan's multiple range test, treatment means with different letters in the same column are significantly different

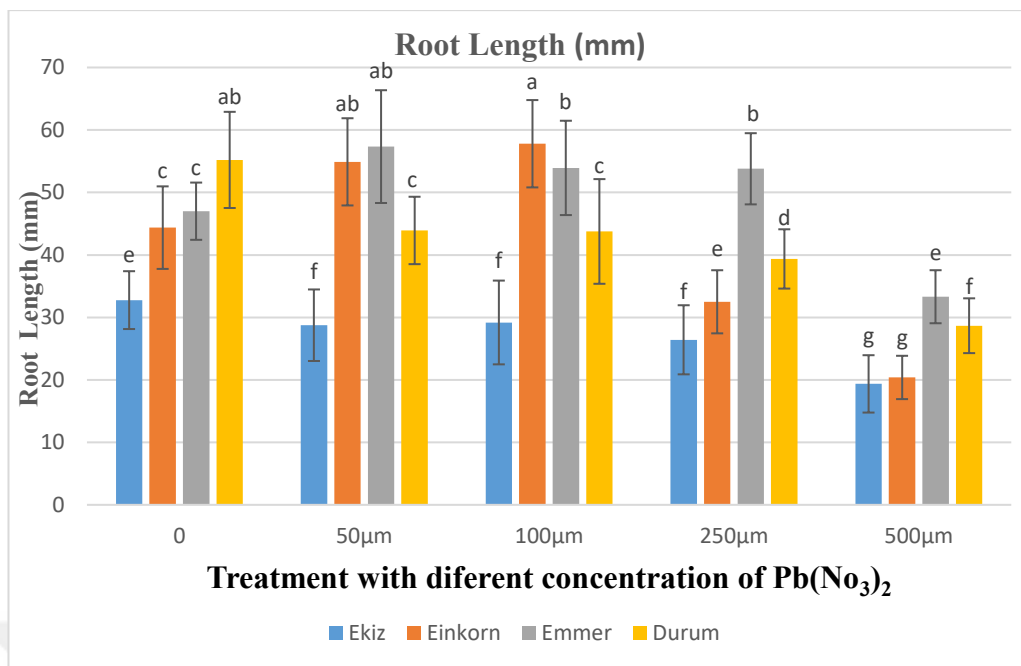


Figure 3.2. Effect of lead concentrations on the root length of wheat landrace in Petri dishes

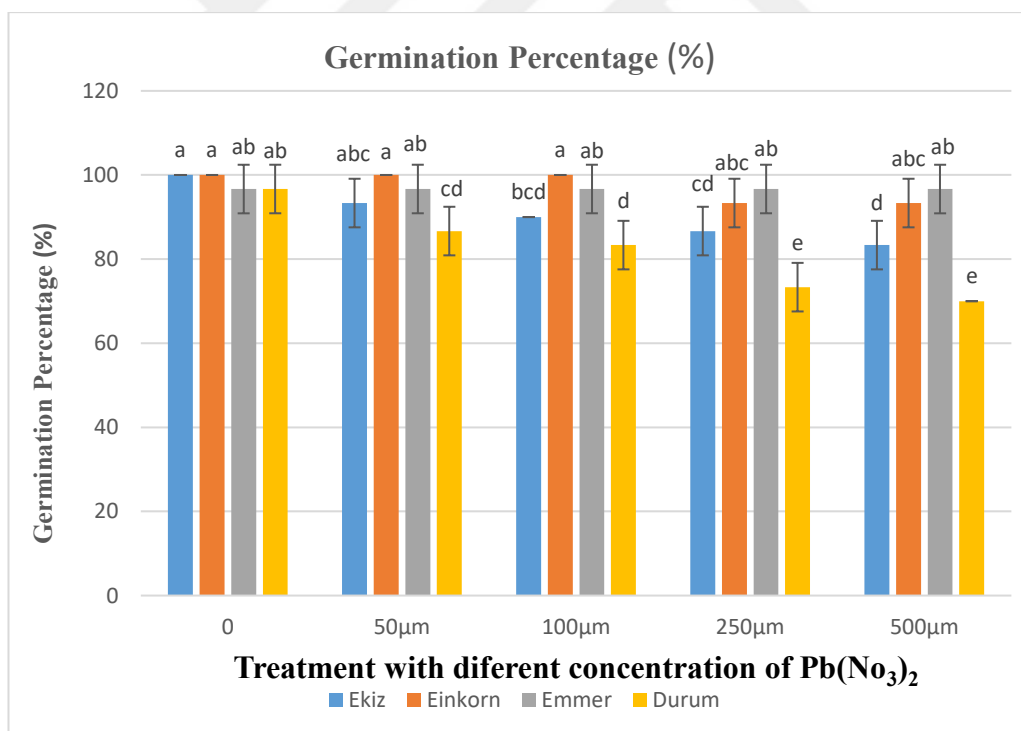


Figure 3.3. Effect of lead concentrations on germination percentage (%) of wheat landraces in petri dishes

The results showed that low concentrations of lead (50 μ M and 100 μ M) increased root length in Einkorn (50 μ M; $54.9^{ab} \pm 6.98$ mm and 100 μ M; $57.8^a \pm 6.99$ mm) compared to the control ($44.37^c \pm 6.61$ mm). On the other hand, Ekiz had reduced root length at 50 μ M ($28.75^f \pm 5.73$ mm) and 100 μ M ($29.19^f \pm 6.70$ mm) compared to the control ($32.77^e \pm 4.63$ mm). Durum also showed a reduction in root length at 50 μ M ($43.92^c \pm 5.40$ mm) and 100 μ M ($43.76^c \pm 8.38$ mm) compared to the control ($55.21^{ab} \pm 7.70$ mm). Similarly, at 250 μ M of lead, the root length was slightly affected across all wheat species (Table 3.1). However, as the lead concentration increased to 500 μ M, root length growth was significantly different with Einkorn (91.66%) and Emmer (89.21%) showing higher root growth compared to Ekiz and Durum (83.21%, 78.77%) (Figure 3.2). Germination percentage was not affected by 50 μ M and 100 μ M lead in all wheat landraces (Figure 3.3). However, at 250 μ M, Ekiz, Einkorn, and Emmer ($86.67^{cd} \pm 5.77$, $100^a \pm 0.00$, and $96.67^{ab} \pm 5.77$, respectively) had significantly different germination percentages compared to Durum ($73.33^e \pm 5.77$). Lead toxicity at 500 μ M significantly affected Durum ($70^e \pm 0.00$) and Ekiz ($83.33^d \pm 5.77$) more than Einkorn ($93.33^{abc} \pm 5.77$) and Emmer ($96.66^{ab} \pm 5.77$) (Table 3.1).

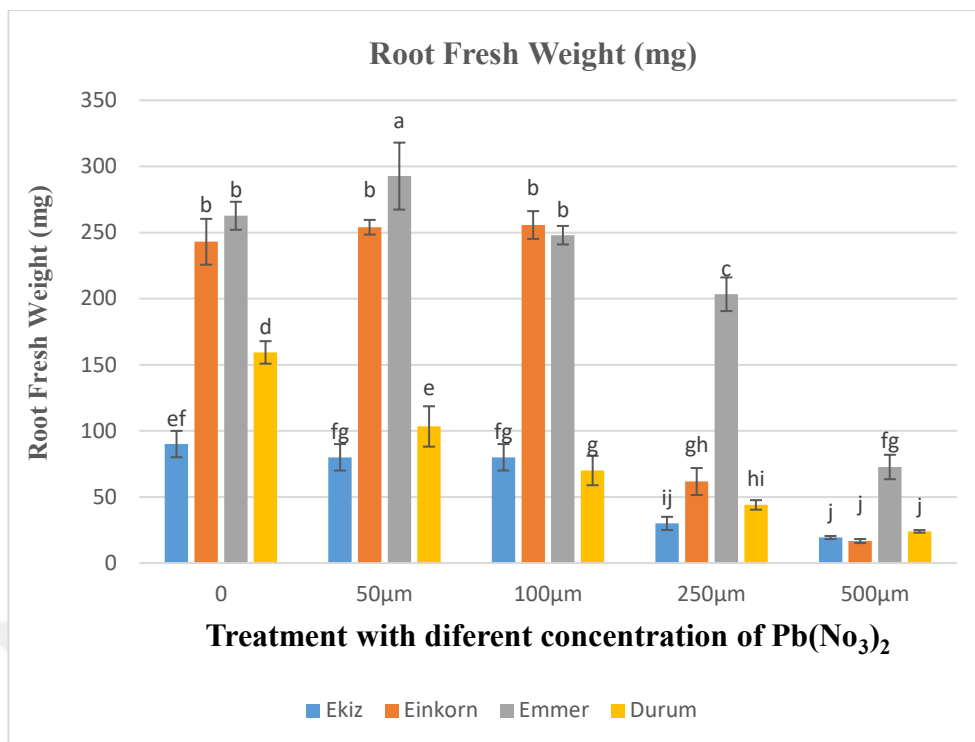


Figure 3.4. Effect of lead concentrations on root fresh weight (mg) of wheat landrace varieties in petri dishes

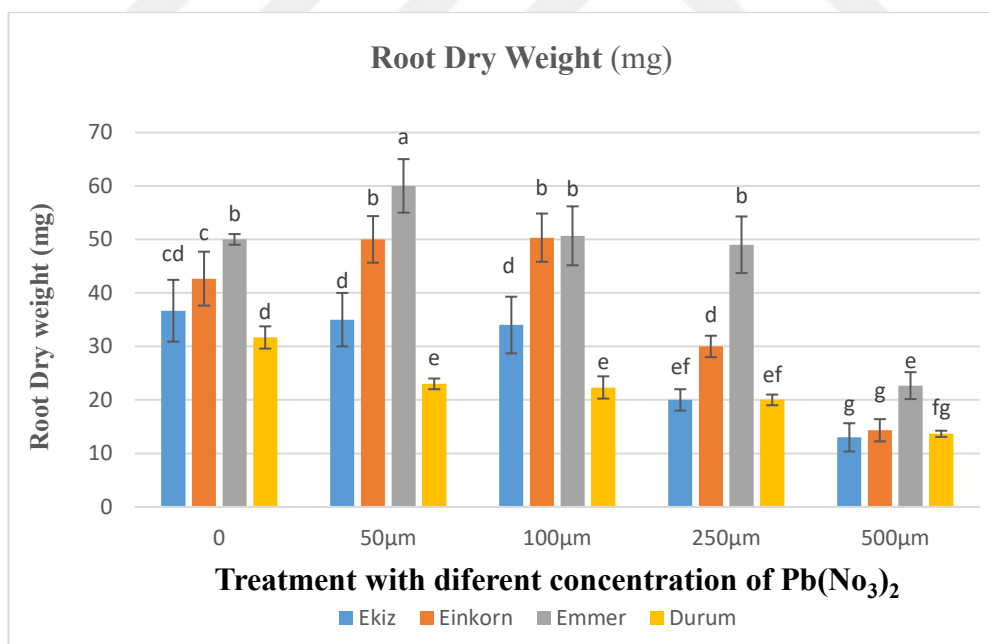


Figure 3.5. Effect of lead on root dry weight (mg) of wheat landraces in petri dishes

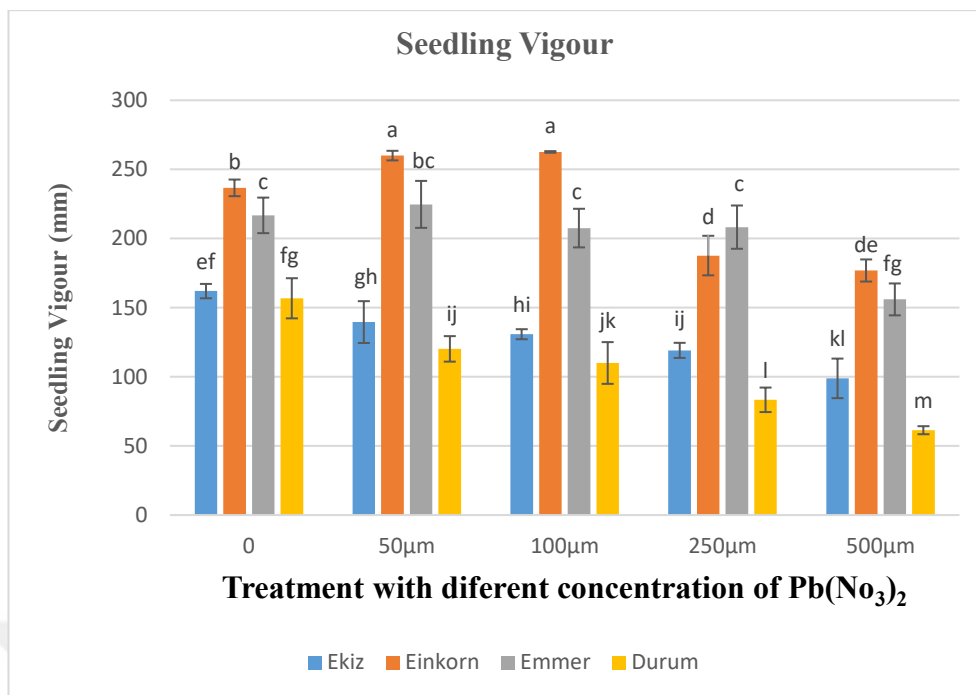


Figure 3.6. Effect of lead nitrate on seedling vigor (mm) of wheat landraces grown in petri dishes

Table 3.2. Effect of different concentrations on morphological features of wheat after 8 days of cultivation in petri dishes

Wheat Landraces	Pb Concentration	S.V.I	Fresh root weight (mg)	Dry root weight (mg)
<i>Triticum aestivum</i> L. (Ekiz)	Control	162 ^{ef} ±5.20	90 ^{ef} ±10.00	36 ^{cd} ± 5.77
	50µm	139.6 ^{gh} ±15.12	80 ^{fg} ±10.00	35 ^d ±5.000
	100µm	130.8 ^{hi} ±3.64	80 ^{fg} ±10.00	34 ^d ±5.29
	250µm	119.13 ^{ij} ±5.46	30 ^{ij} ±5.000	20 ^{ef} ±2.000
	500µm	98.87 ^{kl} ±14.31	19.33 ^j ±1.16	13 ^g ±2.65
<i>Triticum monococcum</i> ssp. <i>monococcum</i> L. (Einkorn)	Control	236.67 ^b ±6.03	243 ^b ±17.35	42.67 ^c ±5.03
	50µm	260 ^a ±3.46	254 ^b ±5.57	50 ^b ±4.36
	100µm	262.67 ^a ±0.58	255.67 ^b ±10.50	50.33 ^b ±4.51
	250 µm	187.7 ^d ±14.29	61.67 ^{gh} ±10.21	30 ^d ±2.000
	500µm	176.9 ^{de} ±7.995	16.67 ^j ±1.53	14.33 ^{fg} ±2.08
<i>Triticum dicoccum</i> Schrank (Emmer)	Control	216.77 ^c ±12.87	262.67 ^b ±10.60	50 ^b ±1.000
	50µm	224.73 ^{bc} ±16.98	292.67 ^a ±25.33	60 ^a ±5.000
	100µm	207.57 ^c ±13.97	248 ^b ±7.000	50.67 ^b ±5.51
	250µm	208.27 ^c ±15.65	203.33 ^c ±12.74	49 ^b ±5.29
	500µm	156 ^{fg} ±11.53	72.67 ^{fg} ±9.24	22.67 ^e ±2.52
<i>Triticum turgidum</i> ssp. <i>durum</i> (Durum)	Control	156.8 ^f ±14.52	159.33 ^d ±8.51	31.67 ^d ±2.08
	50µm	120.2 ^{ij} ±9.22	103.33 ^e ±15.28	23 ^e ±1.000
	100µm	110 ^{jk} ±15.10	70 ^g ±11.14	22.33 ^e ±1.000
	250µm	83.37 ^l ±8.86	44 ^{hi} ±3.61	20 ^{ef} ±1.000
	500µm	61.37 ^m ±2.91	24 ^j ±1.000	13.67 ^{fg} ±0.58

Values indicate means ± SE from three replicates. According to Duncan's multiple range test, treatment means with different letters in the same column are significantly different

3.1.1 Impact of Lead on Seedling Vigor Index (S.V.I), Root Fresh Weight (RFW), Root Dry Weight (RDW) in Wheat Landraces

The findings revealed that low concentrations of Pb (NO₃)₂ (50 µM and 100 µM) slightly increased the root fresh and dry weights of Einkorn and Emmer compared to Ekiz and Durum. The root fresh weight decreased as the concentration of lead increased. The highest percentage of root fresh weight (77.41%) was observed in Emmer at 250µM lead treatment, showing a significant difference compared to Ekiz (33%), Einkorn (25.37%), and Durum (27.62%) (Figure 3.4). Root dry weight was significantly affected at 250 µM of lead, with Ekiz (20^{ef}±2.00 mg), Einkorn (30^d±2.00 mg), and Durum (20^{ef}±1.00 mg) showing lower values compared to Emmer (49^b±5.29 mg) (Table 3.2). At the highest lead concentrations (500 µM), root fresh and dry weights were reduced in all wheat landraces compared to the control. Root fresh weight of Ekiz, Einkorn, and Durum was significantly affected (21.48%, 6.86%, 15.06%) more than Emmer (27.67%) (Figure 3.5). The root dry weight of Emmer (22.67^e±2.52 mg) and Durum (13.67^{fg}±0.58) at 500 µM lead treatment was significantly higher than Ekiz (13^g±2.65 mg), and Einkorn (14.33^{fg}±2.08 mg) (Table 3.2). Seedling Vigor decreased as the concentration of Pb(NO₃)₂ increased. The effect was observed at 50 µM and 100 µM in Ekiz (139.6^{gh} ± 15.12, 130.8^{hi} ± 3.64) and Durum (120.2^{ij} ± 9.22, 110^{ik} ± 15.10) compared to their respective controls (162^{ef} ± 5.20, 156.8^f ± 14.52) (Table 3.2). However, Einkorn and Emmer wheat species showed higher seedling vigor at 50 µM and 100 µM lead treatments compared to the control. The seedling vigor inhibition of Emmer (3.2%) was less negatively affected than that of other wheat species: Ekiz (26.47%), Einkorn (20.69%), and Durum (34.64%) (Figure 3.6).

