Journal of Plant Stress Physiology 2026, 12: 13-31
https://doi.org/10.25081/jpsp.2026.v12.9754

Research Article

Impact of different growth stimulants on growth and biochemical parameters of wheat grown under toxic environment of Pb

Syda Zahra Haider[1]*, Ayesha Batool[1], Rehan Jameel[2]

[1] *Department of Botany, Ghazi University, Dera Ghazi khan, Pakistan*
[2] *Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, UCAS, China*

(Received: October 06, 2025; Revised: February 07, 2026; Accepted: February 13, 2026; Published: February 28, 2026)

*Corresponding author: Syda Zahra Haider, E-mail: zahrabotanist@gmail.com

Abstract

Wheat (*Triticum aestivum* L.) is one of the most important food crops that contributed significantly to human civilization. Wheat production at the global level has increased significantly over the years. Wheat plays a vital role in Pakistan's economy, contributing around 14% to agricultural value addition and about 3% to the national GDP. It accounts for approximately 37% of total food energy intake among the population, underscoring its importance as a dietary staple. Lead (Pb) contamination in agricultural soils poses a significant threat to crop growth and productivity. This study investigates the effects of three growth stimulants *Moringa* leaf extract as treatment (MLE 100, 200 mL), zinc oxide nanoparticles (ZnO NPs, 100 ppm, 200 ppm), and sulfocyclic acid (SCA, 100 ppm, 200 ppm) on the growth and biochemical parameters of wheat grown under Pb-stressed conditions. A controlled experiment was conducted to assess plant growth attributes, chlorophyll content, antioxidant enzyme activity, and oxidative stress markers. Results revealed that Pb toxicity severely impaired wheat growth, reducing biomass accumulation and photosynthetic efficiency while increasing oxidative stress. However, the application of growth stimulants significantly mitigated Pb-induced stress. Among the treatments, MLE enhanced plant height and biomass, ZnO NPs improved chlorophyll content and nutrient uptake, while SCA effectively reduced oxidative damage by boosting antioxidant enzyme activity. These findings suggest that MLE, ZnO NPs, and SCA can be promising strategies for improving wheat growth and resilience under heavy metal stress. Further molecular and genetic studies will be recommended for sustainable wheat production in contaminated soils.

Keywords: Abiotic stress, Nanoparticles, ZnO, Wheat

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops that contributed significantly to human civilization. Based on ploidy levels (number of chromosome sets in a cell), cultivated wheat could be diploids (2n = 2x = 14, AA), tetraploids (2n = 4x = 28, BBAA) and hexaploids (2n = 6x = 42, BBAADD). The chromosome sets in the tetraploids and hexaploidwheats are duplications of different genomes, and hence bread wheat and durum wheats are allopolyploids or exact allotetraploids and allohexaploids, respectively. Bread wheat covers more than 95% of the wheat production at the global level. Wheat can be classified into vernal (winter) and non-vernal (spring) types based on growth habits and the requirement of cold for flowering. The vernal wheat (winter/facultative) accounts 35% while the spring wheat (non-vernal) accounts 65% of the total bread wheat production (Ali *et al.,* 2023).

Since ancient times, wheat has played an important role to in feeding the world. It provides 19% of calories and 21% of proteins. Different food types such as French bread, chapati, biscuits, pasta, macaroni, injera, and porridges are prepared using wheat flour solely or in a mixture with the flour of other cereals. Traditionally wheat culture is dominant in Central and West Asia and North Africa (CWANA), Europe, America and Australia. Because of an increase in urbanization and the change in food habits, the demand for wheat is rising every year across the different regions including Eastern and Southern Africa (5.8%), West and Central Africa (4.7%) and South Asia and Pacific (4.3%), Central Asia (5.6%), Australia (2.2%) and North Africa (2.2%). Almost all the countries in the CWANA and SSA regions are net wheat importers. Egypt is the world's largest wheat importer (9 million tons/year) followed by Algeria (4 million tons/year). Sub-Saharan African countries import 17 billion worth of wheat annually. The annual trade value of wheat in 2016 was about 36 billion dollar for a total volume of 184 million tons of wheat in transaction (Asad *et al.,* 2023).

Wheat production at the global level has increased significantly over the years. According to FAO, about 749.5 million tons of wheat was produced on 220 million ha in 2016 with an average grain yield of 3.4 t/ha^{-1}, a highly significant increase from 1961, which stood at 222 million tons with a productivity level of only 1.2 t/ha^{-1}. The accelerated increase in wheat production is attributed to the adoption of improved technology packages, in particular the adoption of high yielding and disease resistant varieties with better response to inputs (e.g., fertilizers, water), improved irrigation systems, machineries and pesticides as well as better management practices, coupled with conducive policies and strong institutions. Wheat is a widely adapted crop. It is grown from temperate, irrigated to dry and high rainfall areas and from warm, humid to dry, cold environments. Undoubtedly, this wide adaptation has been possible due to the complex nature of the plant's genome, which provides great plasticity to the crop. Wheat is a C3 plant and as such it thrives in cool environments (Naveed *et al.,* 2014).

As of recent reports, Pakistan produces approximately 28 million tons of wheat annually, with Punjab province contributing about 70-75% of this total. The area under wheat cultivation spans around 9 million hectares, which constitutes nearly 40% of the total cultivated land in the country. Despite these figures, average yields remain low at approximately 2.5 to 3 tons per hectare, significantly lagging behind potential yields that could reach up to 6 tons per hectare with improved practices and technologies. Wheat is primarily grown during the Rabi season, which runs from October to April, with sowing typically occurring between October and December and harvesting from April to May. The crop relies heavily on irrigation, as most wheat is cultivated on irrigated lands, making water availability critical for its success (Islam, 2017).

Wheat plays a vital role in Pakistan's economy, contributing around 14% to agricultural value addition and about 3% to the national GDP. It accounts for approximately 37% of total food energy intake among the population, underscoring its importance as a dietary staple. The crop supports livelihoods for millions of farmers, particularly smallholders who dominate the agricultural landscape. Despite its significance, wheat production in Pakistan faces numerous challenges. Changes in weather patterns have led to erratic rainfall and increased temperatures, adversely affecting crop yields. Intensive farming practices have resulted in soil fertility depletion and erosion. Pakistan being a part of South-Asia is very susceptible to calamities presumably caused by the climate change. For the last few decades extensive and frequent disasters have shuddered almost all the sectors specially agriculture sector (Shah *et al.,* 2024).