Under the 500 µM lead treatment, Einkorn and Emmer (176.9^{de} ± 7.995, 156^{fg} ± 11.53) showed significantly higher seedling vigor compared to modern wheat species Ekiz and Durum (98.87^{kl} ± 14.3, 61.37^m ± 2.91) (Table 3.2).

3.2 Effect of Lead on Morphological Attributes of Wheat Species in Soil

The morphological traits of wheat landraces grown in soil, including germination percentage, shoot, root elongation, shoot and root toxicity, tolerance index, root and shoot length, and fresh and dry weights of shoot and root, were significantly affected by lead treatments. Additionally, the ability of these wheat cultivars to cope with lead-induced stress varied depending on the specific cultivar.

Table 3.3. Effect of different treatments of lead nitrate on germination parameters of wheat landraces after 28 days of cultivation in the soil

Wheat Landraces	Pb concentration	Shoot Elongation(mm)	Root Elongation(mm)	Germination(%)
<i>Triticum aestivum</i> L. (Ekiz)	Control	322 ^{bcd} ±44.88	99.15 ^a ±17.15	100 ^a ± 0.00
	50µm	332.17 ^{abcd} ±44.40	94 ^{abc} ±14.29	100 ^a ± 0.00
	100µm	340.67 ^{ab} ±45.73	92.92 ^{bcd} ±15.90	100 ^a ± 0.00
	250µm	319.42 ^{de} ±32.75	89.04 ^{cde} ±9.95	86.67 ^{cd} ±2.89
	500µm	280.2 ^h ±39.15	82.5 ^{fg} ±11.96	85 ^d ±0.00
<i>Triticum monococcum</i> ssp. <i>monococcum</i> L. (Einkorn)	Control	312.33 ^{ef} ±44.32	73 ^{hi} ±12.70	100 ^a ±0.00
	50µm	339.12 ^{abc} ±43.52	82.81 ^{efg} ±16.72	95 ^{ab} ±5.000
	100µm	340.88 ^{ab} ±42.23	82.46 ^{fg} ±17.04	95 ^{ab} ±5.000
	250 µm	297.82 ^{fg} ±30.65	67.31 ^{ij} ±8.68	91.67 ^{abcd} ±2.89
	500µm	294.36 ^{gh} ±26.51	66.91 ^j ±9.55	91.67 ^{abcd} ±2.89
<i>Triticum dicoccum</i> Schrank (Emmer)	Control	331.67 ^{abcd} ±44.42	93.5 ^{abc} ±14.99	100 ^a ±0.00
	50µm	348.07 ^a ±46.88	95.26 ^{ab} ±17.54	95 ^{ab} ±0.00
	100µm	340.52 ^{ab} ±43.100	98.45 ^{ab} ±16.63	96.67 ^{ab} ±5.77
	250µm	328.07 ^{bcd} ±37.25	88.33 ^{cdef} ±14.86	95 ^{ab} ±8.660
	500µm	293.33 ^{gh} ±37.27	83.70 ^{ef} ±12.78	90 ^{bcd} ±8.66
<i>Triticum turgidum</i> ssp. <i>durum</i> (Durum)	Control	319.08 ^{de} ±39.40	87.12 ^{def} ±16.79	98.33 ^{ab} ±2.89
	50µm	297.5 ^{fg} ±41.75	84.90 ^{ef} ±16.32	96.67 ^{ab} ±2.89
	100µm	289.82 ^{gh} ±40.83	83.21 ^{efg} ±14.19	93.33 ^{abc} ±2.89
	250µm	262.62 ⁱ ±42.83	77.38 ^{gh} ±13.63	70 ^c ±5.000
	500µm	245.25 ^j ±37.42	68.62 ^{ij} ±14.19	66.67 ^e ±7.63

Values indicate means ± SE from three replicates. According to Duncan's multiple range test, treatment means with different letters in the same column are significantly different

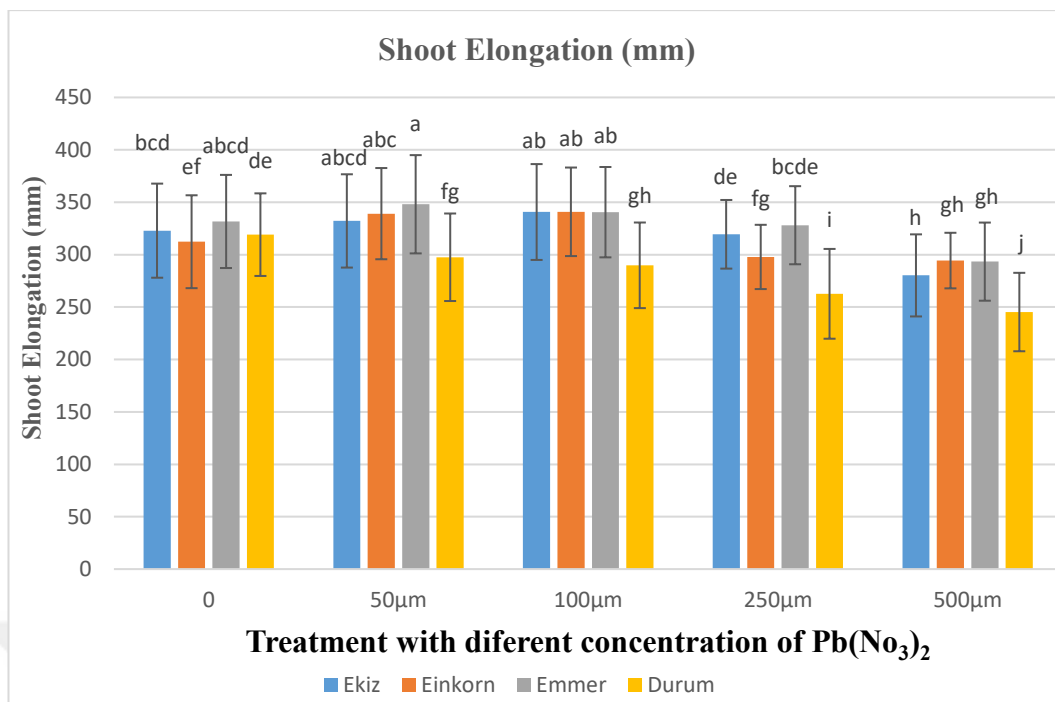


Figure 3.7. Effect of lead concentrations on shoot elongation of wheat landrace cultivars grown in soil

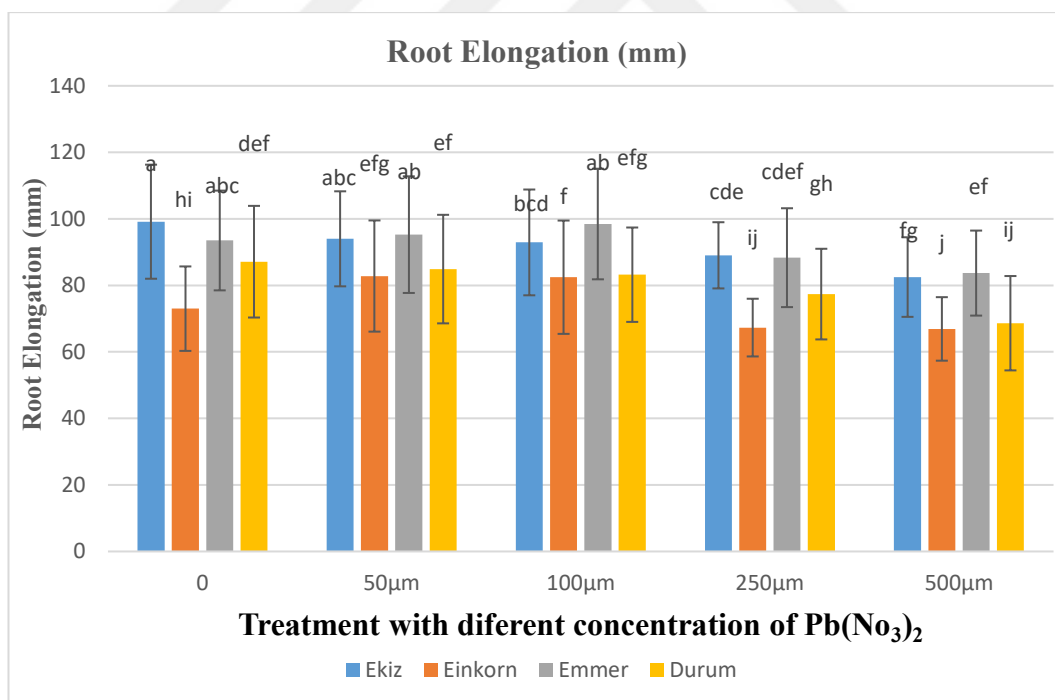


Figure 3.8. Effect of lead concentrations on root elongation of wheat landrace varieties cultivated in soil

3.2.1 Effect of Lead Nitrate on Germination Parameters of Wheat Cultivars in Soil.

Shoot elongation was more affected in Durum than in Ekiz, Einkorn, and Emmer wheat landrace cultivars at $\text{Pb}(\text{NO}_3)_2$ concentrations (50 μM and 100 μM). The shoot length was significantly reduced in Durum by 17.69% compared to Ekiz, Einkorn, and Emmer, which showed reductions of 1.07%, 4.65%, and 1.09% at 250 μM of lead nitrate concentration (Figure 4.7). Lead toxicity at 500 μM significantly affected the shoot elongation of Durum ($245.25^{\text{j}} \pm 37.42$ mm) in comparison to other wheat landraces; Ekiz, Einkorn, Emmer ($280.2^{\text{h}} \pm 39.15$ mm, $294.36^{\text{gh}} \pm 26.51$ mm, $293.33^{\text{gh}} \pm 37.27$) (Table 3.3).

The interaction between wheat landraces and lead treatments (Figure 3.8) revealed that 50 μM and 100 μM lead nitrate concentrations slightly affected Ekiz and Durum wheat cultivars' root elongation compared to the control. On the other hand, Einkorn and Emmer's wheat cultivars were positively affected. Root elongation of cultivars of Ekiz and Durum ($89.04^{\text{cde}} \pm 9.95$ mm, $77.38^{\text{gh}} \pm 13.63$ mm) was significantly affected in comparison to their controls, while Einkorn and Emmer ($297.82^{\text{fg}} \pm 30.65$ mm, $328.07^{\text{bcde}} \pm 37.25$ mm) were less affected than their controls ($73^{\text{hi}} \pm 12.70$ mm, $93.5^{\text{abc}} \pm 14.99$ mm), under 250 μM lead nitrate. Root elongation in Durum wheat significantly decreased ($68.62^{\text{ij}} \pm 14.19$ mm) at 500 μM of lead compared to the other wheat landraces, Emmer, Ekiz, and Einkorn ($83.70^{\text{ef}} \pm 12.78$ mm, $82.5^{\text{fg}} \pm 11.96$ mm, $66.9^{\text{lj}} \pm 9.55$ mm) (Table 3.3).

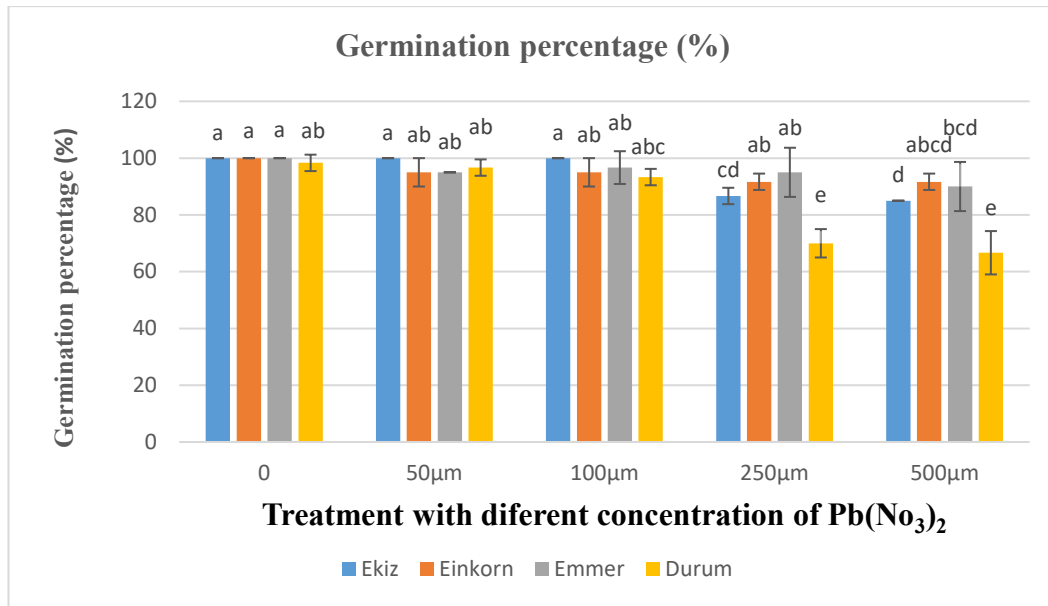


Figure 3.9. Effect of lead nitrate concentration on germination percentage (%) of wheat landrace varieties cultivated in soil

The germination percentage (%) of wheat cultivars was unaffected by low lead nitrate treatments (50 µM and 100 µM). However, at 250 µM of Pb (NO₃)₂, the germination percentage was affected in Emmer (95%), and Einkorn (91.67%) compared to Ekiz (86.67%), Durum (70%) (Figure 3.9). Durum wheat germination percentage ($66.67^{\pm 7.63}$) was significantly affected by lead nitrate at 500 µM compared with Einkorn ($91.67^{abcd \pm 2.89}$), Emmer ($90^{bcd \pm 8.66}$), Ekiz ($85^d \pm 0.00$) (Table 4.3).

3.2.2 Lead phytotoxicity on germination parameters in wheat landraces

Wheat landraces (Ekiz, Einkorn, Emmer) exhibit no shoot toxicity at low lead nitrate concentrations (50 μ M and 100 μ M) as compared to Durum wheat ($6.88^f \pm 0.44$, $9.33^e \pm 0.53$). Similarly, with increasing lead treatment at 250 μ M, shoot toxicity in Durum wheat ($18.05^b \pm 1.42$) was more significant than in Ekiz ($0.87^{ij} \pm 0.19$), Einkorn ($4.63^h \pm 0.60$), and Emmer ($1.10^i \pm 0.13$). The lowest shoot toxicity was observed in Einkorn ($5.75^s \pm 0.31$) compared to Emmer ($11.53^d \pm 0.61$), Ekiz ($13.46^c \pm 0.19$), and Durum ($23.47^a \pm 1.26$) under the 500 μ M lead nitrate concentration (Table 3.4 and Figure 3.10).

Root toxicity was not observed in Einkorn and Emmer at lead nitrate at 50 μ M and 100 μ M compared to Ekiz ($5.05^{ef} \pm 0.51$, $6.25^e \pm 0.74$) and Durum ($2.68^s \pm 0.37$, $4.64^f \pm 0.49$). At 250 μ M lead treatment, Emmer and Einkorn showed less root toxicity ($7.77^d \pm 1.52$, $5.53^{ef} \pm 0.94$) compared to Ekiz and Durum ($10.06^c \pm 0.32$, $11.49^c \pm 2.09$), respectively. Durum and Ekiz wheat ($21.53^a \pm 2.12$, $16.12^b \pm 0.45$) were significantly affected by root toxicity in comparison to Einkorn and Emmer ($8.34^d \pm 0.45$, $10.47^c \pm 0.58$), respectively, under the 500 μ M lead nitrate treatment (Table 3.4 and Figure 3.11).

The tolerance index (%) was slightly impacted in Ekiz ($94.95^{bc} \pm 0.50$, $93.75^{cde} \pm 0.74$), Durum ($97.35^{ab} \pm 0.93$, $95.44^{bc} \pm 0.48$) at 50 μ M and 100 μ M of lead nitrate treatments compared to their control. However, Einkorn and Emmer wheat landraces were not affected. The high concentration of lead (250 μ M) showed an impact on the tolerance index (%) for each of the four wheat species: Emmer ($94.47^{cd} \pm 0.94$), Durum ($88.71^h \pm 2.05$), Einkorn ($92.23^{def} \pm 1.52$), and Ekiz ($89.94^{fgh} \pm 0.32$). Einkorn ($91.66^{efg} \pm 0.44$) and Emmer ($89.46^{gh} \pm 0.66$) showed higher tolerance at 500 μ M compared to Ekiz ($83.88^i \pm 0.45$) and Durum ($76.85^j \pm 5.54$) (Table 3.4 and Figure 3.12).