The minimum water content required in the grain for wheat germination is 35 to 45 percent by weight. Germination may occur between 4 °C and 37 °C, optimal temperature being range of 12 °C to 25 °C. Seed size does not alter germination but affects growth, development and yield. Bigger seeds have several advantages when compared to smaller seeds, such as faster seedling growth, higher number of fertile tillers per plant and a higher grain yield. The advantage of bigger seeds is demonstrated when the crop is grown under environmental stresses, particularly drought (Zahra *et al.,* 2021).

When crop emergence occurs, the seed embryo has three to four leaf primordia and almost half of the leaf primordia are already initiated. During germination, the seminal roots grow first, followed by the coleoptile, which protects the emergence of the first leaf. The length of the coleoptile limits sowing depth and its length changes with genotype, increasing only slightly when seeds are sown deeper. Semi-dwarf wheat has shorter coleoptiles than tall wheat (Elbudony, 2017).

Wheat is the most significant crop in Pakistan, both in terms of area cultivated and its contribution to the economy. It serves as a staple food for the majority of the population and plays a crucial role in ensuring food security. This comprehensive overview will delve into various aspects of wheat production in Pakistan, including historical trends, current practices, challenges faced, and future prospects (Hussain *et al.,* 2022).

Wheat has a key role in human nutrition. It is an essential diet for more than 35% of the world's population, accounting for 20% of daily protein and calorie intake. Wheat accounts for around 40-43% of the daily caloric and protein intake in North Africa and West Asia. Pakistan falls in the list of top 10 wheat producing countries. Wheat is a major staple food crop of Pakistan, dominating all crops in terms of area and production. Wheat accounts for 8.2% of the value added in agriculture and 1.9% of the GDP of Pakistan. Approximately 80% of farmers cultivated wheat on about 8.95 million hectares, which accounts for around 40% of the country's total cultivated land during the winter (Rabi) season. Specifically, 6.62 million hectares (73.9%) of this wheat cultivation was carried out in the Punjab province. Pakistan's day temperature has been experiencing a notable increase during March, April, and May, while the night temperature, specifically, increases in March, coinciding with the grain-filling phase of wheat (Hussain *et al.,* 2021).

Climate change threatens future crop production by temperature shifts, changes in precipitation patterns, and more frequent extreme weather events. The agriculture sector is vulnerable to long-term trends and variations in weather conditions. Identifying the relationships between climatic variables and crop yields is essential for predicting and understanding how long-term weather conditions will affect the growth of plants, as well as for developing appropriate adaptation strategies and responses to climate change. In order to accurately identify the impact of climate change on agriculture, it is essential to develop a model that adequately represents the statistical correlation between climatic factors and crop production (Babula *et al.,* 2008).

During the last hundred years, industrialization has grown at a fast rate. It has thus increased the demand for exploitation of the Earth's natural resources at a careless rate, which has exacerbated the world's problem of environmental pollution. The environment has been seriously polluted by several pollutants such as inorganic ions, organic pollutants, organometallic compounds, radioactive isotopes, gaseous pollutants and nanoparticles. They are defined as heavy metals either due to their high atomic weight or because of their high density. Nowadays, the word 'heavy metal' has been used to describe metallic chemical elements and metalloids which are toxic to the environment and humans. Some metalloids and also lighter metals such as selenium, arsenic and aluminium are toxic (Alloway, 2012). They have been termed heavy metals while some heavy metals are typically not toxic such as the element gold, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, molybdenum, silver, cadmium, tin, platinum, gold, mercury and lead. These heavy metals are found naturally on the Earth's crust since the Earth's formation. Due to the astounding increase of the use of heavy metals, it has resulted in an imminent surge of metallic substances in both the terrestrial environment and the aquatic environment. Heavy metal pollution has emerged due to anthropogenic activity which is the prime cause of pollution, primarily due to mining the metal, smelting, foundries, and other industries that are metal-based, leaching of metals from different sources such as landfills, waste dumps, excretion, livestock and chicken manure, runoffs, automobiles and roadworks. Heavy metal use in the agricultural field has been the secondary source of heavy metal pollution, such as the use of pesticides, insecticides, fertilisers, and more. Natural causes can also increase heavy metal pollution such as volcanic activity, metal corrosion, metal evaporation from soil and water and sediment re-suspension, soil erosion and geological weathering (Jagaba *et al.,* 2024).

Heavy metal toxicity adversely affects various physiological processes in staple crops. High concentrations of heavy metals lead to reduced germination rates, stunted growth, and decreased biomass production. For example, cadmium exposure has been shown to inhibit root and shoot development in crops like wheat. Heavy metals negatively impact photosynthetic activity by damaging chlorophyll synthesis and disrupting the photosynthetic apparatus. This results in lower photosynthetic efficiency and reduced carbohydrate production. Specifically, cadmium and lead stress have been found to significantly inhibit chlorophyll synthesis in various plant species (Sidhu *et al.,* 2017). Heavy metals can compete with essential nutrients at the root level, leading to deficiencies in vital minerals such as calcium (Ca), iron (Fe), and magnesium (Mg). This competition exacerbates growth reductions and negatively impacts crop yield. The presence of heavy metals induces oxidative stress in plants by generating reactive oxygen species (ROS). This stress damages cellular components like membranes and proteins, further impairing growth and development. For instance, oxidative damage can lead to chlorosis (yellowing of leaves) and necrosis (death of tissue). Heavy metal toxicity affects plant water relations by decreasing leaf water potential and disrupting stomatal function. This leads to reduced transpiration rates and impaired nutrient transport within the plant. Exposure to heavy metals alters metabolic processes such as respiration and energy production. Plants under heavy metal stress often allocate more energy towards detoxification mechanisms rather than growth processes, leading to overall reduced biomass production (Kumar *et al.,* 2017).

The accumulation of heavy metals in staple crops poses significant risks for food safety. Crops contaminated with heavy metals can transfer these toxic elements up the food chain, affecting herbivores and ultimately humans who consume these crops. Chronic exposure to heavy metals through contaminated food can lead to serious health issues in humans, including neurological disorders, kidney damage, and increased cancer risk. Various countries have established regulatory limits for heavy metal concentrations in food products to protect public health; however, many regions still report levels exceeding these limits due to inadequate monitoring practices. Lead in the environment can cause serious problems to plants and animals. It has become a major environmental contaminant following rapid industrialization and urbanization (Sarker *et al.,* 2021).