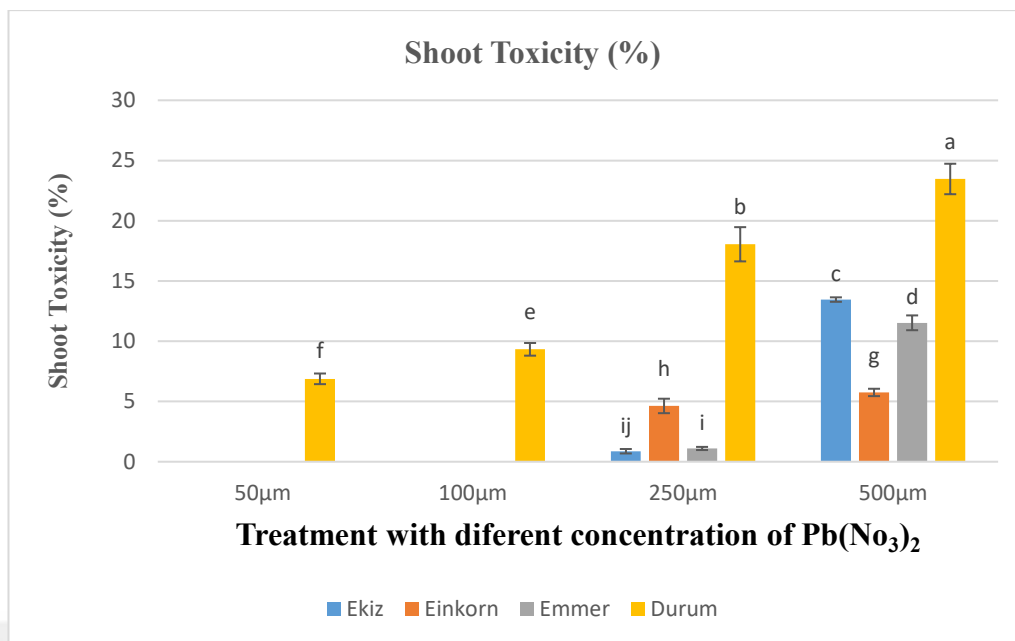


Figure 3.10. Effect of lead nitrate on shoot toxicity (%) of wheat landrace varieties cultivated in soil

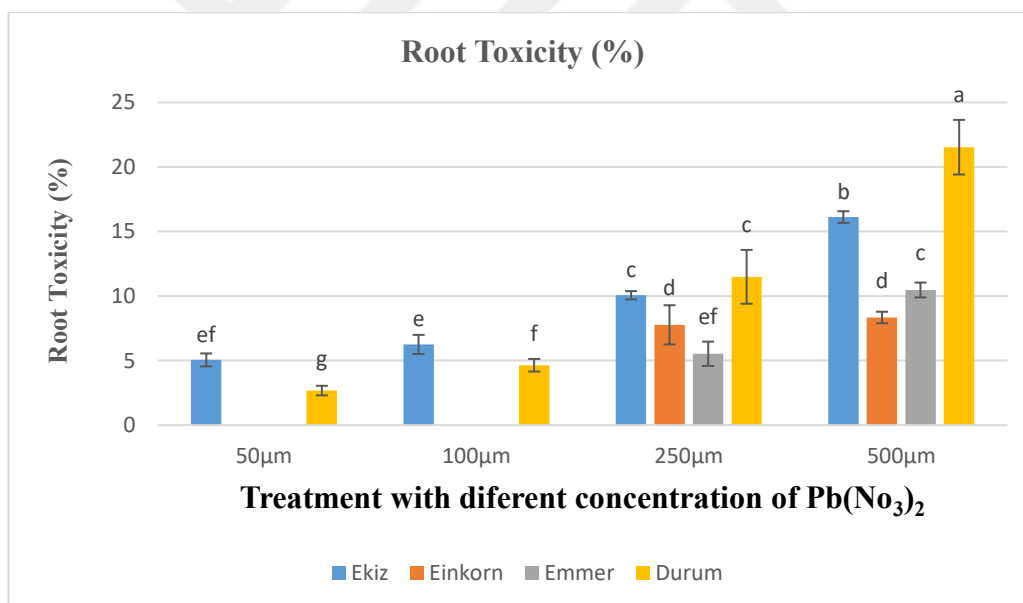


Figure 3.11. Effect of lead nitrate concentration on root toxicity (%) of wheat landrace varieties cultivated in soil

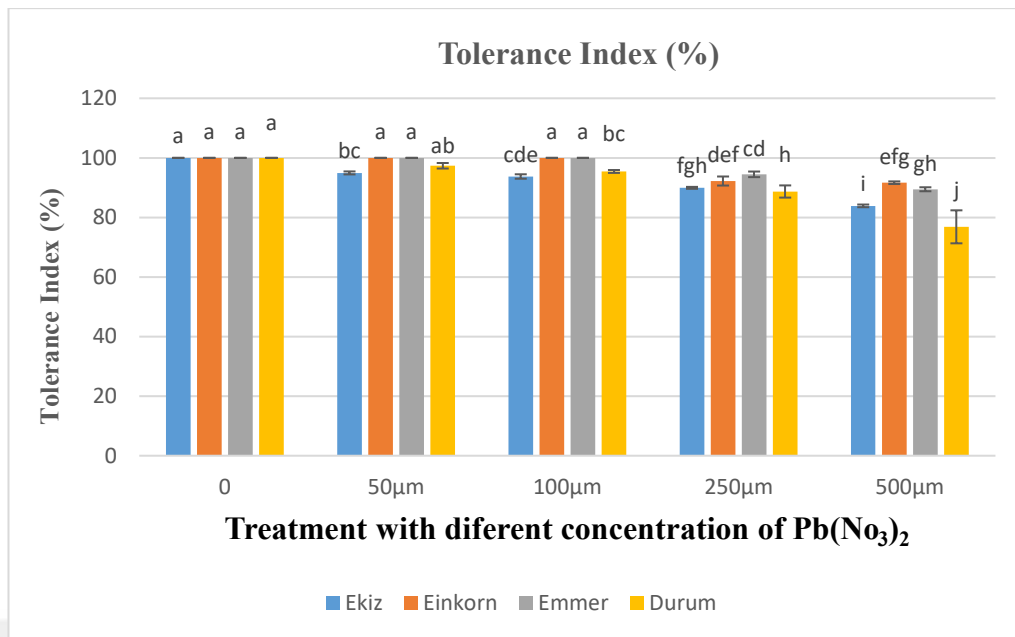


Figure 3.12. Impact of lead nitrate concentration on tolerance index (%) of wheat landrace varieties cultivated in soil

Table 3.4. Impact of different Pb(NO₃)₂ treatments on germination parameters of wheat landraces after 28 days of soil cultivation

Wheat Landraces	Pb Concentration	Shoot toxicity (%)	Root toxicity (%)	Tolerance index (%)
<i>Triticum aestivum</i> L. (Ekiz)	Control	0.00 ⁱ ±0.00	0.00 ^h ±0.00	100 ^a ± 0.00
	50µm	0.00 ⁱ ±0.00	5.05 ^{ef} ±0.51	94.95 ^{bc} ±0.50
	100µm	0.00 ⁱ ±0.00	6.25 ^c ±0.74	93.75 ^{cde} ±0.74
	250µm	0.87 ^{ij} ±0.19	10.06 ^c ±0.32	89.94 ^{fg} ±0.32
	500µm	13.46 ^c ±0.19	16.12 ^b ±0.45	83.88 ⁱ ±0.45
<i>Triticum monococcum</i> ssp. <i>monococcum</i> L. (Einkorn)	Control	0.00 ⁱ ±0.00	0.00 ^h ±0.00	100 ^a ±0.00
	50µm	0.00 ⁱ ±0.00	0.00 ^h ±0.00	100 ^a ±0.00
	100µm	0.00 ⁱ ±0.00	0.00 ^h ±0.00	100 ^a ±0.00
	250 µm	4.63 ^h ±0.60	7.77 ^d ±1.52	92.23 ^{def} ±1.52
	500µm	5.75 ^g ±0.31	8.34 ^d ±0.45	91.66 ^{efg} ±0.44
<i>Triticum dicoccum</i> Schrank (Emmer)	Control	0.00 ⁱ ±0.000	0.00 ^h ±0.00	100 ^a ±0.00
	50µm	0.00 ⁱ ±0.000	0.00 ^h ±0.00	100 ^a ±0.00
	100µm	0.00 ⁱ ±0.000	0.00 ^h ±0.00	100 ^a ±0.00
	250µm	1.10 ⁱ ±0.13	5.53±0.94	94.47 ^{cd} ±0.94
	500µm	11.53 ^d ±0.61	10.47 ^c ±0.58	89.46 ^{gh} ±0.66
<i>Triticum turgidum</i> ssp. <i>durum</i> (Durum)	Control	0.00 ⁱ ±0.000	0.00 ^h ±0.00	100 ^a ±0.00
	50µm	6.88 ^f ±0.44	2.68 ^g ±0.37	97.35 ^{ab} ±0.93
	100µm	9.33 ^e ±0.53	4.64 ^f ±0.49	95.44 ^{bc} ±0.48
	250µm	18.05 ^b ±1.42	11.49 ^c ±2.09	88.71 ^h ±2.05
	500µm	23.47 ^a ±1.26	21.53 ^a ±2.12	76.85 ^j ±5.54

Values indicate means ± SE from three replicates. According to Duncan's multiple range test, treatment means with different letters in the same column are significantly different

Table 3.5. Effect of different treatments of Pb(NO₃)₂ on the Biomass of wheat landraces after 28 days of cultivation in soil

Wheat Landraces	Lead	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)
<i>Triticum aestivum</i> L. (Ekiz)	Control	4960 ^c ±157.16	570 ^{de} ±20.00	1100 ^d ± 100	143.33 ^b ±15.28
	50µm	5006.67 ^{bc} ±61.10	583.33 ^{de} ±55.08	866.67 ^e ± 15.28	133.33 ^b ±11.55
	100µm	5056.67 ^{bc} ±376.47	600 ^d ±62.45	826.67 ^{ef} ± 20.82	110 ^c ±10.00
	250µm	4810 ^c ±60.00	530 ^{def} ±36.06	670 ^{gh} ±60.83	90 ^{cdef} ±10.00
	500µm	3796.67 ^{fg} ±55.08	376.67 ^{hi} ±15.28	613.33 ⁱ ±35.12	80 ^{efg} ±10.00
<i>Triticum monococcum</i> ssp. <i>monococcum</i> L. (Einkorn)	Control	4700 ^{cd} ±546.17	740 ^c ±88.88	926.67 ^e ±64.29	90 ^{cdef} ±10.00
	50µm	5513.33 ^b ±105.99	863.33 ^b ±92.92	1500 ^b ±235.16	140 ^b ±20.00
	100µm	6713.33 ^a ±236.29	1073.33 ^a ±124.23	2290 ^a ±173.21	176.67 ^a ±15.28
	250 µm	4230 ^{def} ±497.59	590 ^{de} ±78.10	710 ^{fg} ±10.00	82.33 ^{def} ±0.58
	500µm	4140 ^{ef} ±370.41	530 ^{def} ±95.39	696.67 ^{fg} ±70.95	82 ^{def} ±6.56
<i>Triticum dicoccum</i> Schrank (Emmer)	Control	4706.67 ^{cd} ±277.37	540 ^{def} ±43.59	1200 ^{cd} ±100.00	96.67 ^{cde} ±5.77
	50µm	4973.33 ^c ±491.66	593.33 ^d ±73.71	1306.67 ^c ±15.28	110 ^c ±10.00
	100µm	4930 ^c ±45.83	580 ^{de} ±10.00	1250 ^c ±26.46	106.67 ^c ±15.28
	250µm	3986.67 ^{fg} ±73.71	443.33 ^{fghi} ±20.82	940 ^e ±43.59	90 ^{cdef} ±10.00
	500µm	3903.33 ^{fg} ±240.28	400 ^{ghi} ±45.83	806.67 ^{efg} ±75.72	80 ^{efg} ±10.00
<i>Triticum turgidum</i> ssp. <i>durum</i> (Durum)	Control	4590 ^{cde} ±138.92	520 ^{def} ±26.46	936.67 ^e ±15.28	100.67 ^c ±8.33
	50µm	4580 ^{cde} ±180.00	500 ^{defg} ±36.06	653.33 ^h ±77.67	83 ^{def} ±7.810
	100µm	24560 ^{cde} ±377.23	473.33 ^{efgh} ±50.33	600 ^{hi} ±45.83	69.67 ^{fgh} ±8.08
	250µm	3406.67 ^g ±188.24	406.67 ^{ghi} ±20.82	486.67 ^{ij} ±30.55	62 ^{gh} ±7.550
	500µm	2580 ^h ±516.43	333.33 ⁱ ±66.55	386.67 ^j ±40.41	55 ^h ±3.606

Values indicate means $\pm$ SE from three replicates. According to Duncan's multiple range test, treatment means with different letters in the same column are significantly different

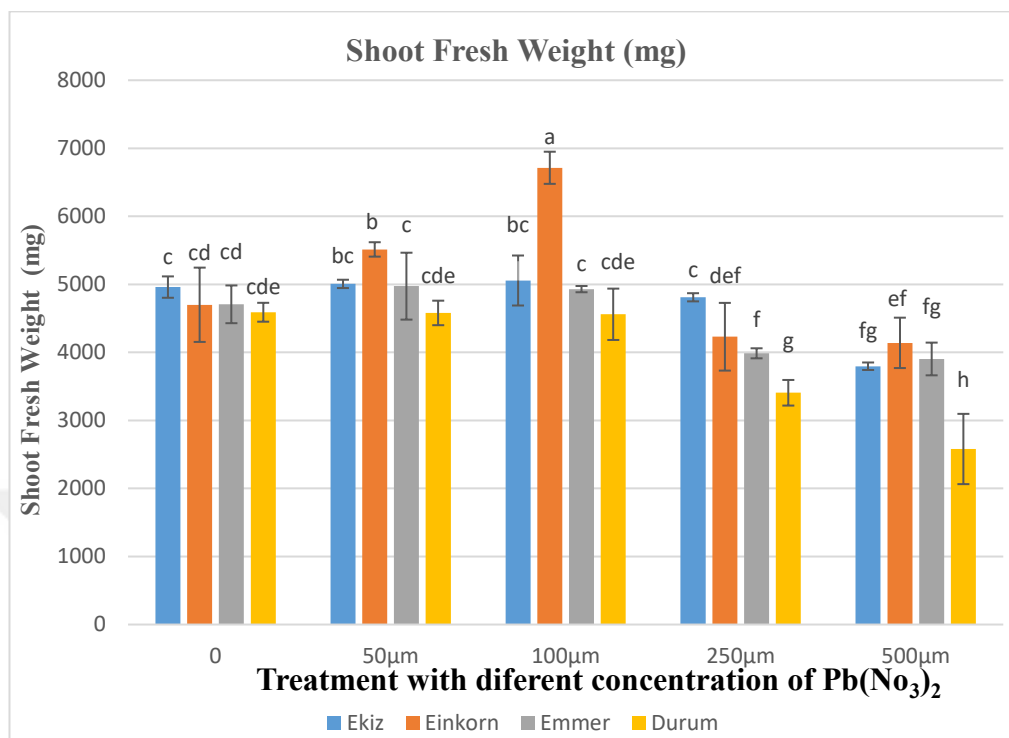


Figure 3.13. Effects of lead nitrate concentrations on shoot fresh weight (mg) of wheat landrace varieties cultivated in soil

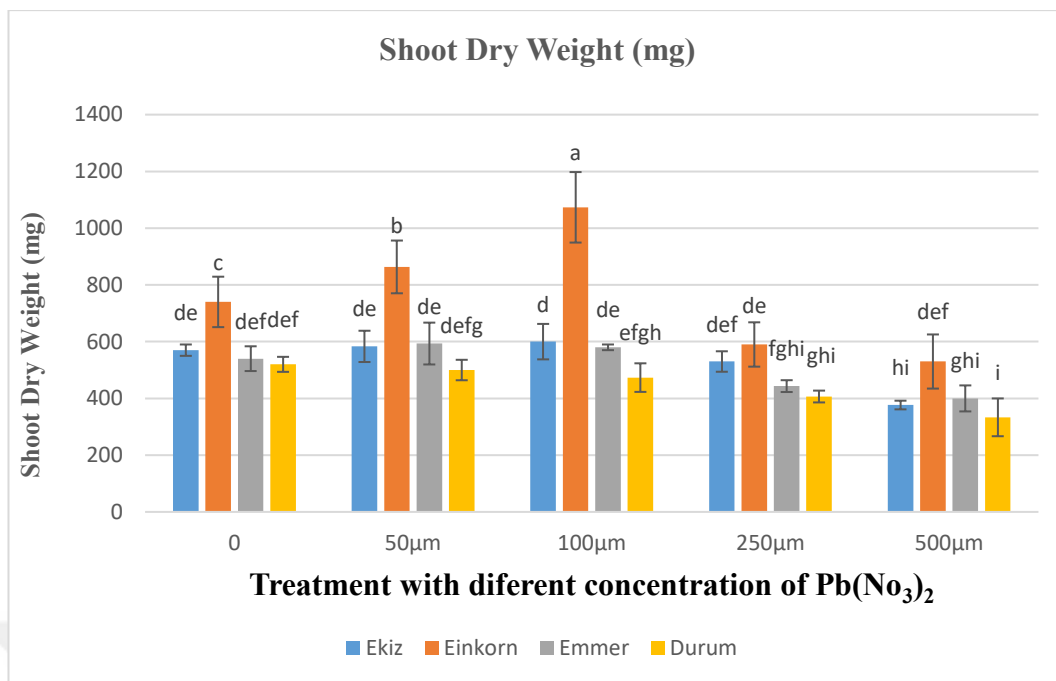


Figure 3.14. Impacts of lead nitrate concentrations on the shoot dry weight (mg) of wheat landrace cultivars grown in soil

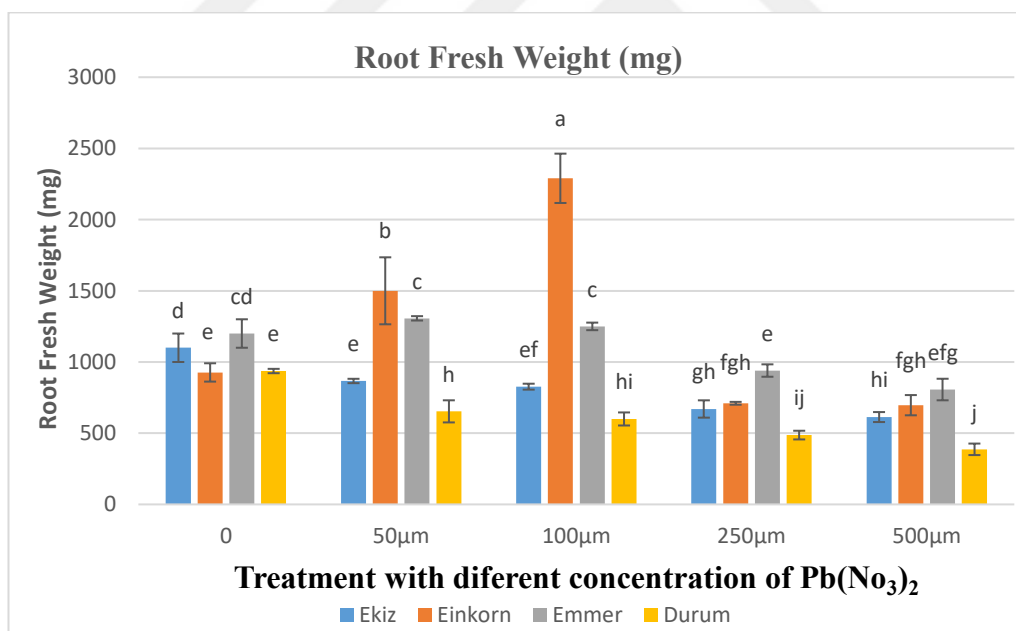


Figure 3.15. Effect of lead nitrate concentrations on root fresh weight (mg) of wheat landrace cultivars grown in soil