Lead is not amongst the essential elements for plants, but they absorb this metal if it is present in their environment, especially in rural areas where the soil is polluted by automotive exhaust and in fields contaminated with fertilizers which contain heavy metals as impurities (Chopra *et al.,* 2009).

According to the US Environmental Protection Agency, lead is one of the most common heavy metal contaminants in aquatic and terrestrial ecosystems and can have adverse effects on the growth and metabolism of plants, owing to its direct release into the atmosphere (Kiran *et al.,* 2022). Lead effects on plants have been described in several reviews (Sharma & Dubey, 2005).

Plants absorb Pb from solution in the soil through their roots and, subsequently, the largest proportion of Pb^{+2} is accumulated within roots in an insoluble form (Li *et al.,* 2007). Lead accumulation in plants increases with an increase in the exogenous lead level. Lead can cause a broad range of physiological and biochemical dysfunctions on seed germination, plant growth, water status and nitrate assimilation (Rahman *et al.,* 2024). Although lead transport from plant roots to shoots is usually limited (Sharma & Dubey, 2005), photosynthesis is especially affected by lead exposure (Qin *et al.,* 2023); chlorophyll and carotenoid contents, photosynthetic rate and CO_2 assimilation are strongly decreased. Ca, Fe and Zn levels decrease in the root tips after lead exposure (Ahmad *et al.,* 2011). In Norway spruce, lead application inhibits growth, and this effect is related to a decrease of Ca^{+2} and Mn^{+2} levels (Rout & Das, 2003). A decreased uptake of K^+, Ca^{+2}, Mg^{+2}, Fe^{+3} and Na^+ was also observed in *Picea abies* treated with lead (Godbold & Kettner, 1991). Thus, the inhibition of mineral ion uptake appears to be a general consequence of lead exposure. Conversely, increased provision of certain inorganic salts can antagonize lead effects to some extent (Lamhamdi *et al.,* 2013).

Plant growth stimulants, also known as biostimulants are natural or synthetic substances that enhance plant growth, development, and overall health. They function by improving nutrient uptake, boosting metabolism, and increasing tolerance to abiotic stresses such as drought or heavy metal toxicity. Common categories of biostimulants include humic acids, seaweed extracts, beneficial microorganisms, and plant hormones like cytokinins and auxins. For instance, seaweed extracts are rich in growth-promoting compounds and have been shown to improve crop yield and quality. Similarly, cytokinins are plant hormones that regulate cell division and growth, playing a crucial role in plant development.

Moringa leaf extract (MLE) is renowned for its rich composition of phytohormones, vitamins, and antioxidants, which can enhance plant growth and stress tolerance. While specific studies on MLE's effect on wheat under Pb stress are limited, research on other crops under heavy metal stress provides valuable insights. For instance, the synergistic application of MLE and zinc oxide nanoparticles (ZnO NPs) has been shown to alleviate cadmium (Cd) stress in linseed plants by enhancing antioxidant enzyme activities and reducing oxidative damage (Ramzan *et al.,* 2022).

Zinc oxide nanoparticles (ZnO NPs) have emerged as potential agents in enhancing plant growth under abiotic stresses, including heavy metal toxicity. Although direct studies on ZnO NPs' effect on wheat under Pb stress are scarce, related research offers promising perspectives. For example, foliar application of ZnO NPs improved the growth of wheat and reduced cadmium concentration in plant tissues, indicating a potential mechanism for mitigating heavy metal uptake and toxicity (Nazir *et al.,* 2024).

Additionally, the combined application of ZnO NPs and *Moringa oleifera* leaf extract alleviated Cd stress in linseed plants through enhanced antioxidant enzyme activity, suggesting a synergistic effect that could be explored in wheat under Pb stress.

Sulfocyclic acid (SCA) is known for its chelating properties and potential to enhance plant tolerance to heavy metal stress by binding to metal ions, thereby reducing their bioavailability and toxicity. While specific studies on SCA's impact on wheat under Pb stress are limited, its general role in heavy metal chelation suggests potential benefits. Further research is necessary to elucidate SCA's effectiveness in mitigating Pb toxicity in wheat and its influence on growth and biochemical parameters.

Despite the importance of lead contamination in North Africa, it remains unclear as to which economical species are able to resist Pb-stress. Wheat is grown on 17% of all crop areas and represents the staple food for 40% of the world's population and is the primary food staple in North Africa (Maccaferri *et al.,* 2009). Wheat is important agricultural specie, so it appeared to be of interest to compare the effects of lead exposure on these two species. In the present study, we have investigated the effects of lead stress on mineral content (Na, K, Ca, P, Mg, Fe, Cu, Zn and Mn), and its consequences on biomass, chlorophyll, soluble proteins and proline contents in leaves and roots of spinach and wheat. Lead is not included in essential elements for plants, but they absorb it when it is present in their environment, especially in rural areas where the soil is polluted by automotive exhaust and in fields contaminated with fertilizers containing heavy metal ingredients. The effect of lead depends on the concentration, type of salt, soil properties and plant species (Zulfiqar *et al.,* 2019).

Toxic levels of lead affect plant processes, as the metal reacts with important functional groups in macromolecules, and the activity of several enzymes is modified, some of which are important in photosynthesis, plant water status and mineral nutrition. The major processes affected are seed germination, seedlings growth, tolerance index, dry mass of roots and shoots. When lead enters the plant cells, like various heavy metals, it induces an oxidative stress in growing plant parts due to enhanced production of reactive oxygen species (ROS), and cell damages result in a reduction of plant productivity. Plants have antioxidant systems to protect them against oxidative damage. Those detoxification processes are complex and highly compartmentalized in plant cells. The level of ROS in wheat seedlings is controlled by an antioxidative system that consists of antioxidative enzymes and non-enzymatic low molecular mass antioxidants (Riaz *et al.,* 2022). Superoxide dismutase (SOD, EC 1.15.1.1) is a key antioxidative enzyme that catalyzes disproportionation of superoxide anion (O_2^-) to H_2O_2 and O_2. Catalase (CAT; EC 1.11.1.6) localized in peroxysomes, scavenges H_2O_2 by converting it to H_2O and O_2. Peroxidase (POD; EC 1.11.1.7) reduces H_2O_2 using several reductants such as phenolic compounds. POD is also the key enzyme in lignin biosynthesis participating in the formation of radicals of lignin units before their polymerization. The two enzymes Ascorbate peroxidase (APX; EC 1.11.1.1) and glutathione S-transferase (GST; EC 2.5.1.18) play a pivotal role in scavenging ROS and maintaining the level of antioxidants ascorbate and glutathione. In addition, GST is involved in the detoxification (conjugation) of lipid peroxidation products (unsaturated alkenals) (Hossain *et al.,* 2006).

Therefore, the current study was performed to evaluate the effect of various growth stimulators to reduce the effect of Pb stress in wheat, To Investigate the role of stimulants in physiological parameters of wheat in response of Pb and to assess the impact of stimulants on biochemical and antioxidant attributes of Pb-stressed wheat.

Materials and methods

Experimental site and soil

All the necessary facilities for the research were provided by the laboratory of the Department of Botany. The pot experiment was conducted at the Department of Botany, Ghazi University, Dera Ghazi Khan. The wheat used was *Triticum aestivum* L. (common wheat), obtained from the Ayoub Research Centre Faisalabad. The specific variety data, such as the name of the variety (e.g., "Sonalika" or "PBW343"), was used. In order to precisely assess the impact of several growth stimulants in the presence of lead (Pb) stress, the wheat plants were cultivated under a carefully controlled environment.

Collection of Moringa leaves and extract preparation

Moringa plant leaves were collected from around the Dera Ghazi Khan. To produce MLE, 200 grams of young leaves were weighed, dried it in a shady place for 5 day then crushed with a pestle in a mortar to make powder.

Moringa and *Kachnar* leaves were collected from completely grown plants around the Dera Ghazi Khan. To produce KLE, 100 grams of young leaves were weighed and then crushed with a pestle in a mortar with a tiny amount of water (10 milliliters for 100 grams of new material). The juice was extracted by hand pressure and filtered through cheesecloth. The fluid was filtered one more using No. 1 Whatman filter paper. Using the Fuglie (2000) approach, the extract was diluted 1:32 (v/v) with distilled water and sprayed directly onto the wheat plants. After cutting and extracting, the KLE were put to work in five hours. The extracted product was stored at 0 °C in a refrigerator.

Preparation of salt solution

By dissolving 1.8 grams in 200 mL of distilled water, a 0.1 M solution of Zn acetate salt was used. On a magnetic heating plate, stir the salt solution until it dissolves completely. To adjust the pH and make a ZnO solution, add 0.1 molar solution drops by gradually. For a 0.1 M solution, 1.8 g sulfocyclic acid salt was dissolved in 200 mL distilled water and proceeded for further applications.

Synthesis of zinc oxide nanoparticle

Add 5 mL *Kachnar* leaves extract into ZnO solution in every five minutes with measuring cylinder stirring continuously for 40 to 50 minutes until turn in white color. Centrifuge the solution at 10,000 rpm for 30 minutes. Remove the supernatant liquid and introduced acetone into the pellet. Transfer the nanoparticles mixture into a china dish and place it in a dry oven at 80 °C for 2 days. After drying, gently grind the resulting material to ensure uniform particle distribution. Collect the ground nanoparticles and stored them in Eppendrof tubes for further analysis and application.

Characterization of ZnO NPS

Scanning electron microscopy (SEM)

Morphological prototype and nanoscale ZnO NPs distribution were studied through SEM imaging. The Aid of Scanning Electron Microscope (SEM) SIGMA model as MIRA3 (TESCAN Brno and the Czech Republic) that was sourced from the Institute of UAF, Faisalabad was used to perceive the biogenic ZnO NPs profile. Samples for SEM preparation were made by only keeping smaller quantities of samples on carbon coated zinc substrates for SEM. The films then were had air dried before SEM micrographs were established at different magnifications by using a scanning electron microscope.

Foliar application of ZnO NPs, Sulfosaylicylic acid and Moringa extract solution on wheat under lead stress

The ZnO NPs of different concentrations were applied to the Wheat plants. Foliar sprayers were used to spray of ZnO nanoparticles. ZnO nanoparticles with KLE were applied to different developmental stages based on the methods of treatment. *Moringa* powder solution of different concentrations with ZnO NPs applied on wheat under lead stress. Extra care was taken to ensure that the plants were completely covered with the spray ingredients. The pots were arranged in a complete randomized block design (CRBD) for the experiment. Wheat variety (Sonalika) was selected for pots experiments. The lead treatment (100 ppm) was applied after three weeks of sowing. Pb treated plant was regarded as the negative control while the one without regarded as the control. All treatments were initiated as *Moringa* (100 mL and 200 mL), ZnO nanoparticles (100 ppm and 200 ppm) and Sulfosaylicylic acid (100 ppm and 200 ppm). ZnO nanoparticles synthesized from *Kachnar* leaf extract were used as treatments. The different treatments were applied at the vegetative stage after 30 days of sowing. After that different morphological and physio-biochemical attributes were analyzed (Table 1).

Data collection

The crop was harvested when it was fully grown. While the straw yield was recorded on a sun-dry basis, the grain yield was determined using a 14% moisture basis. A variety of data were gathered about root length, shoot length, fresh and dry weight of roots and physico-chemical parameters.

Morphological parameters

Scale and weight balance were used to determine every parameter, including fresh shoot weight, dry weight root, root fresh weight and dry weight of shoot among others.

Measurement of root, shoot length (cm)

Seedlings were removed from the ground at various intervals. Roots and shoots were measured precisely from the beginning (Lopushinsky & Max, 1990).

Determination of fresh root and shoot weight (gm)

Plants weight was determined by using weighing balance like root fresh weight and shoot fresh weight (Ahmad *et al.,* 2011).

Determination of dry root and shoot weight (gm)

Plants weight was determined by using spring balance like dry shoot weight, root dry weight, etc., (Liu & Li, 2005).

Determination of spike weight

Spike weight was calculated as the normalized peak-to-peak amplitude of detected spikes, accounting for baseline noise. This metric was used to analyze spike intensity and validate signal reliability across experimental conditions.

Number of trillars

To count wheat tillers, randomly dig up plants, count stems from the base, and calculated the average number of tillers per plant used a shovel, ruler, and notebook to note.