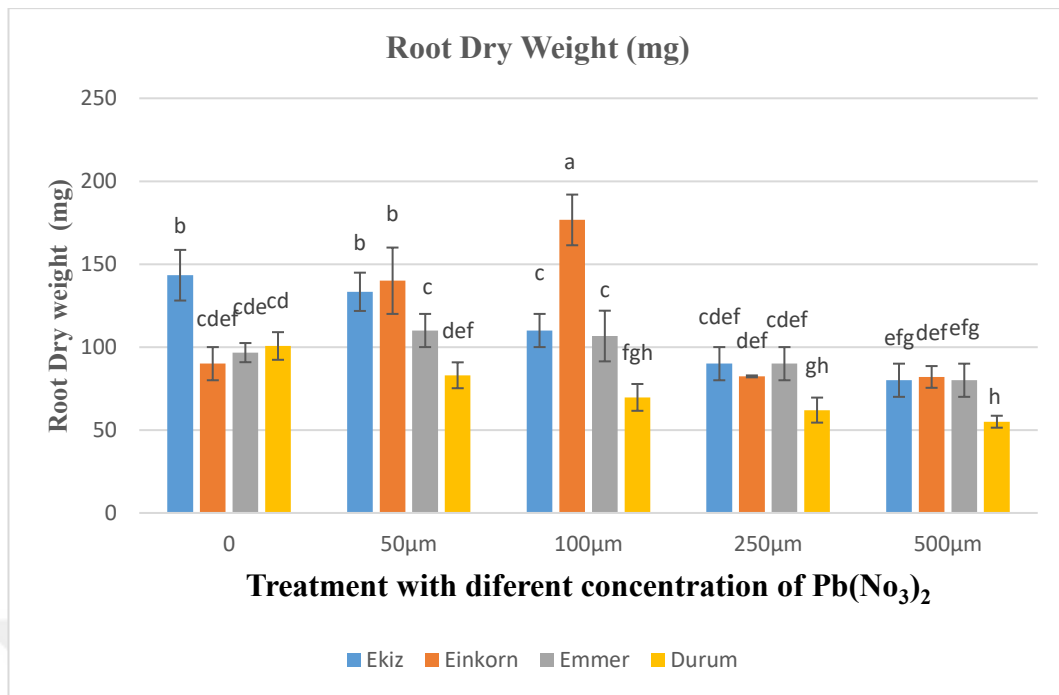


Figure 3.16. Effect of lead nitrate concentrations on root dry weight (mg) of wheat landrace cultivars grown in soil

3.2.3 Impact of Lead on Shoot Fresh Weight (SFW), Shoot Dry Weight (SDW), Root Fresh Weight (RFW), and Root Dry Weight (RDW) in Wheat Landraces

Increased lead nitrate concentrations in the medium at 50µM and 100µM affected shoot fresh weight (by 0.22% and 0.65%) and shoot dry weight (3.85% and 8.97%) in Durum wheat. On the other hand, these concentrations positively affect the shoot fresh and shoot dry weight of Ekiz, Einkorn, and Emmer. The results showed that Pb(NO₃)₂ (250 µM) significantly reduced fresh weight by 31.17 %, and shoot dry weight by 21.79% in Durum wheat, compared to Ekiz (decrease of 3.02%, and 7.02%), Einkorn (10% and 79.73%), Emmer (15.30% and 17.90%), respectively (Figure 3.13, 3.14). High lead nitrate concentrations reduced shoot fresh and dry weights (mg) at 500µM. Einkorn wheat shoot fresh weight (4140^{ef}±370.41 mg), shoot dry wheat (530^{def}±95.39 mg) were slightly affected compared to Emmer shoot fresh weight (3903.33^{fg}±240.28 mg), shoot dry weight (400^{ghi}±45.83 mg), Ekiz shoot fresh weight (3796.67^{fg}±55.08 mg), shoot dry weight (376.67^{hi}±15.28 mg), and Durum shoots fresh weight (2580^h±516.43 mg), shoot dry weight (333.33ⁱ±66.55 mg) (Table 3.5).

Root fresh and root dry weight were negatively affected by the increased Pb (NO₃)₂ concentrations in the medium. Root fresh weight of Ekiz (866.67^e± 15.28 mg, 826.67^{ef}± 20.82 mg), Durum (653.33^h±77.67 mg, 600^{hi}±45.83 mg) at 50 µM and 100 µM were reduced compared to control (1100^d± 100 mg, 936.67^e±15.28 mg), respectively. On the other hand, the root fresh weight of Einkorn (1500^b±235.16 mg, 2290^a±173.21 mg) and Emmer wheat (1306.67^c±15.28 mg, 1250^c±26.46 mg) was higher than their respective controls: Einkorn (926.67^e±64.29 mg), and Emmer (1200^{cd}±100 mg) (Table 3.5). The root fresh weight of Ekiz and Durum was significantly affected by 39.09%, and 48.04%, respectively, compared to Einkorn by 23.38%, and Emmer by 21.67% at 250 µM lead nitrate concentration. Ekiz and Durum root fresh weights were considerably affected by 55.76 % and 58.72% to lead nitrate at 500 µM lead nitrate concentration compared to Einkorn (24.82%) and Emmer (32.78%) (Figure 3.15).

The root dry weight of Einkorn and Emmer wheat was positively affected by 50 µM and 100 µM lead nitrate compared to Ekiz and Durum. Einkorn had the highest root dry weight compared to other wheat species under 100 µM of lead nitrate. Ekiz and Durum root dry weight was significantly reduced by 37.20% and 38.41%, respectively, compared to Einkorn and Emmer (8.52% and 6.90%) under 250 µM of lead. The root dry weight of Durum (44.18% and 45.37%) was more affected and decreased compared to Einkorn and Emmer wheat (8.89% and 17.24%) at 50µM of lead (Figure 3.16).

Table 3.6. Impact of Pb(NO₃)₂ at different concentrations on pigment content of wheat landraces after 28 days of cultivation in soil

Wheat Landraces	Pb Concentration	Chl a mg/L	Chl b mg/L	Carotenoids mg/L
<i>Triticum aestivum</i> L. (Ekiz)	Control	1.844 ^{ij} ±0.007	3.090 ^{hij} ±0.008	214.93 ^{hi} ± 0.487
	50µm	1.838 ^{ijk} ±0.006	3.071 ^{jkl} ±0.014	216.42 ^{fg} ±0.576
	100µm	1.883 ^{def} ±0.008	3.142 ^{def} ±0.008	220.06 ^d ±0.840
	250µm	1.894 ^{bcd} ±0.008	3.060 ^{kl} ±0.001	219.48 ^{de} ±1.141
	500µm	1.848 ⁱ ±0.009	3.050 ^l ±0.020	215.99 ^{gh} ±1.169
<i>Triticum monococcum</i> ssp. <i>monococcum</i> L. (Einkorn)	Control	1.834 ^{jk} ±0.007	3.094 ^{hij} ±0.009	215.99 ^{fgh} ±0.958
	50µm	1.876 ^{efg} ±0.005	3.160 ^{cd} ±0.004	218.40 ^e ±0.421
	100µm	1.897 ^{bc} ±0.013	3.189 ^b ±0.022	219.53 ^{de} ±0.240
	250 µm	1.904 ^b ±0.008	3.195 ^b ±0.013	219.53 ^{de} ±0.557
	500µm	1.923 ^a ±0.001	3.233 ^a ±0.004	223.40 ^a ±0.178
<i>Triticum dicoccum</i> Schrank (Emmer)	Control	1.866 ^{gh} ±0.008	3.096 ^{hi} ±0.014	216.40 ^{fg} ±0.547
	50µm	1.872 ^{fg} ±0.011	3.150 ^{de} ±0.020	220.58 ^{cd} ±0.822
	100µm	1.933 ^a ±0.003	3.174 ^{bc} ±0.013	222.53 ^{ab} ±0.640
	250µm	1.903 ^b ±0.001	3.131 ^{efg} ±0.003	219.61 ^{de} ±0.195
	500µm	1.887 ^{cde} ±0.0005	3.111 ^{gh} ±0.020	221.43 ^{bc} ±0.170
<i>Triticum turgidum</i> ssp. <i>durum</i> (Durum)	Control	1.830 ^k ±0.008	3.077 ^{ijk} ±0.013	213.81 ⁱ ±0.258
	50µm	1.859 ^h ±0.0003	3.122 ^{fg} ±0.006	216.94 ^f ±0.171
	100µm	1.871 ^g ±0.005	3.121 ^{fg} ±0.006	218.54 ^e ±0.689
	250µm	1.829 ^k ±0.0004	2.923 ^m ±0.019	211.29 ^j ±1.266
	500µm	1.829 ^k ±0.002	2.887 ⁿ ±0.011	211.28 ^j ±0.740

Values indicate means ± SE from three replicates. According to Duncan's multiple range test, treatment means with different letters in the same column are significantly different

3.2.4 Effect of Lead on Chlorophyll (a), Chlorophyll (b), and Carotenoids in Wheat Species

Results of lead stress on chlorophyll (a), showed an increase at 50 μM and 100 μM of lead nitrate in all wheat varieties: Ekiz ($1.838^{\text{ijk}} \pm 0.006$ mg/L, $1.838^{\text{ijk}} \pm 0.006$ mg/L), Einkorn ($1.876^{\text{efg}} \pm 0.005$ mg/L, $1.897^{\text{bc}} \pm 0.013$ mg/L), Emmer ($1.872^{\text{fg}} \pm 0.011$ mg/L, $1.933^{\text{a}} \pm 0.003$ mg/L), Durum ($1.859^{\text{h}} \pm 0.0003$ mg/L, $1.871^{\text{g}} \pm 0.005$ mg/L) compared to the control ($1.844^{\text{ij}} \pm 0.007$ mg/L, $1.834^{\text{jk}} \pm 0.007$ mg/L, $1.866^{\text{gh}} \pm 0.008$ mg/L, $1.830^{\text{k}} \pm 0.008$ mg/L), respectively. The content of chlorophyll (a) was higher in Ekiz ($1.894^{\text{bcd}} \pm 0.008$ mg/L), Einkorn ($1.903^{\text{b}} \pm 0.001$ mg/L), and Emmer ($1.903^{\text{b}} \pm 0.001$ mg/L) than in Durum wheat ($1.829^{\text{k}} \pm 0.0004$ mg/L) at 250 μM of lead nitrate. At 500 μM lead nitrate, chlorophyll (a) significantly increased in Einkorn ($1.923^{\text{a}} \pm 0.001$ mg/L) and Emmer ($1.887^{\text{cde}} \pm 0.0005$ mg/L) compared to Ekiz ($1.848^{\text{i}} \pm 0.009$ mg/L) and Durum ($1.829^{\text{k}} \pm 0.002$ mg/L) (Table 3.6 and Figure 3.17).

Chlorophyll (b) at 50 μM and 100 μM of lead nitrate was positively affected in all wheat landraces, with Ekiz ($3.071^{\text{ijkl}} \pm 0.014$ mg/L, $3.142^{\text{def}} \pm 0.008$ mg/L), Einkorn ($3.160^{\text{cd}} \pm 0.004$ mg/L, $3.189^{\text{b}} \pm 0.022$ mg/L), Emmer ($3.150^{\text{de}} \pm 0.020$ mg/L, $3.131^{\text{efg}} \pm 0.003$ mg/L), and Durum ($3.122^{\text{fg}} \pm 0.006$ mg/L, $3.121^{\text{fg}} \pm 0.006$ mg/L) showing increases compared to the control ($3.090^{\text{hij}} \pm 0.008$ mg/L, $3.094^{\text{hij}} \pm 0.009$ mg/L, $3.096^{\text{hi}} \pm 0.014$ mg/L, $3.077^{\text{ijk}} \pm 0.013$ mg/L), respectively. The chlorophyll (b) content of Einkorn and Emmer at 250 μM lead nitrate was significantly increased ($3.195^{\text{b}} \pm 0.013$ mg/L, $3.131^{\text{efg}} \pm 0.003$ mg/L) while Ekiz and Durum ($3.060^{\text{kl}} \pm 0.001$ mg/L, $2.923^{\text{m}} \pm 0.019$ mg/L) were adversely affected. At 500 μM lead nitrate, chlorophyll (b) content was highest in Einkorn ($3.233^{\text{a}} \pm 0.004$ mg/L) compared to Emmer ($3.111^{\text{gh}} \pm 0.020$ mg/L), Ekiz ($3.050^{\text{l}} \pm 0.020$ mg/L), and Durum ($2.887^{\text{n}} \pm 0.011$ mg/L) (Table 3.6 and Figure 3.18).

Carotenoid levels increased at 50 μM and 100 μM concentrations of $\text{Pb}(\text{NO}_3)_2$ in wheat cultivars, with Ekiz ($216.42^{\text{fg}} \pm 0.576$ mg/L, $220.06^{\text{d}} \pm 0.840$ mg/L), Einkorn ($218.40^{\text{e}} \pm 0.421$ mg/L, $219.53^{\text{de}} \pm 0.240$ mg/L), Emmer ($220.58^{\text{cd}} \pm 0.822$ mg/L, $222.53^{\text{ab}} \pm 0.640$ mg/L), Durum ($216.94^{\text{f}} \pm 0.171$ mg/L, $218.54^{\text{e}} \pm 0.689$ mg/L) showing higher values compared to the control ($214.93^{\text{hi}} \pm 0.487$ mg/L, $215.99^{\text{fgh}} \pm 0.958$ mg/L, $216.40^{\text{fg}} \pm 0.547$ mg/L, $213.81^{\text{i}} \pm 0.258$ mg/L), respectively. The carotenoid content in Ekiz, Einkorn, and Emmer was significantly higher at 250 μM ($219.48^{\text{de}} \pm 1.141$ mg/L, $219.53^{\text{de}} \pm 0.557$ mg/L, and

219.61^{de}±0.195 mg/L, respectively), compared to Durum wheat (211.29^j±1.266 mg/L). At 500 μ M lead nitrate, carotenoid levels in Ekiz (215.99^{gh}±1.169 mg/L) and Durum (211.28^j±0.740 mg/L) were lower compared to Einkorn and Emmer (223.40^a±0.178 mg/L, 221.43^{bc}±0.170 mg/L) (Table 3.6 and Figure 3.19).

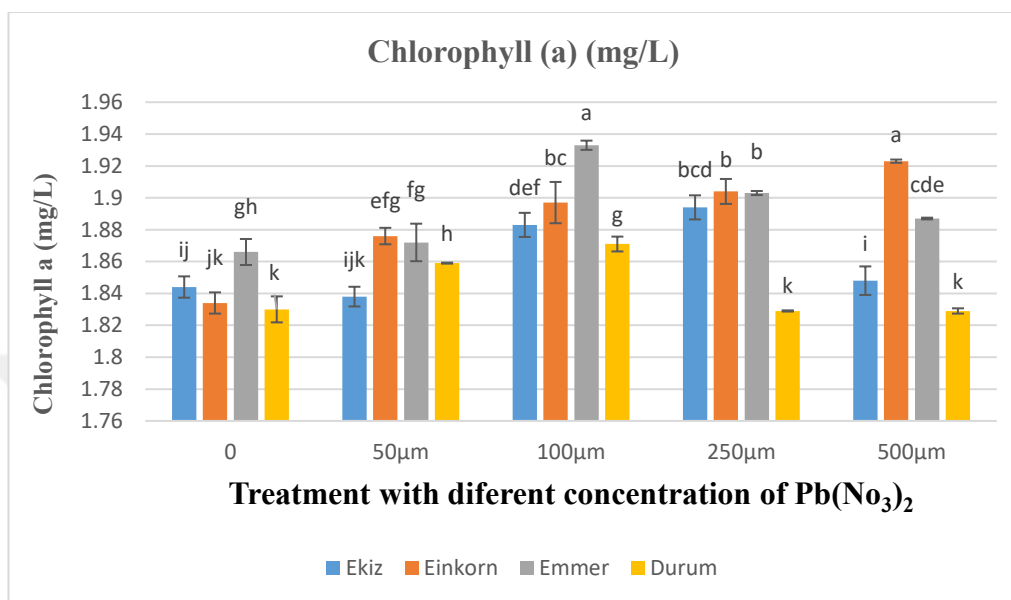


Figure 3.17. Effect of lead nitrate concentrations on chlorophyll (a) (mg/L) content of wheat landrace varieties cultivated in soil

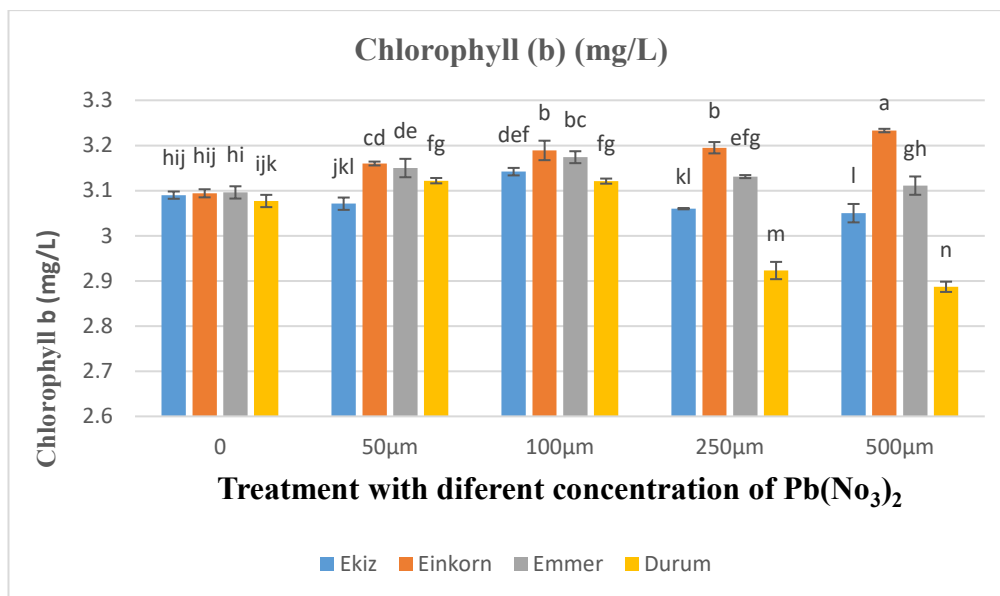


Figure 3.18. Effect of lead nitrate concentrations on chlorophyll (b) (mg/L) content of wheat landrace cultivars