Table 1: Experimental layouts

Treatments	Concentration
T0	Control
T1	Pb (100 ppm)
T2	*Moringa* (100 mL) + Pb (100 ppm)
T3	ZnO (100 ppm) + Pb (100 ppm)
T4	SCA (100 ppm) + Pb (100 ppm)
T5	*Moringa* (200 mL) + Pb (100 ppm)
T6	ZnO (200 ppm) + Pb (100 ppm)
T7	SCA (200 ppm) + Pb (100ppm)

Physiological parameters

Measurement of a, b and total chlorophyll contents (ug/g)

Twenty milliliters of 80% acetone were added to 0.5 grams of finely chopped, fresh leaf material, which was then mashed for five minutes in a sanitized mortar. The resultant extract was filtered through a layer of Whatman No. 1 filter paper using a Buchner funnel and suction. For a further five minutes, the pulp is ground again using 15 milliliters of 80% acetone. A suitable amount of 80% acetone was added to the filtrate to get the final volume down to 5 mL using a spectrophotometer, the extract's absorbance was measured at 663 nm, 645 nm, and 440 nm (Yang *et al.,* 1998). The contents of chla, b and carotenoids were calculated using the following formulae:
Chlorophyll a (mg/L) = 12.7(A663) – 2.7(A645)
Chlorophyll b (mg/L) = 22.9(A645) – 4.7(A663)
Total chlorophyll content = (A652x1000/34.5)

Biochemical parameters

Proline contents, soluble sugar, Catalase activity, superoxide dismutase (SOD) activity, etc., were determined.

Proline content (µg/g)

First take 2 g of leaves of all treatments and grind these leaves in 7 mL distilled water and put this abstract in small beaker according to treatments of both varieties. New leaf specimens In 4 mL of sulfocyclic acid, 0.2 g was ground. 2 mL of filtrate is taken and reacted with 4 mL of Ninhydrin reagent and 2 mL of glacial acetic acid in separate test tubes. And put this material on water bath for one hour until color change and put these test tubes in ice. After half hour 4 mL of toluene was added and mixture mixed properly until upper color layered is appeared. This layer then separated from mixture in another set of test tubes and absorbance was observed at 520 nm wavelength.

Proline Content= (Sample absorbance * Dilution *k value)/fresh weight of plant tissue

Phenolic content determination (mg/g)

Determination of phenolic was described by julkumn-Titto R (1985). Extract 40-50 mg of ground fresh material in 1 ml 80% acetone at 50 °C for 1 hour. Take an aliquote and dilute with distilled water to 1 mL in test tube then add 0.5 mL of folin-crocaltus phenol reagent and shake vigorously then immediately add 2.5 mL of Na_2CO_3 an make the volume to 5 mL and vortex vigorously for 5-10 seconds. Wait for 20 minutes and measure the absorbance at 750 nm by using spectrophotometer.

Superoxide dismutase (SOD activity) (units/mL)

Determination of SOD was assayed by photochemical method as described by Ginnopolitics and Ries (1977). First take abstract of fresh leaves and used 130 mM methionine, 1 mM EDTA 5.0 mM, phosphate buffer, 0.02 mM riboflavin and 0.75 mM NBT. This mixture then put it in fluorescent light for 7 minutes and then the absorbance was recorded at 560 nm. Activity of enzymes was calculated applying Lambert-Beer law.

$$A = \varepsilon LC$$

Where A = absorbance
ε = extinction coefficient
L = length of each wall

Measurement of catalase activity (µM)

The absorbance at 240 nm, which was used to measure CAT activity, decreased when H_2O_2 was removed (Cakmak & Marschner, 1992). The reaction mixture (2 mL) consisted of 10 mM H_2O_2, 0.2 mL of enzyme extract, and 25 mM phosphate 0buffer (pH 7.0). An extinction coefficient of 39.4 mM^{-1} cm^{-1} will be used to calculate the activity of the enzyme. It was discovered that one unit of particular enzyme activity was needed to degrade one micromole of H_2O_2 min^{-1} mg^{-1} protein at 25 °C.

Total soluble sugar (µg/mL)

The Phenol-Sulfuric Acid Method was utilized to evaluate soluble sugar (Chapman & Craigie, 1978). Ten milliliters of 80% ethanol were used to homogenize fresh leaf material (0.5 g), which was then cooked for an hour at 80 °C in a water bath. Test tubes were filled with a 0.5 mL sample extract, which was then ground in a blended 10 mL of ethanol. NaCl heated the water in the bath for an hour. A 0.5 mL sample extract was placed in a test tube, to which 1 ml of 18% phenol was added. The combination was then allowed to incubate at room temperature. 2.5 mL of sulfuric acid was added after an hour, and the mixture was thoroughly shaken. Using a spectrophotometer, each sample was ultimately absorbed at a wavelength of 490 nm.

The soluble sugar concentration (µg/mL) was calculated using the following formula:

Sugar (µg/mL) = sample absorbance × dilution factor × K value/weight of fresh plant tissue

Total carbohydrates

Total soluble carbohydrates were extracted according to a modified procedure described by Wardlaw and Willenbrink (1994). Total amounts of wsc (mg wsc/100 mg dry weight) were determined as fructose equivalents using the anthrone colorimetric assay (Yemm & Willis, 1954) at 620 nm on an lG-721spectrophotometer.

Free amino acids

The total amount of free amino acids was calculated utilizing the method of (Hamilton & Van-Slyke, 1943). Fresh leaves weighing 0.5 grams were homogenized in 10 ml of a pH 7.0, 50 mM phosphate buffer, then centrifuged at 10,000 rpm. The amount of free amino acids in the supernatant was measured. One gram of ninhydrin was added to 50 milliliters of distilled water to create acidic ninhydrin. First, 0.2 grams of ninhydrin must be dissolved in 10 milliliters of either acetone or ethanol to make a 2% solution of the compound. The pyridine solution was made by mixing 5mL of pyridine with 45 mL of distilled water. In order to prepare the reaction mixture, 0.5 mL of the extracted material, 0.5 mL of ninhydrin, and 0.5mL of pyridine solution were combined. For 30 minutes, this mixture was heated in a water bath to 60 °C. After that, each test tube received 25 mL of distilled water. After 5 minutes in the ice breaker, these test tubes were removed, and the final absorption value was measured at 570 nm using a spectrophotometer (UV-1100).

Membrane stability index

From each treatment of the variety, fresh leaves weighing 0.1 g were taken and placed in test tubes with 25 mL of distilled water. These test tubes were submerged in a 40 °C water bath for 30 minutes. The initial conductivity (C1) was noted using a conductivity meter. The samples were kept at 100 °C for 10 minutes, according to Premachandra *et al.* (1990), as updated by Sairam (1994), during which time the final conductance (C2) was determined.