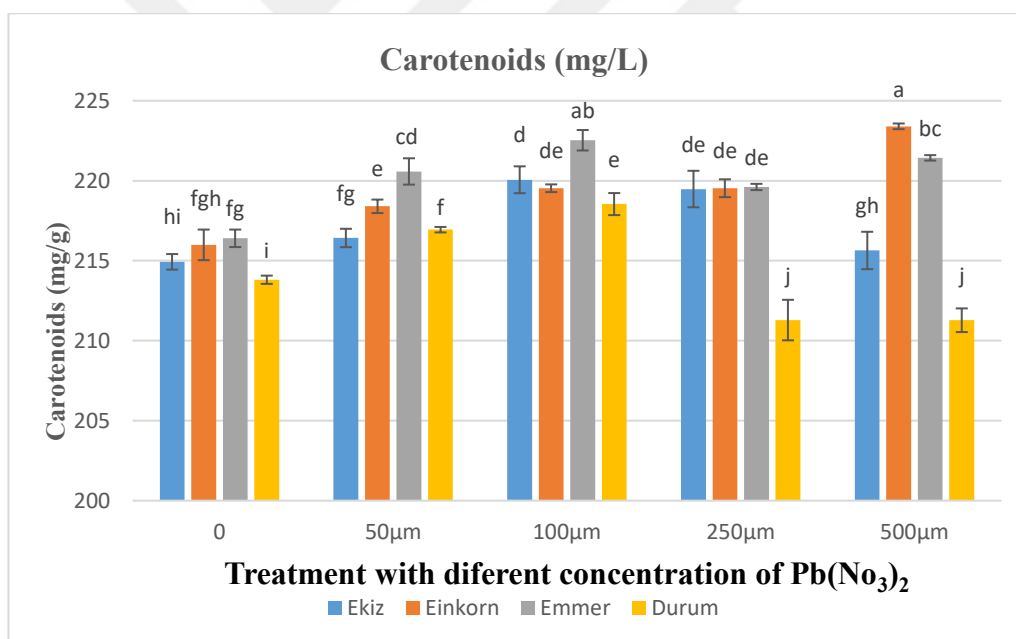


Figure 3.19. Effect of lead nitrate concentrations on carotenoid content (mg/L) of wheat landrace varieties cultivated in soil

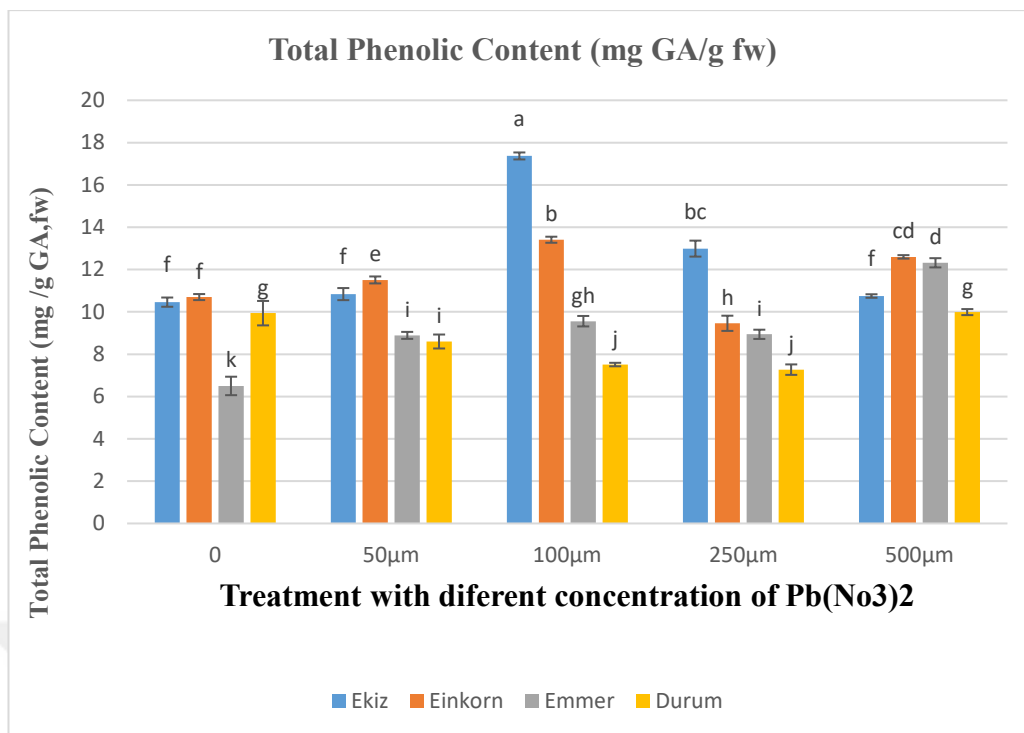


Figure 3.20. Effect of lead nitrate concentrations on total phenolic content (mg GA/g fw) of wheat landrace varieties

Table 3.7. Impact of various concentrations of Pb (NO₃)₂ on phenolic, proline, and protein contents of wheat landraces after 28 days of cultivation in soil

Wheat Landraces	Lead(Pb) Treatment	Total Phenolic content mg GA/g	Proline μ mol/g	Protein content mg/g
<i>Triticum aestivum</i> L. (Ekiz)	Control	10.46 ^g ±0.22	0.57 ^a ±0.025	19.07 ^m ± 1.36
	50 μ m	10.84 ^f ±0.29	1.91 ^b ±0.007	23.94 ^{ikl} ±2.25
	100 μ m	17.37 ^a ±0.16	2.60 ^a ±0.005	9.87 ⁿ ±0.70
	250 μ m	12.99 ^c ±0.38	1.57 ^e ±0.002	49.21 ^d ±0.95
	500 μ m	10.75 ^f ±0.08	1.46 ^{gl} ±0.005	58.74 ^b ±1.71
<i>Triticum monococcum</i> ssp. <i>monococcum</i> L. (Einkorn)	Control	10.7 ^f ±0.14	1.03 ^l ±0.016	23.07 ^{kl} ±1.10
	50 μ m	11.51 ^e ±0.16	1.30 ^h ±0.016	21.74 ^l ±0.20
	100 μ m	13.41 ^b ±0.14	1.84 ^c ±0.002	26.01 ^{hij} ±0.70
	250 μ m	9.46 ^h ±0.36	1.26 ⁱ ±0.018	25.94 ^{hij} ±0.72
	500 μ m	12.60 ^d ±0.08	1.53 ^f ±0.001	31.34 ^f ±2.60
<i>Triticum dicoccum</i> Schrank (Emmer)	Control	6.50 ^k ±0.44	0.93 ^l ±0.022	28.14 ^g ±1.00
	50 μ m	8.89 ^l ±0.16	0.85 ⁿ ±0.003	32.34 ^f ±0.87
	100 μ m	9.56 ^{gh} ±0.25	0.84 ^o ±0.008	16.94 ^m ±2.43
	250 μ m	8.94 ⁱ ±0.22	0.90 ^m ±0.005	24.34 ^{ik} ±2.60
	500 μ m	12.32 ^d ±0.22	1.64 ^d ±0.005	24.67 ^{ijk} ±0.50
<i>Triticum turgidum</i> ssp. <i>durum</i> (Durum)	Control	9.94 ^g ±0.58	0.36 ^t ±0.005	35.07 ^e ±1.10
	50 μ m	8.60 ⁱ ±0.33	0.35 ^t ±0.005	68.41 ^a ±1.70
	100 μ m	7.51 ^j ±0.08	0.96 ^k ±0.005	51.74 ^c ±1.04
	250 μ m	7.27 ^j ±0.25	0.66 ^p ±0.041	27.27 ^h ±0.61
	500 μ m	9.99 ^h ±0.14	0.37 ^r ±0.015	26.47 ^{hij} ±0.70

Values indicate means $\pm$ SE from three replicates. According to Duncan's multiple range test, treatment means with different letters in the same column are significantly different

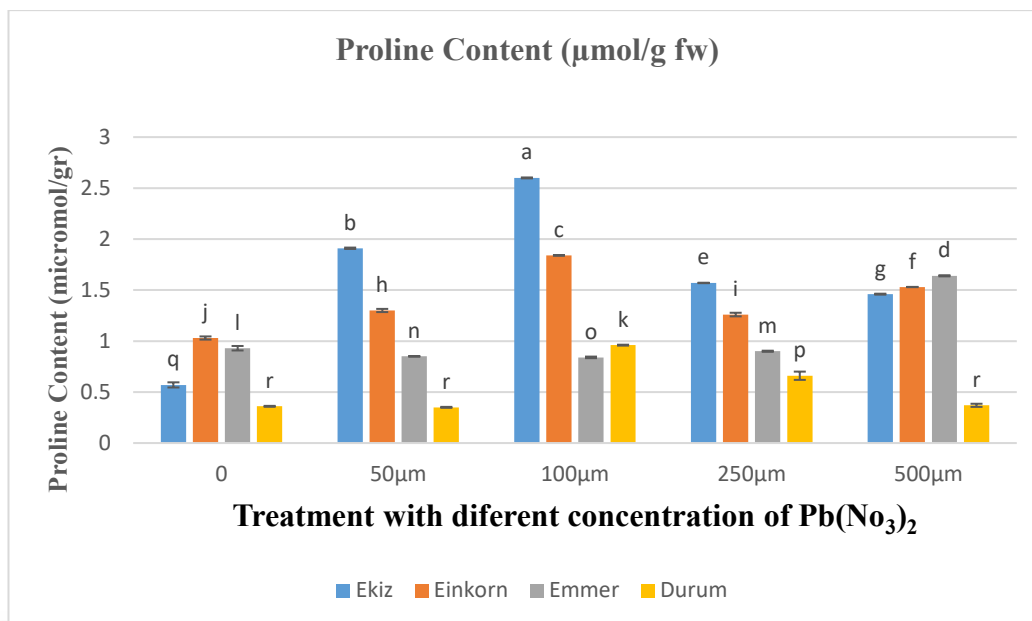


Figure 3.21. Impact of lead nitrate concentrations on proline content in wheat landrace cultivars in soil

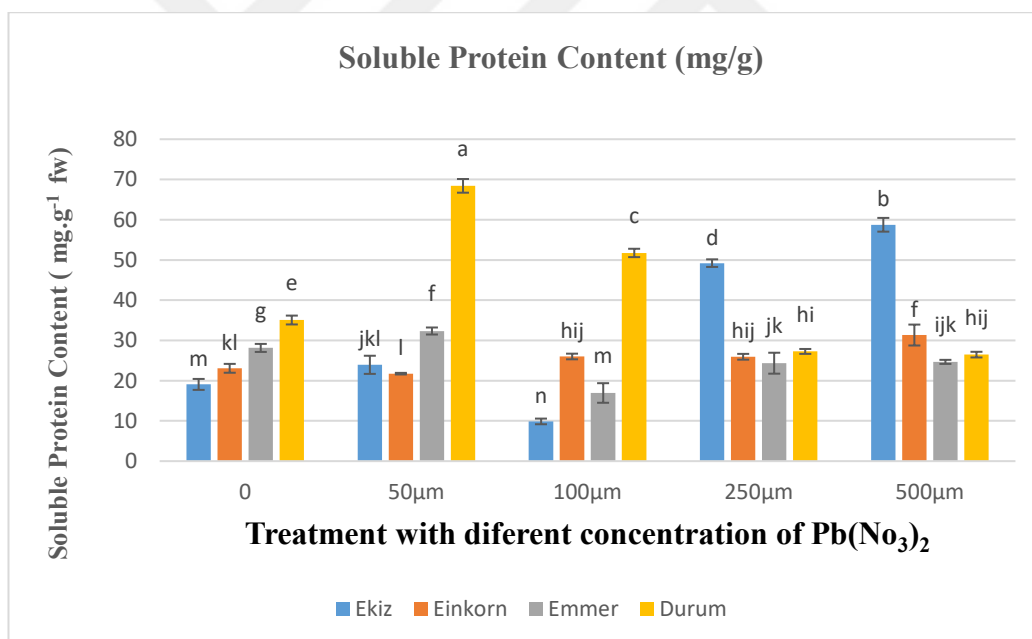


Figure 3.22. Effect of lead nitrate concentrations on soluble protein content in wheat landrace cultivars in soil

3.2.5 Effect of Lead on Phenolic, Proline, and Soluble Protein Content in Wheat Landraces

The phenolic contents of Ekiz ($10.84^f \pm 0.29$ mg GA/g), Einkorn ($11.51^e \pm 0.16$ mg GA/g), and Emmer ($8.89^l \pm 0.16$ mg GA/g) were higher than the control ($10.46^g \pm 0.22$ mg GA/g, $10.7^f \pm 0.14$ mg GA/g, $6.50^k \pm 0.44$ mg GA/g), respectively. However, Durum wheat had a phenolic content of $8.60^j \pm 0.33$ mg GA/g, which was lower than the control ($9.94^g \pm 0.58$ mg GA/g) under 50 μ M of lead nitrate. The highest amounts of phenolic contents with 100 μ M of Pb (NO₃)₂ were observed significantly in Ekiz ($17.37^a \pm 0.16$ mg GA/g), Einkorn ($13.41^b \pm 0.14$ mg GA/g) compared to Emmer ($9.56^{gh} \pm 0.25$ mg GA/g), Durum ($7.51^i \pm 0.08$ mg GA/g). At 250 μ M of lead nitrate treatment, the phenolic content in Ekiz significantly increased compared to other species: Einkorn ($9.46^h \pm 0.36$ mg GA/g), Emmer ($8.94^i \pm 0.22$ mg GA/g), and Durum ($7.27^j \pm 0.25$ mg GA/g). At 500 μ M lead nitrate, phenolic contents in Einkorn ($12.60^d \pm 0.08$ mg GA/g), Emmer ($12.32^d \pm 0.22$ mg GA/g) were significantly higher compared to Ekiz ($10.75^f \pm 0.08$ mg GA/g), and Durum ($9.99^h \pm 0.14$ mg GA/g) (Table 3.7 and Figure 3.20).

Proline content in Ekiz wheat significantly increased at 50 μ M and 100 μ M of lead nitrate treatment with values of, $1.91^b \pm 0.007$ μ mol/g, $2.60^a \pm 0.005$ μ mol/g, respectively, compared to Einkorn ($1.30^h \pm 0.016$ μ mol/g, $1.84^c \pm 0.002$ μ mol/g), Emmer ($0.85^n \pm 0.003$ μ mol/g, $0.84^o \pm 0.008$ μ mol/g), and Durum ($0.35^f \pm 0.005$ μ mol/g, $0.96^k \pm 0.005$ μ mol/g). The proline content in Ekiz ($1.57^e \pm 0.002$ μ mol/g) at 250 μ M Pb (NO₃)₂ was the highest among wheat landraces, followed by Einkorn ($1.26^i \pm 0.018$ μ mol/g), Emmer ($0.90^m \pm 0.005$ μ mol/g), and Durum ($0.66^p \pm 0.041$ μ mol/g). At 500 μ M lead nitrate, Durum wheat's proline content ($0.37^r \pm 0.015$ μ mol/g) was significantly lower than that of other wheat cultivars: Emmer ($1.64^d \pm 0.005$ μ mol/g), Einkorn ($1.53^f \pm 0.001$), and Ekiz ($1.46^g \pm 0.005$ μ mol/g) (Table 3.7 and Figure 3.21).

The effect of lead nitrate on soluble protein content (%) in wheat landraces at the two-leaf stage, 8 days after germination, showed that soluble protein content in Durum ($68.41^a \pm 1.70$ mg/g, $51.74^c \pm 1.04$ mg/g) was dramatically increased compared to Ekiz ($23.94^{kl} \pm 2.25$ mg/g, $9.87^n \pm 0.70$ mg/g), Einkorn ($21.74^l \pm 0.20$ mg/g, $26.01^{hij} \pm 0.70$ mg/g), Emmer ($32.34^f \pm 0.87$ mg/g, $16.94^m \pm 2.43$ mg/g) at 50 μ M and 100 μ M of Pb (NO₃)₂. At 250 μ M and 500 μ M lead nitrate concentrations, the protein content of Ekiz ($49.21^d \pm 0.95$ mg/g, $58.74^b \pm 1.71$ mg/g) showed

significant differences compared to Einkorn ($25.94^{hij} \pm 0.72$ mg/g, $31.34^f \pm 2.60$ mg/g), Emmer ($24.34^{jk} \pm 2.60$ mg/g, $24.67^{jk} \pm 0.50$ mg/g), Durum ($27.27^h \pm 0.61$ mg/g, $26.47^{hij} \pm 0.70$ mg/g) (Table 3.7 and Figure 3.21).

Table 3.8. Oxidative stress and antioxidant enzymes in wheat seedlings under lead nitrate stress in wheat landraces after 28 days of cultivation in soil

Wheat Landraces	Pb Concentration	CAT ($\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$)	SOD Activity (U/mg protein)	H ₂ O ₂ ($\mu\text{mol/g}$)
<i>Triticum aestivum</i> L. (Ekiz)	Control	$3.54^{def} \pm 0.17$	$14.64^i \pm 0.87$	$40.45^j \pm 1.12$
	50 μm	$4.00^{cd} \pm 0.47$	$56.70^c \pm 1.77$	$41.19^j \pm 0.85$
	100 μm	$9.92^a \pm 0.63$	$96.24^a \pm 0.88$	$114.70^{ef} \pm 8.77$
	250 μm	$3.26^{efg} \pm 0.09$	$22.61^h \pm 0.57$	$185.75^d \pm 16.37$
	500 μm	$2.39^{ij} \pm 0.07$	$13.68^{jk} \pm 0.24$	$268.57^b \pm 35.41$
<i>Triticum monococcum</i> ssp. <i>monococcum</i> L. (Einkorn)	Control	$2.90^{ghi} \pm 0.09$	$15.76^j \pm 2.01$	$72.55^{hi} \pm 3.37$
	50 μm	$4.89^b \pm 0.07$	$16.19^j \pm 2.82$	$75.98^{hi} \pm 0.85$
	100 μm	$4.02^{cd} \pm 0.10$	$28.88^g \pm 0.37$	$82.35^{ghi} \pm 1.53$
	250 μm	$3.99^{cd} \pm 0.25$	$31.66^f \pm 1.93$	$137.48^e \pm 1.85$
	500 μm	$3.58^{cde} \pm 0.09$	$40.57^d \pm 0.37$	$174.73^d \pm 1.47$
<i>Triticum dicoccum</i> Schrank (Emmer)	Control	$3.49^{def} \pm 0.10$	$20.54^{hi} \pm 1.09$	$72.06^{hi} \pm 2.78$
	50 μm	$2.49^{hij} \pm 0.23$	$20.47^{hi} \pm 1.24$	$73.53^{hi} \pm 2.25$
	100 μm	$4.89^b \pm 0.91$	$79.13^b \pm 1.43$	$80.64^{hi} \pm 2.21$
	250 μm	$4.12^c \pm 0.27$	$33.53^{ef} \pm 1.27$	$108.33^{fg} \pm 2.25$
	500 μm	$3.48^{def} \pm 0.09$	$33.43^{ef} \pm 1.16$	$224.22^c \pm 15.11$
<i>Triticum turgidum</i> ssp. <i>durum</i> (Durum)	Control	$3.00^{fgh} \pm 0.05$	$15.59^j \pm 1.48$	$56.87^{ij} \pm 1.85$
	50 μm	$2.25^i \pm 0.09$	$19.03^i \pm 2.18$	$56.87^{ij} \pm 2.25$
	100 μm	$2.26^i \pm 0.07$	$20.93^{hi} \pm 2.36$	$93.87^{fgh} \pm 7.01$
	250 μm	$3.07^{efg} \pm 0.01$	$34.75^e \pm 2.42$	$225.45^c \pm 17.69$
	500 μm	$2.00^i \pm 0.04$	$11.28^k \pm 2.69$	$341.84^a \pm 49.37$

Values indicate means $\pm$ SE from three replicates. According to Duncan's multiple range test, treatment means with different letters in the same column are significantly different

3.2.6 Impact of Lead Nitrate on H₂O₂ Content and Antioxidant Enzymes (CAT and SOD)

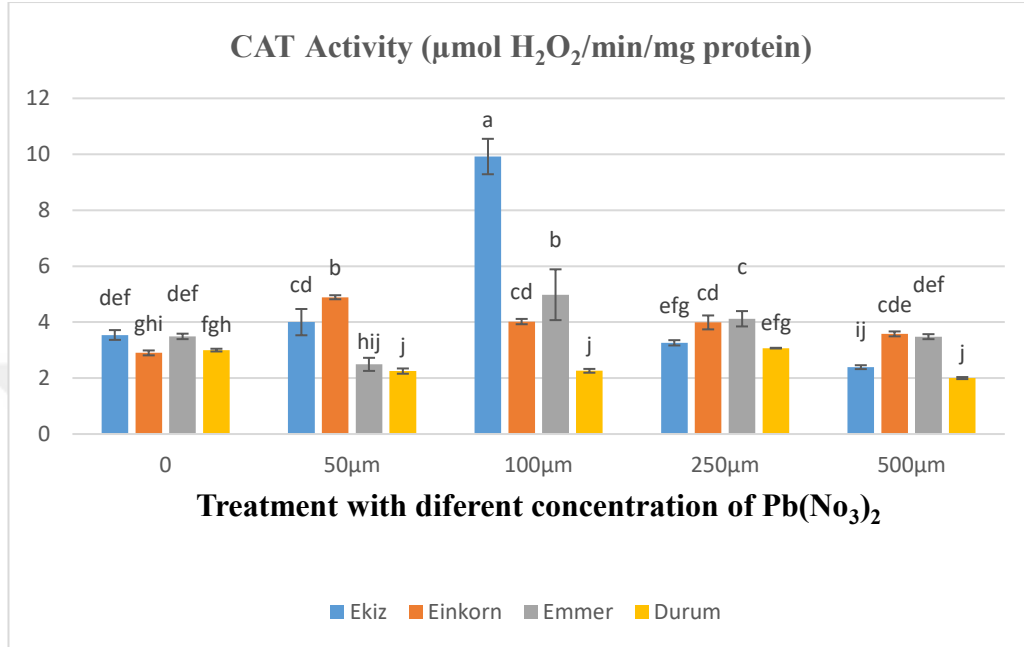


Figure 3.23. Effect of lead concentrations on CAT activity of wheat landrace varieties cultivated in soil

The findings demonstrated that the levels of antioxidant enzymes (SOD and CAT) increased in wheat landraces exposed to toxic doses of lead nitrate. Ekiz and Einkorn's cultivars showed higher CAT activity ($4.00^{\text{cd}} \pm 0.47 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$ and $4.89^{\text{b}} \pm 0.07 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$, respectively) at 50 μM of $\text{Pb}(\text{NO}_3)_2$. On the other hand, CAT activity in wheat cultivars Emmer ($2.49^{\text{hij}} \pm 0.23 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$), and Durum ($2.25^{\text{j}} \pm 0.09 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$) was decreased compared to the control ($3.49^{\text{def}} \pm 0.10 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$, $3.00^{\text{fgh}} \pm 0.05 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$). Ekiz CAT activity ($9.92^{\text{a}} \pm 0.63 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$) at 100 μM lead nitrate concentration was significantly increased compared to other wheat landrace cultivars: Einkorn ($4.89^{\text{b}} \pm 0.07 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$), Emmer ($4.89^{\text{b}} \pm 0.91 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$), Durum ($2.26^{\text{j}} \pm 0.07 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$). At 250 μM of lead nitrate treatment, the CAT activity of Einkorn ($3.99^{\text{cd}} \pm 0.25 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$) and Emmer ($4.12^{\text{c}} \pm 0.27 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$) showed a significant increase compared

to Ekiz ($3.26^{efg} \pm 0.09 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$) and Durum ($3.07^{efg} \pm 0.01 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$) (Table 3. 8). The CAT activity of Ekiz and Durum dramatically decreased at 500 μM lead nitrate compared to Einkorn and Emmer (Figure 3.23).

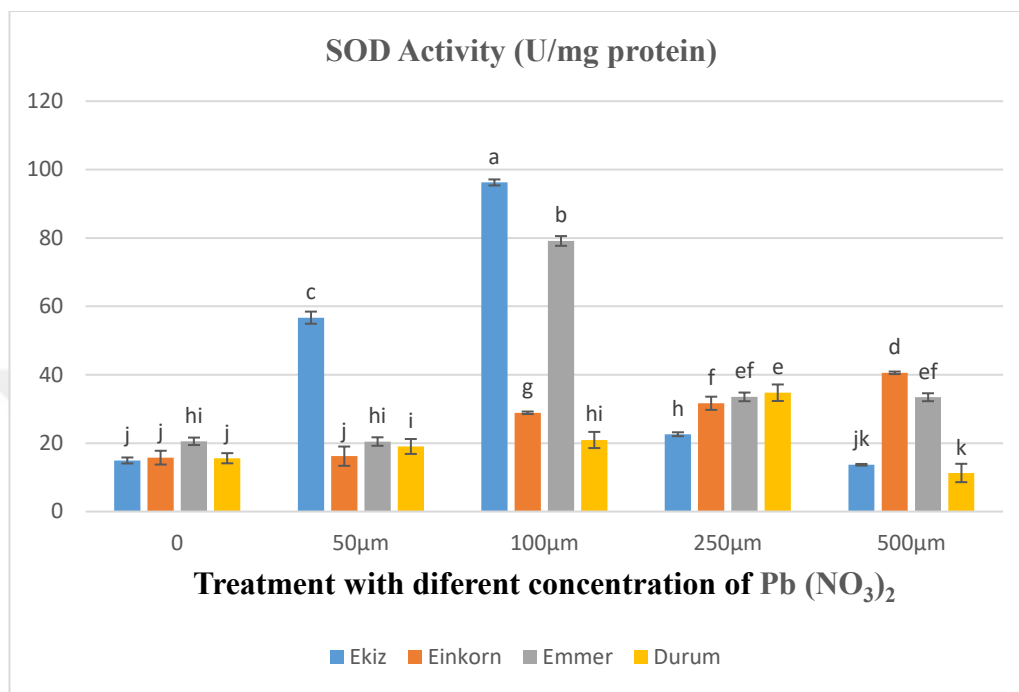


Figure 3.24. Effect of lead concentrations on SOD activity of wheat landrace varieties cultivated soil

The SOD activity of Ekiz wheat ($56.70^c \pm 1.77 \text{ U/mg protein}$) at 50 μM Pb (NO₃)₂ showed a significant increase compared to Einkorn ($16.19^j \pm 2.82$), Emmer ($20.47^{hi} \pm 1.24 \text{ U/mg protein}$), and Durum ($19.03^i \pm 2.18 \text{ U/mg protein}$). At 100 μM of lead nitrate, SOD activity in Ekiz and Emmer ($96.24^a \pm 0.88 \text{ U/mg protein}$, $79.13^b \pm 1.43 \text{ U/mg protein}$) drastically increased compared to Einkorn ($28.88^g \pm 0.37 \text{ U/mg protein}$), Durum ($20.93^{hi} \pm 2.36 \text{ U/mg protein}$). In contrast to Einkorn ($31.66^f \pm 1.93 \text{ U/mg protein}$), Emmer ($33.53^{ef} \pm 1.27 \text{ U/mg protein}$), and Durum ($34.75^e \pm 2.42 \text{ U/mg protein}$), the SOD activity of Ekiz was considerably lower at 250 μM Pb (NO₃)₂ concentration (Table 3.8). At 500 μM lead nitrate, SOD activity in Einkorn, Emmer, showed a dramatic increase of 157.42%, and 62.76%,

respectively compared to Ekiz and Durum whose SOD activity decreased by 8.43%, 27.65% respectively (Figure 4.24).

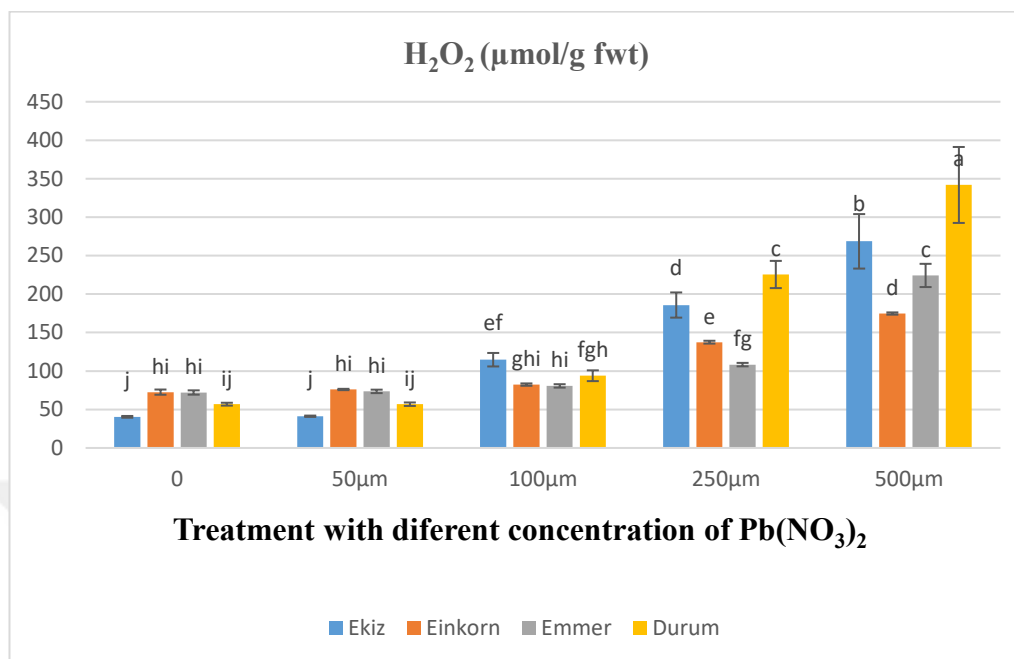


Figure 3.25. Effect of lead nitrate concentrations on H₂O₂ of wheat landrace varieties cultivated in soil

Hydrogen peroxide content increased in all wheat landraces due to exposure to high concentrations of lead nitrate. However, there was no significant difference in hydrogen peroxide levels at a 50 μM concentration of lead nitrate in Ekiz, Einkorn, Emmer, and Durum cultivars compared to the control groups (Figure 3.25). At 100 μM Pb (NO₃)₂, the wheat cultivars Ekiz (114.70^{ef}±8.77 μmol/g) and Durum (93.87^{fgh}±7.01 μmol/g) exhibited higher hydrogen peroxide content than Einkorn (82.35^{ghi}±1.53 μmol/g) and Emmer (80.64^{hi}±2.21 μmol/g).

At 250 μM and 500 μM lead nitrate, the hydrogen peroxide content in Ekiz (185.75^d±16.37 μmol/g, 242.11^c±1.85 μmol/g) and Durum (225.45^c±17.69 μmol/g, 341.84^a±49.37 μmol/g) was significantly higher compared to Einkorn (137.48^e±1.85 μmol/g, 174.73^f±1.47 μmol/g), and Emmer (108.33^{fg}±2.25 μmol/g, 224.22^c±15.11 μmol/g) (Table 3.8).

3.3 Evaluation of Soil Water Holding Capacity (SWHC)

Soil water holding capacity (SWHC) is a crucial factor for understanding a plant's ability to uptake the necessary water and nutrients required for its physiological and biochemical processes. The results indicated that the mean value of soil water holding capacity for the soil used to cultivate the wheat landrace cultivars was 38.66% (Table 3.9).

Table 3.9. SWHC analysis results

Soil samples	Sample weight (g)	Volume of water through V1 (ml)	Volume of water collected in the cylinder V2 (ml)	Volume of water retained by soil (V1-V2)	Percentage of SWHC
R1	25 (g)	50 (ml)	41 (ml)	9 (ml)	36%
R2	25 (g)	50 (ml)	40 (ml)	10 (ml)	40%
R3	25 (g)	50 (ml)	40 (ml)	10 (ml)	40%

Table 3.10. Effect of lead stress on elemental concentrations (mg/kg) in wheat landrace cultivars

Wheat cultivars	Treatments Pb(NO ₃) ₂	Pb (mg/kg)	Ca (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	P (mg/kg)	K (mg/kg)
Ekiz	Control	0.032	9869.316	142.653	2290.176	12306.660	61503.368
	100µM	0.244	13693.359	88.632	2907.063	15958.239	70330.472
	500µM	0.249	9793.017	80.352	2570.029	14642.132	66393.184
Einkorn	control	0.000	6396.044	69.268	2190.309	6959.281	36513.455
	100µM	0.134	10068.006	68.422	2380.168	11131.615	46231.546
	500µM	0.193	7579.987	55.289	2224.373	8185.136	38733.590
Emmer	Control	0.035	8266.418	79.001	1961.148	10957.506	56389.838
	100 µM	0.128	11451.184	292.744	2197.719	9125.17	58515.897
	500 µM	0.148	9137.540	137.072	2648.88	10245.63	58294.255
Durum	Control	0.077	7849.930	128.710	1789.714	10196.347	51714.784
	100µM	0.162	10491.238	66.681	2153.579	10097.220	52305.225
	500µM	0.242	6424.927	60.368	1586.507	8460.799	42489.157

3.4 Effect of Lead on Essential Elements

The results revealed the concentrations of microelements, microelements, and toxic elements (Pb, Ca, Fe, Mg, P, and K) in wheat landraces from several provinces of Türkiye. The levels of each element in the wheat species may either increase or decrease depending on the wheat cultivar and the lead treatments applied.

3.4.1 Lead Accumulation in Wheat Landraces.

The lead concentration at 100 µM was significantly higher in Ekiz and Durum (0.244 mg/kg, 0.162 mg/kg, respectively) compared to other cultivars, Einkorn (0.134 mg/kg) and Emmer (0.128 mg/kg). Additionally, at 500 µM lead nitrate concentration, Ekiz and Durum (0.249 mg/kg, 0.242 mg/kg, respectively) had higher lead concentrations than Einkorn (0.193 mg/kg) and Emmer (0.148 mg/kg) (Table 3.9).

3.4.2 Variations in Essential Elements

Lead nitrate-treated at 100 μ M resulted in increased phosphorus (P) content in Ekiz (15958.239 mg/kg) and Einkorn (11131.615 mg/kg) compared to the control groups, which had 12306.660 mg/kg, 6959.281 mg/kg, respectively. However, the P content of Emmer (9125.169 mg/kg), and Durum (10097.220 mg/kg) slightly decreased compared to the control 10957.506 mg/kg, and 10097.220 mg/kg, respectively. Under 500 μ M of lead nitrate, the P content in Ekiz (14642.132 mg/kg) was significantly higher than in the other cultivars, including Einkorn (8185.136 mg/kg), Emmer (10245.631 mg/kg), and Durum (8460.799 mg/kg) (Table 4.9).

Pb (NO₃)₂ stress at 100 μ M Pb (NO₃)₂ also led to increased potassium (K) content in the wheat landraces: Ekiz, Einkorn, Emmer, Durum 70330.472, 46231.546, 58515.897, 52305.225 (mg/kg) compared to the control groups 61503.368, 36513.455, 56389.838, 51714.784 (mg/kg). Additionally, it increased P content in the wheat landraces: Ekiz, Einkorn, Emmer, Durum 70330.472, 46231.546, 58515.897, 52305.225 (mg/kg) compared to the control groups, which had 61503.368, 36513.455, 56389.838, 51714.784 (mg/kg), respectively.