The following formula was used to determine the membrane stability index (MSI):

$$MSI = [1 - (C1/C2)] \times 100$$

Hydrogen peroxide

Spectrophotometric measurements of hydrogen peroxide (H_2O_2) were made following the reaction with potassium iodide. Using a pestle and mortar, 0.20 g of fresh leaves were ground in 1% Trichloroacetic Acid (TCA) and centrifuged at 12000 g for 15 min. Potassium iodide solution 10 mM was made. The reaction mixture included 1mL of potassium phosphate buffer (pH.7.0, 10 mM), 1 mL of extracted material, and 1 mL of 10 mM KI_2. The optical density of hydrogen peroxide (H_2O_2) was measured at 390 nm as the control standard (Velikova et al., 2000).

Total soluble protein

Protein content could be calculated using the Bradford Method, published in 1976 (Jones *et al.,* 1989). Fresh leaf samples weighing 0.2 g were crushed in 4 mL of a 50 mM potassium phosphate buffered solution (pH 7.0), and then centrifuged for 15 minutes at 10,000 rpm. 0.1 mL of the extracted sample and 2.5 mL of Bradford dye were mixed in test tubes. A spectrophotometer was used to detect the absorbance at 595 nm after waiting 10 minutes.

Catalyze activity

The absorbance at 240 nm, which was used to measure CAT activity, decreased when H_2O_2 was removed (Cakmak & Marschner, 1992). The reaction mixture (2 mL) consisted of 10 mM H_2O_2, 0.2 mL of enzyme extract, and 25 mM phosphate buffer (pH 7.0). An extinction coefficient of 39.4 mM^{-1} cm^{-1} will be used to calculate the activity of the enzyme. It was discovered that one unit of particular enzyme activity was needed to degrade one micromole of H_2O_2 min^{-1} mg^{-1} protein at 25 °C.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the mean value of each treatment, through statistic software 8.1, and for mean deviation. Least significance differences were calculated at <0.05%, 0.01% and 0.0015 level.

Results

Characterization of nanoparticle ZnO

Biogenic Zinc Oxide Nanoparticles (ZnO NPs) were shaped using scanning electron microscopy (SEM), as seen in Figure 1, the micrograph of the copper oxide nanoparticles revealed a spherical form and particles size.

Morphological parameters

Effect on shoot length

Analysis of variance (ANOVA) presented in Table 2 depicts the significant variation ($P \leq 0.05$) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on shoot length. The heavy metal Pb treated plant showed decreased shoot length indicating the toxic effects of lead as compared to control. The effect of different treatments on shoot length (cm) under exposure to lead (Pb, 100 ppm). The

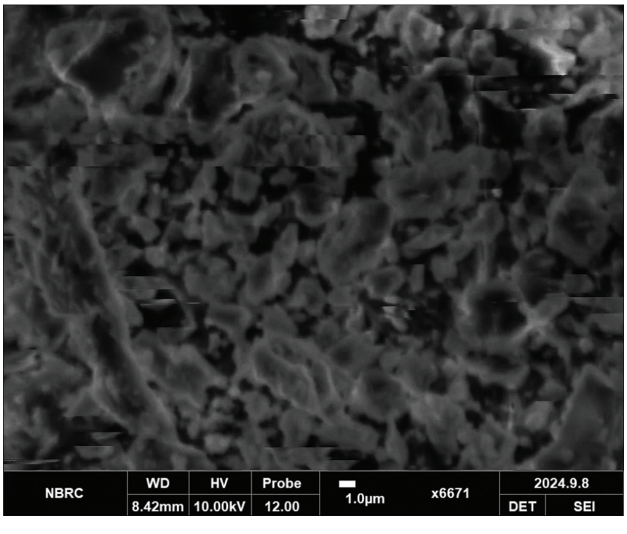

Figure 1: Depicted that SEM analysis for ZnO nanoparticle

Table 2: Analysis of variance table for shoot length

Source	DF	SS	MS	F	P
Treatment	7	1530.5	218.64	0.42	0.8769
Replicate	2	2605.6	1302.79		
Error	14	7365.7	526.12		
Total	23	11501.8			

control (T0) has moderate shoot length, while Pb alone (T0-) significantly reduces it, highlighting its toxicity. Treatments with *Moringa*, ZnO, and SCA at 100 ppm (T1, T2, T3) improve shoot length compared to Pb alone, with *Moringa* showing the most substantial effect, followed by ZnO and SCA. At 200 ppm concentrations (T4, T5, T6), all treatments further enhance shoot length, demonstrating a dose-dependent mitigation of Pb toxicity. Among all treatments, *Moringa* at 200 ppm (T4) has the strongest positive impact, followed by ZnO (T5) and SCA (T6). This indicates that higher concentrations of these agents are more effective in counteracting the negative effects of Pb (Figure 2).

Effect on root length

Analysis of variance (ANOVA) presented in Table 3 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on root length. The heavy metal Pb treated plant showed decreased root length indicating the toxic effects of lead as compared to control. The effect of various treatments on Root Length under Pb stress conditions. The control group (T0) shows moderate root length, while T0- (Pb 100 ppm) results in a significant reduction, indicating Pb toxicity. Treatments T1 to T6, which combine *Moringa*, ZnO, and SCA at varying concentrations with Pb (100 ppm), exhibit variable improvements. Among them, T5 (ZnO 200 ppm + Pb 100 ppm) achieves the highest root length, suggesting ZnO at 200 ppm effectively mitigates Pb stress. T1 (*Moringa* 100 ppm + Pb) and T4 (*Moringa* 200 ppm + Pb) also show notable increases, while T6 (SCA 200 ppm + Pb) has the lowest root length among the treatments, highlighting its reduced efficacy in alleviating Pb toxicity (Figure 3).

Effect on shoot fresh weight

Analysis of variance (ANOVA) presented in Table 4 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on shoot fresh weight. The heavy metal Pb treated plant showed decreased shoot fresh weight indicating the toxic effects of lead as compared to control. The effect of different treatments on shoot fresh weight under Pb stress conditions. The control group (T0) shows the lowest shoot weight, while T0- (Pb 100 ppm) further reduces it, highlighting Pb toxicity. Treatments T1 to T6, which combine *Moringa*, ZnO, and SCA at varying concentrations with Pb (100 ppm), show improved shoot weights compared to T0-. Among these, T5 (ZnO 200 ppm + Pb 100 ppm) exhibits the highest shoot fresh weight, suggesting ZnO at 200 ppm is the most effective in alleviating Pb stress. T4 (*Moringa* 200 ppm + Pb) and T6 (SCA 200 ppm + Pb) also demonstrate significant improvements, but slightly lower than T5, indicating ZnO's superior role in mitigating Pb toxicity (Figure 4).