Wheat landrace cultivars Ekiz (70330.472 mg/kg), Einkorn (46231.546 mg/kg), Emmer (58515.897 mg/kg), and Durum (52305.225 mg/kg) exhibited elevated K content when exposed to 100 μ M Pb nitrate. The potassium (K) content of the Durum (42489.157 mg/kg) significantly decreased at 500 μ M of lead nitrate concentration compared to the control (51714.784 mg/kg). On the other hand, k content in the other wheat species: Ekiz (66393.184), Einkorn (38733.59), and Emmer (58294.255 mg/kg) was higher than the values in their respective control groups.

4.DISCUSSION

4.1 Seed Germination and Seedling Vigor

Lead nitrate $\text{Pb}(\text{NO}_3)_2$ effect on the growth and development of wheat cultivars, including the percentage of wheat species, has been thoroughly investigated. Depending on the quantity and genotype of wheat, researchers have reported increases and decreases in the germination rate across various wheat species when different $\text{Pb}(\text{NO}_3)_2$ concentrations (ranging from 50 μM to 500 μM) are applied. The findings of this study are consistent with Sędzik et al. (2015) results that high concentrations of $\text{Pb}(\text{NO}_3)_2$ reduce the germination percentage of wheat seedlings.

Pb negatively affected the morphological traits of the test plants. Compared to the control group, three species of wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), and pumpkin (*Cucurbita maxima*) showed greater tolerance to lead than the other test plants. On the other hand, low concentrations of lead (Pb) stimulated growth in *Tetraena qataranse* by enhancing root and shoot elongation. The increased activity of antioxidant enzymes, which reduce oxidative stress and support general plant health and resistance to Pb toxicity, is likely the cause of this response. Additionally, phenolic chemicals may be involved in this reaction (Usman et al., 2020). Similarly, in our research, a concentration of lead nitrate at 100 μm increased the morphological traits of wheat species, whereas higher concentrations reduced growth, consistent with the findings of Yang et al. (2010). In their study, the germination percentage of seeds slightly increased in response to 1 mM lead stress compared to the control but dramatically reduced in response to 4 mM lead treatment, particularly in the growth of shoots and roots.

The diversity of genes across all cultivars may be responsible for the observed variations in seed germination, whether via stimulation or inhibition, as noted by Idrees et al. (2015). This contrast assists in identifying the precise impacts of cadmium and confirms that, as the study findings suggest, the observed decrease in germination is directly related to cadmium stress. These findings align with research conducted by Mehboob et al. (2018) on the effects of different concentrations (0, 20, 40, 60, 80, and 100 ppm) of lead on seed germination and seedling growth performance of wheat (*Triticum aestivum*) as compared to the control. *T. aestivum* seed germination and seedling growth were inhibited by lead

acetate treatment at 100 ppm as compared to the control. *T. aestivum* showed high tolerance (92.50%) and a seedling vigor index (1586) at 20 ppm, with the lowest tolerance (64.50%) and SVI (302) at 80 ppm of Pb. In line with Lamhamdi et al. (2013), who reported that the Pb accumulation in seedlings negatively correlated with morphological parameters of plant growth and positively correlated with external concentrations when evaluating phytotoxicity in wheat seeds grown in a solution with lead nitrate (0.05, 0.1, 0.5, and 1 g/L) for six days.

4.2 Effect of Lead on Shoot and Root Elongation

Coleoptile length, root length, and fresh and dry weight were reduced in the seedling germination stage compared to the control at a high concentration of $\text{Pb}(\text{NO}_3)_2$ (Table 4.1, Table 4.2). The ancient cultivars Einkorn and Emmer exhibited greater tolerance to lead than modern species Ekiz and Durum. Similarly, as reported by Bhatti et al. (2013), morphological attributes, such as root and shoot length, shoot fresh and dry weights, and the number of tillers, decreased in two wheat varieties, "Chakwal-97" and "Sehar-2006" when exposed to 0, 40, and 60 ppm lead concentrations. According to Aicha et al. (2020), seedlings of *Cleome amblyocarpa* Barr. & Murb stressed by lead nitrate were exposed to six diverse concentrations (0.50, 100, 150, 200, and 250 ppm) five times. Increasing lead concentrations caused a significant decrease in the seedlings' tigelles, radicle length, as well as a notable increase in phytotoxicity (%). Similar findings were reported by Moosavi et al. (2012) who studied Canola (*Barassica napus*), Safflower (*Carthamus tinctorious*), and *Triticum aestivum* germinated in liquids with different levels of heavy metal content. Heavy metal concentrations (BiNO_3 , CdNO_3 , $\text{Sr}(\text{NO}_3)_2$, and ZnNO_3) at 50, 200, 350, 500, and 1000 ppm were used. Seed germination, root length, and branch length all experienced a decline in percentages as the concentrations of the solutions increased across all treatments.

In line with research conducted by Kumar et al. (2018), there was a progressive and significant effect of higher lead concentration on several physiological features in *Triticum aestivum* L. exposed to $\text{Pb}(\text{NO}_3)_2$ concentrations of 0, 50, 100, 150, 200, 250, and 500 μM in a petri dish. These effects include reductions in fresh and dry weight, germination %, and shoot and root length. Consistent with this, Al-Ghzawi et al. (2019) reported that lead negatively impacted seed germination, root, and shoot systems for every genotype and changes in

biochemical composition of two barley genotypes and seven wheat landraces grown in controlled growth environments and exposed to two concentrations of $\text{Pb}(\text{NO}_3)_2$ (0 and 7 mM).

Our research findings demonstrated that lead harmed root elongation more than shoot elongation in wheat landraces. This aligns with Mesmar & Jaber (1991), who reported that *Triticum aestivum* and *Lens esculanta*, when cultivated on wet filter paper containing $\text{Pb}(\text{NO}_3)_2$, showed significantly reduced seed germination (approximately 60% at 20 mM $\text{Pb}(\text{NO}_3)_2$). The development of both plant species was impeded by the Pb, with root growth inhibition being more pronounced than stem growth.

According to Ерофеева (2020), when exposed to $\text{Pb}(\text{NO}_3)_2$ and copper sulfate, the concentrations of the toxicants induced a reduction in *Triticum aestivum* seed germination relative to the control by 19–38% and 23–58%, respectively, which indicated the development of harmful effects. In *Triticum aestivum* seedlings, all investigated concentrations of both toxicants significantly decreased the root system length (69 % to 25 times) and shoot height (25–76%) compared to the control levels. Pea (*Pisum sativum*) seedlings exhibited significantly higher resistance to the investigated concentrations (0.15, 0.30, and 0.60 g/L of copper sulfate; 0.5, 1.0, and 1.5 g/L of $\text{Pb}(\text{NO}_3)_2$) than *Triticum aestivum*. In line with research conducted by Ghanghro et al. (2022), the "SKD-1" variety outperformed all other varieties in terms of root and shoot length at 50, 30, and 70 ppm lead acetate stress in wheat types such as "Imdad", "Abadgar", "SKD-1", "Anmol-91", "Tj-83", and "Sonalika".

4.3 Effect of Lead Stress on Dry and Fresh Weight

Our research findings revealed that lead toxicity significantly decreases biomass production, resulting in a reduction in root and shoot fresh weight and dry weight in wheat varieties exposed to high concentrations of lead (250 μM and 500 μM) (Table 3.5). In line with research conducted by Kopittke et al. (2007), *Brachiaria decumbens* (Signal grass) showed a 50% decrease in fresh mass of shoots at 5 μM Pb^{2+} and roots at 3 μM Pb^{2+} . In contrast, *Chloris gayana* (Rhodes grass) was more sensitive, with a 50% reduction in shoots and roots at just 0.5 μM Pb^{2+} . Similarly, according to Faheed (2005), the various lead concentrations affected germination and seedling development in *Eruca sativa* plants. Increased

lead concentrations caused a significant decrease in the percentage of seed germination, as well as in fresh and dry matter root, and shoot length. Root length was more sensitive to lead concentration than shoot length. Consistent with Srivastava et al.(2014) exposure to lead also resulted in a significant reduction in root/shoot length, fresh weight, relative water content, and photosynthetic pigments, while increasing ROS ($O_2^{\cdot-}$ and H_2O_2) production. This was observed in rice seedlings (*Oryza sativa* L.) grown in sand culture and exposed to 150 μ M $Cd(NO_3)_2$ or 600 μ M $Pb(CH_3COO)_2$ individually or in combination, for 8–16 days.

According to Kang et al.(2009), lead (Pb) significantly inhibited the germination of wheat seeds. The negative impact of increased Pb^{2+} content led to a decrease in the number, length, and height of roots, the fresh weight of seedlings, and the vigor index. However, Pb^{2+} concentrations below 50 mg/L accelerated wheat seed germination and increased the number of roots.

Shi et al. (2014) reported that Pb stress had varying impacts on Lettuce (*Lactuca sativa* L.) and Rapeseed (*Brassica campestris* L.). The investigation revealed that higher Pb concentrations increased the fresh weight and dry weight of roots while decreasing the number, area, and fresh weight of leaves in *Lactuca sativa* L.. At higher concentrations of Pb treatment, the growth indices of *Brassica campestris* L. were reduced. In line with the research conducted by Jelea et al. (2015), the toxicity of Pb inhibits seed germination and seedling development in *Lactuca sativa* var. *capitata* L. and Lolium (*Lolium perenne* L.) when exposed to various concentrations of $Pb(NO_3)_2$ (0.05 g/L, 0.1 g/L, 0.5 g/L, and 1 g/L). The Pb decreased strain length, root length, and germination percentage.

Our results show that biomass production and seedling development of *T. aestivum* were significantly affected by 100 ppm Pb acetate treatment, which is consistent with Shafiq (2018), evaluation of the effects of various Pb concentrations (0, 20, 60, 80, and 100 ppm) on the growth performance of common wheat seedlings (*Triticum aestivum*) compared to the control. Various lead treatments considerably inhibited root development and growth, with shoot length significantly reduced at 40 ppm of lead concentration. Additionally, the number of leaves, leaf area, dry weight, and shoot length of *Triticum aestivum* significantly decreased at 20 ppm of lead treatment.

This study examines the impacts of varying $\text{Pb}(\text{NO}_3)_2$ concentrations on the fresh and dry weight of Einkorn and Emmer biomass for the first time, and previous studies have not reported this.

4.4 Toxicity and Tolerance of Wheat Landraces

The restriction of cell division in root meristems Pb to restricted root development, which is one of the main impacts of Pb toxicity in plants (Eun et al., 2000). Additionally, Pb induces oxidative stress mediated by ROS, which causes cellular damage (Liu et al., 2009; Malecka et al., 2009). Our research highlighted that wheat landrace varieties, *T. monoccocum* and *T. dicocum*, were more tolerant to lead stress compared to *T. aestivum* and *T. turgidum* in terms of shoot and root toxicity. Similarly, Erdem et al. (2012) reported that durum wheat was more sensitive to Zn deficiency and Cd toxicity than bread wheat. In their study, durum (Kızıltan) and bread (Bezostaja-I) wheat were germinated in Zn-deficient calcareous soil and treated with increasing Cd (0, 2.5, and 10 mg kg⁻¹ soil) concentrations and Zn (0 and 5 mg kg⁻¹ soil) concentrations. High Cd concentrations led to the formation of necrotic patches on the base and sheath portions of the oldest leaves in both wheat cultivars; however, the symptoms were more noticeable in durum wheat, especially in the presence of zinc deficiency. Shoot growth was significantly ($P < 0.01$) reduced in durum wheat treated with high Cd (10 mg kg⁻¹).

4.5 Photosynthetic Pigment Content

Our findings demonstrated that the effect of lead led to an increase in chlorophyll (a) in all wheat varieties -Ekiz, Einkorn, Emmer, and Durum- compared to the control at 50 μM and 100 μM of lead nitrate. At 250 μM of lead nitrate, chlorophyll (a) levels in Ekiz, Einkorn, and Emmer significantly increased compared to the Durum cultivar. Chlorophyll (a) in the ancient species Einkorn and Emmer significantly increased compared to modern cultivars Ekiz and Durum at 500 μM of lead nitrate. Chlorophyll (b) was positively affected in all wheat landraces -Ekiz, Einkorn, Emmer, and Durum- when exposed to 50 μM and 100 μM of lead nitrate compared to the control. At 250 μM lead nitrate, Einkorn and Emmer chlorophyll (b) content was dramatically increased compared to Ekiz and Durum. With 500 μM lead nitrate treatment, Einkorn's chlorophyll (b) was highest

compared to Emmer, Ekiz, and Durum. This finding is consistent with Gomes et al. (2014), who revealed that lead-contaminated soil led to increased synthesis of carotenoid pigments, total chlorophyll, chlorophyll (a), and chlorophyll (b) in vetiver (*Vetiveria zizanioides* (L.) Nash), castor beans (*Ricinus communis* L.), sunflower (*Helianthus annuus* L.), and maize (*Zea mays* L.) species, which were reliable markers of soil lead concentrations, indicating plant stress caused by metal toxicity.

Carotenoids are the substrate for producing strigolactones and abscisic acid and are crucial to the photosynthetic process and photo-oxidative defense. In addition to their antioxidant functions and their role as precursors to vitamin A, carotenoids significantly influence wheat by affecting the grain's color (whiteness versus yellowness) (Colasuonno et al., 2017). Consistent with the study by Bhatti et al. (2013), it was reported that Pb stress (0, 40, and 60 ppm affected the photosynthetic pigments in *Triticum aestivum* L. varieties Chakwal-97 and Sehar-2006, reducing levels of chlorophyll (a, b), which are essential for photosynthesis. However, carotene contents increased, possibly as a response to the stress conditions to mitigate the damage caused by lead in line with the study by Malik et al. (2021). This study assessed nine cultivars of *T. durum* L. ("CBD25", "CBD46", "CBD58", "CBD59", "CBD63", "CBD66", "CBD68", "CBD69", and "CBD82") exposed to different Pb concentrations (10, 20, and 40 mg kg⁻¹). Chlorophyll (a) did not change considerably in response to Pb concentrations, although cultivars exhibited significant variability. CBD82 exhibited the most marked decrease in chlorophyll (50%) at the highest Pb level. CBD66 cultivated in the control group showed the lowest levels of chlorophyll (a). The responses of the cultivars to increasing Pb levels for chlorophyll b were considerably different, with only CBD82 showing a continuous reduction with rising Pb levels. Carotenoid content in all *T. durum* cultivars was less affected by Pb applications, except CBD63 and CBD69, which exhibited higher reductions at all lead concentrations.

In line with our findings, carotenoids increased in all wheat varieties compared to the control groups with 50 μ M and 100 μ M of lead nitrate. At 250 μ M of lead nitrate treatment, carotenoid levels in Ekiz, Einkorn, and Emmer significantly increased compared to the Durum cultivar. The carotenoids of Einkorn and Emmer significantly increased compared to those in Ekiz and Durum at 500 μ M concentration of lead nitrate (Table 4.6). According to (Pazoki et al., 2014),

lead concentrations harmed the pigment content of *Triticum aestivum* L. with the maximum reduction of chlorophylls occurring at higher doses of lead (900 mg/kg) while carotene and xanthophyll content increased.

4.6 Phenolic Compounds (mg GA/g)

Phenolics are one significant class of secondary metabolites in wheat, essential for scavenging radical oxygen species produced during stress and mitigating damage under conditions of heavy metal exposure. Due to their carboxyl and hydroxyl groups, phenolic compounds can chelate heavy metals, removing reactive oxygen species (ROS) and preventing the peroxidation caused by heavy metals and ROS (Noor et al., 2023).

Our results demonstrate that the total phenol in Ekiz significantly increased compared to Einkorn, Emmer, and Durum cultivars at 50 μM of $\text{Pb}(\text{NO}_3)_2$. The total phenolic content in Ekiz and Emme species dramatically increased with 100 μM of $\text{Pb}(\text{NO}_3)_2$ treatment compared to Einkorn, Durum. At 250 μM of Pb nitrate, the phenolic levels in Ekiz significantly increased compared to those in Einkorn, Emmer, and Durum. At 500 μM of Pb, the phenolic content in Einkorn, Emmer, was considerably higher than in Ekiz and Durum. As reported by Pazoki, (2015), treating mycorrhiza and PGPR at the highest Pb level (900 mg Pb/kg) increased wheat phenolic content to a level higher than the control group. This finding is consistent with Muszyńska et al. (2014), who observed a significant increase in phenolic compound concentration in *Hippophaë rhamnoides* L. leaves compared to control samples. The increase in phenolic compounds in plants might explain their defense mechanism against oxidative stress caused by heavy metal ions. Moreover, Jańczak-Pieniążek et al. (2022) revealed that winter wheat cultivars "Hyacinth", "Hyvento", and "Hyking" exhibited higher accumulation of phenolic compounds due to heavy metal stress. They also observed an enhancement in the activity of tyrosine ammonia-lyase (TAL) and phenylalanine ammonia-lyase (PAL). No significant differences were found in total phenols between the "Hyvento" and "Hyking" cultivars in response to increasing Pb concentrations compared to the "Hyacinth" cultivar at 1000 ppm concentration. Similarly, Sanal (2019) reported that the wheat variety treated "Saban" with combined concentrations of 15 μM , 30 μM , and 60 μM showed both an increase and a decrease in the quantities of compounds that exhibited phenolic characteristics compared to the control group.

4.7 Proline (μmol/g)

Our results showed that the proline content of Ekiz wheat was dramatically enhanced with 50 μM and 100 μM of Pb nitrate treatment compared to Einkorn, Emmer, and Durum cultivars. The proline content in Ekiz, affected by 250 μM of Pb(NO₃)₂, was the highest among the other wheat landraces, including Durum, Emmer, and Einkorn. On the other hand, the proline content of Durum significantly decreased compared to Emmer, Einkorn, and Ekiz varieties at 500 μM of lead nitrate. In line with the study by Doğan & Çolak (2009) who reported that lead one of the toxic metals, affected various physiological features in the bread wheat variety Tosunbey (*Triticum aestivum* L) the increases in proline levels in roots and shoots, along with non-protein SH groups, may indicate their role in the plant's response to lead concentrations (0, 10 and 100 mg/L) in a hydroponic medium. This is consistent with the findings of Slimani et al. (2017), who observed a significant impact of lead on germination. The highest Pb concentration resulted in a complete absence of germination in both Durum wheat varieties (Gta and Vitron). Additionally, the significant impairment of cell biochemical markers due to lead (Pb) stress highlights the negative impact of lead on the biochemical processes in the seedlings. The disruption affects cellular metabolism and leads to alterations in pigment content, proteins, proline, and glutathione levels in seedlings. In line with the study by Tripathi et al. (2016) which found that *Triticum aestivum* (wheat) exhibited significant proline hyperaccumulation when exposed to excess copper (Cu) and cadmium (Cd), Our findings indicate that plants redirect their resources toward the induction of survival mechanisms rather than increasing growth and development during stress. Consistent with Feiyi et al. (2002), who reported that proline content and ethylene production increased in (*Cucumis sativus*) and bread wheat (*Triticum aestivum*) under stress from combined Cd and Pb. Our findings also support the idea that proline is often associated with stress tolerance.

According to the findings of Ouaini et al. (2023), a significant reduction in chlorophyll levels and an increase in proline content were observed in *Atriplex canescens* when being subjected to varying concentrations of NaCl (0.50% and 3%) combined with different levels of Pb(NO₃)₂ (800, 1600, and 2400 ppm).μmol/g

4.8 Protein Content (mg/g)

The soluble protein content (%) mg in Durum at 50 μM and 100 μM of $\text{Pb}(\text{NO}_3)_2$ was significantly higher in Ekiz, Einkorn, and Emmer. A significant difference in protein content was observed between Einkorn, Emmer, and Durum compared to the Ekiz cultivar at 250 μM and 500 μM $\text{Pb}(\text{NO}_3)_2$. This aligns with Souahi et al. (2021), who reported that *Triticum durum* Desf showed increased total protein content in leaves and roots when treated with 0.3 and 0.6 g/l of Pb, compared to *Triticum aestivum* L. In line with the research by Kanwal et al. (2020), treatment negatively impacted physiological measures such as stomatal conductance (-82%), transpiration rate (-72%), and photosynthetic rate (-74%). Biochemical markers were also negatively affected, including carotenoids (-41), total chlorophyll (-43), chlorophyll a (-42), and chlorophyll b (-53). Wheat (Cv. Shafaq-2006) had significantly reduced protein levels (-81%), P levels (-60%), and K levels (-55%) under the highest lead concentration (T6). Consistent with Olteanu et al. (2011), who revealed that SOD activity significantly increased across all Pb^{2+} concentrations with increases ranging from 24.59% to 65.19% for Pb acetate and 20.88% to 175.40% for $\text{Pb}(\text{NO}_3)_2$ compared to the control group, our findings also show increased antioxidative enzyme activity. However, despite this increase, Pb exposure led to a decline in the level of soluble proteins in the wheat seedlings (*Triticum aestivum* cv. Maruca).

According to Ergün & Öncel (2012), who investigated the effects of heavy metals (Pb, Zn, and Cd) and their interactions with hormones (abscisic acid and gibberellin) on wheat seedlings (*Triticum aestivum* L. cv. Gün 91) over 5 and 10 days, the amount of soluble protein increased in seedlings treated with lead and cadmium. However, soluble protein levels decreased in the 10-day samples of seedlings treated with Zn, Zn + GA_3 , AAA, and Cd + GA_3 . Janmohammadi et al. (2013) reported that Pb stress reduced the grain yield of wheat cultivars by an average of 41.4%. It also increased lipid peroxidation and proline accumulation in leaves, while protein content decreased by 8-25.4% across different cultivars in Pb-contaminated soils. This is consistent with Datta et al.(2009), who found that increasing Pb concentration significantly inhibited chlorophyll and protein synthesis in all wheat cultivars (*Triticum aestivum* L.).

4.9 Oxidative Stress and Antioxidant Enzymes in Wheat Seedlings

Pb to Stress in Wheat Cultivars

Lead (Pb) is a non-essential heavy metal and does not play a role in metabolic activities. However, it can easily be absorbed and accumulate in various parts of plants (Nas & Ali, 2018). In the current study, we investigated the toxic effects of different lead concentrations on oxidative stress (H_2O_2) and antioxidant enzymes (CAT and SOD) in wheat landrace cultivars.

4.9.1 CAT Activity ($\mu\text{mol } H_2O_2/\text{min}/\text{mg protein}$)

Our findings showed that the activity of CAT in the Einkorn and Ekiz cultivars was higher than in other species at a 50 μM lead concentration. At a 100 μM lead concentration, the Ekiz cultivar exhibited higher CAT activity than the other cultivars. When the lead nitrate was adjusted to 250 μM and 500 μM , there was a significant difference in CAT activity between the cultivars: Einkorn, Emmer, Ekiz, and Durum wheat. According to Sobhy et al. (2019), the activities of SOD, CAT, and POX all increased in *Triticum aestivum* under lead stress. This is consistent with the findings of Liu et al. (2010), who observed that Pb stress increases the content of GSH in wheat (cultivar 'Jinan No. 17'), thereby affecting the activity of CAT. Similarly, Murtaza et al. (2019) reported an increase in lipid peroxidation and antioxidant enzyme activities (SOD, CAT, APX and peroxidase (POD)) under individual and combined metal treatments (Cd and Pb). However, wheat's chlorophyll content and membrane stability index decreased in the Galaxy-2013 cultivar. In alignment with the findings of Nasr (2013), the activity of studied enzymes (APX, CAT, GR and SOD) in both root and shoot was affected by Cd concentration (0-300 μM) in two wheat cultivars: Darab (Cd-sensitive) and Maroon (Cd-tolerant). The catalase activity in the roots was significantly influenced by both the genotype and the interaction between the genotype and Cd concentration.

According to Oudah et al. (2017), radish (*Raphanus sativus*) and cress (*Lepidium sativus*) showed significantly increased activity of antioxidant enzymes (CAT and POD) after 30 days of exposure, particularly at lead concentrations of 30 ppm and 50 ppm. On the other hand, proline accumulation and enzyme activity responses were similar to control treatments at high lead concentrations. Consistent with the findings of Nessim & El-Shenody (2018), found that carotenoid concentrations, total soluble sugars, Na and Fe content, ascorbic acid,

malondialdehyde, and the activities of POD and CAT increased in *Triticum aestivum* (cv. Gemmeza R2) leaves. In contrast, lead nitrate significantly decreased the total chlorophyll, photosynthetic activity, photosynthetic pigments (Chl a, Chl b), root length, shoot height, and the fresh and dry masses of both roots and shoots in the 21-day-old plants.

4.9.2 SOD Activity (U/mg protein)

The increase in SOD activity can be attributed to two factors: reducing oxidative stress and protecting the plant's defense system from oxidative damage (Malecka et al., 2009). The SOD enzyme is the first line of defense against ROS in many cellular organelles, converting superoxide radicals into oxygen and hydrogen peroxide (Gill & Tuteja, 2010).

Our findings showed that the SOD activity in Ekiz wheat at 50 μM of Pb was dramatically higher compared to other wheat cultivars. Ekiz and Emmer exhibited significant differences in SOD activity compared to Einkorn and Durum Cultivars at 100 μM of Pb. However, Ekiz was significantly reduced by 250 μM of lead compared to other cultivars. The SOD activity in Einkorn and Emmer was significantly different from Ekiz and Durum under 500 μM of $\text{Pb}(\text{NO}_3)_2$. Similarly, Navabpour et al. (2020) reported that in *Triticum aestivum* L., the CAT, and SOD enzyme activities increased with higher lead concentrations in both leaves and roots. Consistent with the findings obtained by Pang et al. (2001), the activity of SOD significantly increased in both the shoot and root of the wheat seedlings, and the activity of CAT was also improved in the roots when exposed to Pb stress. In contrast, Yang et al. (2011) found that low concentrations of 2 and 4 mM of $\text{Pb}(\text{NO}_3)_2$ did not significantly change SOD activity in wheat seedlings of Xihan and Ningchun compared to the control; however, CAT activity significantly increased. Following Al-Yasari et al. (2023), the Iraq variety (*Triticum aestivum* L.) had a significant increase in the activities of POD, SOD, CAT, and proline compared to the Jandohl variety (*Triticum durum* L.) under different concentration of Cd and Pb. In line with our findings, we found that antioxidant enzyme levels (SOD and CAT) in the Durum cultivar were the lowest compared to other cultivars under Pb stress.

The investigation of Taspinar et al. (2023) found that Pb exposure caused significant physiological and biochemical damage in *Triticum aestivum* L. SOD

activity significantly increased in response to Pb and the protective treatments, indicating a defense mechanism against oxidative stress. On the other hand, other wheat species produced smaller amounts of antioxidant enzymes (SOD and CAT) in response to heavy metals stress, suggesting less tolerance to cope with the stress. Similarly, research by Dey et al. (2007), showed that wheat seedlings exposed to Cd and Pb showed significant growth depression when treated with these metals at various concentrations (Cd: 0-200 μ M; Pb: 0-2000 μ M), SOD activity in roots was undetectable even at the lowest Cd concentration (50 μ M), while in shoots. It declined sharply with increasing Cd levels.

4.9.3 Hydrogen Peroxide (H₂O₂)

It is the most prevalent substance in aerobic biological systems in higher plants due to its strong reactivity and toxicity. In plants, H₂O₂ serves as a signaling molecule, facilitating responses to biotic and abiotic stimuli (Petrov & Van Breusegem, 2012). Other studies have shown that H₂O₂ plays two significant functions as a signal molecule and a harmful chemical in wheat seedlings cultivated under H₂O₂ stress. Whether the effect on plants is negative or positive depends on the concentration of H₂O₂, whether low or high (Ge et al., 2013).

The findings obtained by Giannakoula et al. (2021) reported an increase in leaf H₂O₂ and MDA with the treatment of Pb and Cu in *Citrus aurantium* L. Toxicity symptoms of both Pb and Cu-treated plants included stunted growth and decreased pigment concentration. Our findings revealed that wheat landrace cultivars demonstrated higher levels of H₂O₂ due to exposure to high lead nitrate concentrations. However, when lead nitrate was present at a 50 μ M concentration, there was no significant difference in hydrogen peroxide levels between the Ekiz, Einkorn, Emmer, and Durum cultivars and the controls. Whereas with treatment at 100 μ M, 250 μ M, and 500 μ M Pb(NO₃)₂, the Ekiz and Durum cultivars showed a dramatic increase in H₂O₂ compared to Einkorn and Emmer.

Our results confirmed previous investigations (Malar et al., 2014), which found that CAT is the main enzyme produced in plant cells that reduces the level of H₂O₂. Our findings supported previous studies (Kaur et al., 2013) demonstrating that exposure to Pb at concentrations ranging from 50 μ M to 500 μ M elevated H₂O₂ levels and enhanced CAT and SOD activity in *Triticum aestivum*.

According to Dođru (2020), Tokak 157/37 barley cultivar's leaves treated with 15 mM lead exhibited an increase in H₂O₂, potentially indicating oxidative stress. With 15 mM Pb toxicity, the SOD activity in the leaves of two barley cultivars (Namely, Tokak 157/37 and Tarm-92) was considerably higher by 441% and 350%, respectively. These results confirm our findings, the cultivars Einkorn and Emmer possessed antioxidants enzymes (SOD and CAT) and a lower amount of H₂O₂ compared to Ekiz and Durum species under lead concentration (250µM and 500µM), indicating these cultivars were more resistant to Pb stress than other species.

4.10 Effect of Pb (NO₃)₂ Treatment on Elements in Wheat

Landraces

CAT is a crucial antioxidant enzyme that responds to biotic and abiotic stresses. The divalent cations (Mn²⁺, Mg²⁺, Ca²⁺, and Fe²⁺) are essential cofactors in regulatory mechanisms that enhance CAT activity in wheat when exposed to stress (Ghorbel et al., 2023). Consistent with our results, the elements (Ca, Fe, Mg, P, and K) and the CAT enzyme were highest in Emmer and Einkorn compared to other wheat species at 500 µM of lead nitrate.

Iron plays a significant role in the activity of SODs, particularly iron-containing SODs (Fe-SODs). These enzymes are critical for mitigating plant oxidative stress by converting superoxide radicals (O₂^{-•}) into H₂O₂ and O₂. Fe-SODs are primarily located in chloroplasts and are essential in managing ROS generated during photosynthesis. Studies highlight the importance of Fe-SODs in enhancing plant tolerance to various stresses, including heavy metal toxicity like Pb. Fe supplementation can enhance Fe-SOD activity, improving plants' ability to detoxify ROS (Bowler et al., 1994; Demirevska-Kepova et al., 2004). Consistent with our findings, the Fe levels were higher in Emmer and Ekiz species, resulting in a higher amount of SOD enzyme activity compared to Einkorn and Durum cultivars at 100 µM lead. Emmer and Einkorn had higher Fe levels and SOD enzymes than Ekiz and Durum at 500 µM.

Tripathi et al. (2016) reported that exposure to Pb increased oxidative stress indicators such as MDA levels and H₂O₂. Silicon (Si) addition reduced these indicators of oxidative stress and increased antioxidant levels, such as total phenolic compounds (TPCs) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

capacity. Pb stress affected nutritional status, causing a decrease in Ca, P, Mg, Zn, and Ni while increasing K, S²⁻, B, Cu, Fe, Mn, and Na in wheat (*Triticum aestivum* L.) seedlings.

Liu et al. (2010) revealed that the amount of Pb in soil increased with high concentrations of Pb, and it accumulated in wheat leaves before producing visible phytotoxic and oxidative damage effects. Ca²⁺ levels in wheat leaves increased with increasing concentrations of Pb²⁺, affecting Ca²⁺ signal transduction pathways. In line with the research conducted by Bush (1995) and Webb et al. (1996), Ca can act as a second messenger in response to most external stimuli in plants. Similar to our results, the Ekiz and Durum cultivars had higher Pb content than the Einkorn and Emmer cultivars at Pb(NO₃)₂ concentrations of 100 μM and 500 μM. The amount of Ca²⁺ was significantly higher in Einkorn and Emmer than in other cultivars.

Lamhamdi et al. (2013) reported a decrease in the growth and uptake of all mineral ions when spinach (*Spinacia oleracea*) and bread wheat (*Triticum aestivum* L.) were exposed to Pb(NO₃)₂ at concentrations of 1.5, 3, and 15 mM. However, the Mn concentration increased. The deficiency of mineral nutrients caused a reduction in chlorophyll (a), chlorophyll (b), and proline levels in these species, with wheat being more affected than spinach. At Pb concentrations, spinach had a higher soluble protein content than wheat. Consistent with our findings, the cultivar Durum showed lower levels of chlorophyll (a), chlorophyll (b), and proline compared to other wheat landraces due to mineral deficiency (K, Ca, P, Mg, and Fe) at 500 μM Pb concentration. In line with the research conducted by Din et al. (2023), the Pb treatments caused severe oxidative damage to rye and wheat compared to triticale, with major antioxidant enzymes (SOD, POD, CAT, and GPX) showing maximum activity. Pb concentrations in the roots of triticale were the highest and the concentrations of mineral ions (Mg, Ca, and K) in its leaves were greater than those in rye and wheat. Pb exposure consistently resulted in increased photosynthetic activity in triticale.

5. CONCLUSION

The effect of various concentrations of Pb (NO₃)₂ on seedling growth and seed germination inhibition in wheat landrace varieties, namely Ekiz, Einkorn, Emmer, and Durum wheat, was evaluated. The experiments were conducted on soil and petri dishes under controlled conditions in growth chambers. The investigation revealed that when exposed to high concentrations of lead, the tolerance index for seedling development, along with the fresh and dry weights of shoots and roots, gradually decreased. Incubation in the growth chamber was regulated under conditions. The morphological and physiological attributes of wheat cultivated in petri dishes, including coleoptile length, root elongation, and seed germination, were negatively impacted by Pb, particularly at high concentrations of Pb(NO₃)₂. The cultivars grown in soil under lead stress exhibited impaired seedling germination and growth. Durum and Ekiz wheat varieties were significantly more affected by Pb(NO₃)₂ in terms of fresh and dry shoot and root weights, as well as root and shoot elongation, compared to Einkorn and Emmer. Durum was the most susceptible to root toxicity among all treatments. However, shoot and root toxicity was more noticeable in all wheat landraces at increased Pb concentrations.

The tolerance index decreased as the Pb concentrations rose, with Ekiz showing the highest tolerance across all Pb(NO₃)₂ concentrations, followed by Emmer and Einkorn, respectively. The study also examined the accumulation of H₂O₂ in wheat landraces and the impact of Pb stress on antioxidant enzyme activity (CAT and SOD). Chlorophyll a and b levels decreased, while carotenoid content increased with increasing lead treatments, depending on the wheat cultivar. The findings showed that different wheat varieties exhibited variations in oxidative enzyme activity, which may be linked to protein content and nutrient elements (Ca, Fe, Mg, P, and K) involved in defense systems.

Pb is a toxic heavy metal that disrupts physiological processes by producing reactive oxygen species (ROS). Lead nitrate treatments significantly increased the total amount of phenolic compounds and proline in wheat species under lead stress. The accumulation of phenolics and proline is an adaptive strategy for plants to cope with environmental challenges. Proline plays a crucial role in reducing oxidative stress by directly scavenging ROS, increasing the activity of vital antioxidant enzymes that protect cell membranes, and enhancing the plant's defenses. The

results indicate that exposure to lead causes a notable increase in the phenolic content of wheat plants. Given that phenolics have strong antioxidative properties, this increase likely represents a defensive reaction to mitigate oxidative stress caused by Pb.

The ancient wheat landrace cultivars, Einkorn and Emmer, were higher in phenolic compounds compared to the modern Ekiz and Durum wheat varieties, especially with increased lead treatment. This study demonstrates that Pb exposure significantly alters wheat cultivars' protein synthesis, phenolic content, antioxidant defense system, and antioxidant enzyme activity. The findings suggest that the reactions involved in Pb detoxification and stress adaptation are more complex than previously understood.

This part is essential to the thesis. Future research should focus on determining the molecular genetic foundation of these reactions and exploring the possibility of genetic engineering or programs to increase the resistance of wheat to lead. This data is critical for maintaining crop viability and productivity in heavy metal-contaminated conditions. Understanding the underlying genetic processes regulating Pb tolerance will assist researchers in establishing targeted breeding strategies to generate wheat cultivars with more resistance to Pb stress. Ultimately, this may help preserve food security and agricultural production in regions impacted by heavy metal pollution.

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