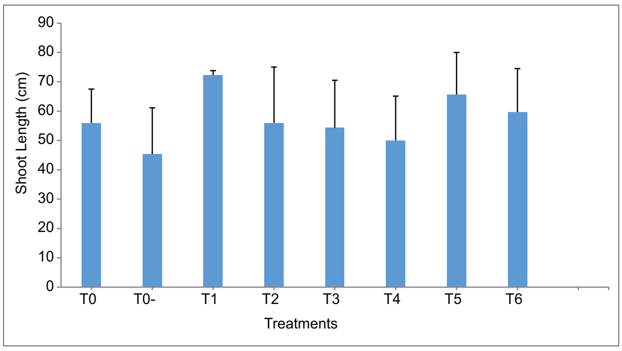

Figure 2: Effect of different growth parameters on shoot length of wheat under heavy metal stress

Table 3: Analysis of variance table for root length

Source	DF	SS	MS	F	P
Treatment	7	532.67	76.0952	1.14	0.3952
Replicate	2	33.33	16.6667		
Error	14	937.33	66.9524		
Total	23	1503.33			

Effect on root fresh weight

Analysis of variance (ANOVA) presented in Table 5 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on root fresh weight. The heavy metal Pb treated plant showed decreased shoot dry weight indicating the toxic effects of lead as compared to control. The root fresh weight of plants under different treatments involving lead (Pb) stress and different regulators. The control group (T0) had the lowest root weight, while the Pb-only treatment (T0-) further reduced growth, indicating Pb toxicity. Treatments T4 (*Moringa* 200 ppm + Pb) and T5 (ZnO 200 ppm + Pb) resulted in the highest root fresh weights, suggesting that higher concentrations of *Moringa* and ZnO are more effective in mitigating Pb stress. Other treatments (T1, T2, T3, T6) showed moderate improvements compared to Pb-only, but their effects were less pronounced. Overall, *Moringa* and ZnO at higher concentrations appear to significantly enhance root growth under Pb stress conditions (Figure 5).

Effect on shoot dry weight

Analysis of variance (ANOVA) presented in Table 6 the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on shoot dry weight. The heavy metal Pb treated plant showed decreased shoot dry weight

Table 4: Analysis of variance table for shoot fresh weight

Source	DF	SS	MS	F	P
Treatment	7	36.3737	5.19625	2.84	0.0459
Replicate	2	0.7731	0.38653		
Error	14	25.6316	1.83083		
Total	23	62.7784			

Table 5: Analysis of variance table for root fresh weight

Source	DF	SS	MS	F	P
Treatment	7	46.499	6.64271	0.75	0.6334
Replicate	2	8.02	4.00988		
Error	14	123.409	8.81496		
Total	23	177.928			

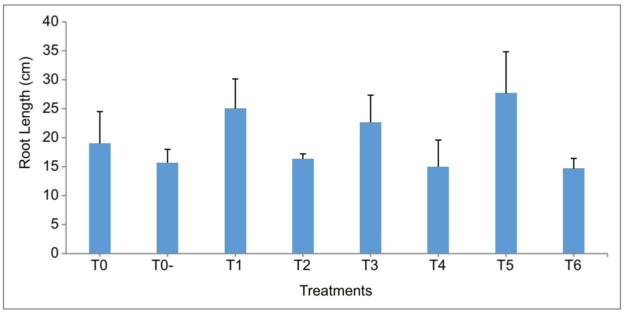

Figure 3: Effect of different growth parameters on root length of wheat under heavy metal stress

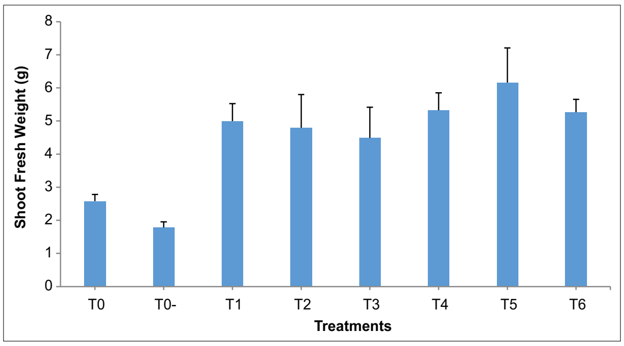

Figure 4: Effect of different growth parameters on shoot fresh weight of wheat under heavy metal stress

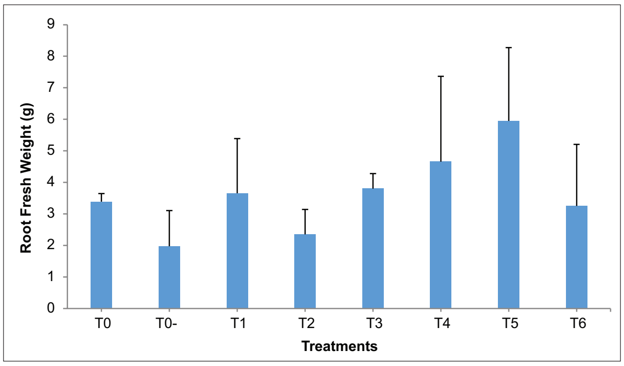

Figure 5: Effect of different growth parameters on root fresh weight of wheat under heavy metal stress

indicating the toxic effects of lead as compared to control. The effects of different treatments on shoot dry weight under varying concentrations of additives and lead (Pb, 100 ppm). The control (T0) showed a baseline measurement, while T0- (Pb only) displayed reduced shoot dry weight, highlighting Pb's negative impact. Among the treatments, T5 (ZnO 200 ppm + Pb) resulted in the highest shoot dry weight, indicating a strong mitigating effect of ZnO at higher concentrations. Treatments T1 (*Moringa* 100 ppm + Pb) and T2 (ZnO 100 ppm + Pb) also showed improved growth compared to T0-, whereas T4 (*Moringa* 200 ppm + Pb) and T3 (SCA 100 ppm + Pb) exhibited relatively lower values, suggesting their mitigating effects were less pronounced. Overall, T5 demonstrated the most substantial improvement, emphasizing ZnO's potential in alleviating Pb-induced stress (Figure 6).

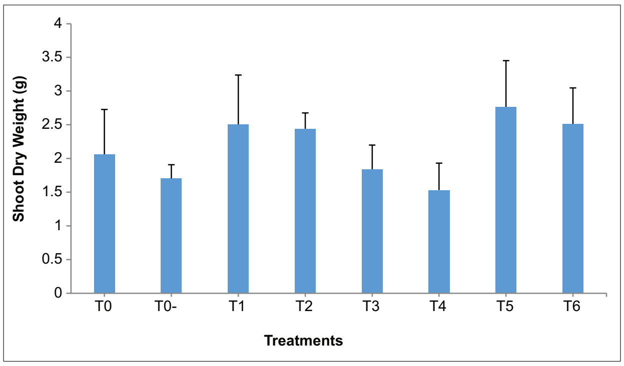

Figure 6: Effect of different growth parameters on shoot dry weight of wheat under heavy metal stress

Table 6: Analysis of variance table for shoot dry weight

Source	DF	SS	MS	F	P
Treatment	7	4.2234	0.60334	0.85	0.569
Replicate	2	2.7325	1.36625		
Error	14	9.9894	0.71353		
Total	23	16.9453			

Table 7: Analysis of variance table for root dry weight

Source	DF	SS	MS	F	P
Treatment	7	32.0461	4.57802	3.35	0.0259
Replicate	2	5.0424	2.52118		
Error	14	19.1439	1.36742		

Effect on root dry weight

Analysis of variance (ANOVA) presented in Table 7 depicts the significant variation ($P \leq 0.05$) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on root dry weight. The bar chart illustrates the effect of various treatments on root dry weight under lead (Pb) stress. The control treatment (T0) and lead stress alone (T0-, Pb 100 ppm) show lower root dry weights, with T0- being significantly reduced compared to T0. Treatments combining lead with *Moringa*, ZnO, or SCA at 100 ppm (T1, T2, T3) show slight improvements, while the higher concentrations of *Moringa* (T4) and ZnO (T5) at 200 ppm notably enhance root dry weight, demonstrating their potential to mitigate the adverse effects of lead. Among all treatments, T4 and T5 yield the highest root dry weights, highlighting the effectiveness of these higher concentration amendments in promoting growth under lead stress (Figure 7).

Effect on spike weight

Analysis of variance (ANOVA) presented in Table 8 depicts the significant variation ($P \leq 0.05$) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* Extract and their combined interaction on spike weight. The heavy metal Pb treated plant showed decreased spike weight indicating the toxic effects of lead as compared to control. The addition of at both 100 ppm (T1) and 200 ppm (T4) moderately mitigated the toxicity, with T4 showing slightly better results. Zinc oxide nanoparticles (ZnO) were *Moringa oleifera* highly effective, with 100 ppm (T2) nearly restoring the spike weight to control levels and 200 ppm (T5) producing the highest spike weight, exceeding all treatments. Similarly, sulfocyclic acid (SCA) at 100 ppm (T3) and 200 ppm (T6) improved spike weights, but their effectiveness was limited compared to *Moringa* and ZnO. Overall, ZnO at 200 ppm (T5) was the most effective treatment in countering Pb-induced toxicity and enhancing spike weight, followed by *Moringa* and SCA, indicating the potential of these agents in alleviating heavy metal stress (Figure 8).

Effect on number of tillers

Analysis of variance (ANOVA) presented in Table 9 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* Extract and their combined interaction on number of tillers. The heavy metal Pb treated plant showed decreased spike weight indicating the toxic effects of lead as compared to control.The number of tillers under various treatments involving lead (Pb, 100 ppm) and combinations of *Moringa*, ZnO, and SCA at different concentrations. The control (T0) shows the highest number of tillers, indicating optimal growth conditions. Lead alone (T0-) significantly reduces the number of tillers, reflecting its inhibitory effects. Among treatments at 100 ppm, SCA (T3) shows the highest recovery in tiller number, followed by ZnO (T2) and *Moringa* (T1). However, at 200 ppm, the number of tillers does not improve further, as seen with *Moringa* (T4), ZnO (T5), and SCA (T6), suggesting limited or no additional benefit of increased concentrations. Overall, SCA at 100 ppm appears to be the most effective in mitigating the toxic effects of Pb on tiller production (Figure 9).

Physiological parameters

Effect on membrane stability index

Analysis of variance (ANOVA) presented in Table 10 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on membrane stability Index. The heavy metal Pb treated plant showed decreased membrane stability Index indicating the toxic effects of lead as compared to control. The membrane stability index under various treatments (T0 to T6) involving lead (Pb) stress and different concentrations of *Moringa* extract, ZnO, and SCA. The control group (T0) shows a baseline index, while Pb treatment alone (T0-) results in a decline. Treatments with 100 ppm of *Moringa*, ZnO, and SCA (T1–T3) slightly improve membrane stability compared to Pb alone. However, treatments with 200 ppm (T4–T6) of the same substances show a significant increase, with T6 (SCA at 200 ppm + Pb) achieving the highest stability index, followed by T4 (*Moringa*) and T5 (ZnO). Error bars indicate variability, and overall, higher concentrations of treatments are more effective in mitigating Pb-induced damage to membrane stability (Figure 10).

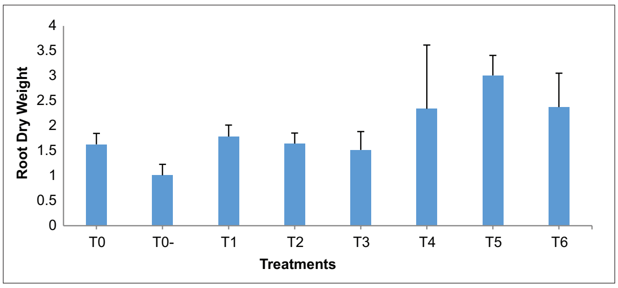

Figure 7: Effect of different growth parameters on root dry weight of wheat under heavy metal stress

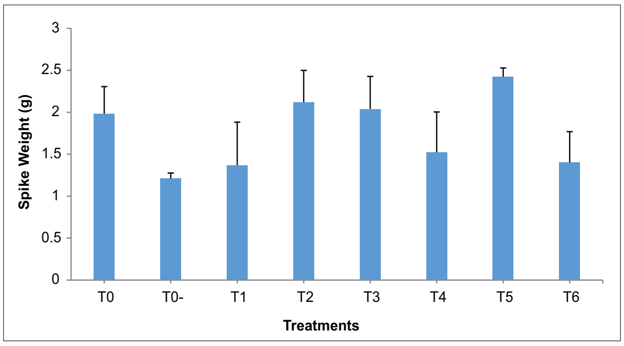

Figure 8: Effect of different growth parameters on spike weight of wheat under heavy metal stress

Effect on chlorophyll a

Analysis of variance (ANOVA) presented in Table 11 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on chlorophyll a. The heavy metal Pb treated plant showed decreased chlorophyll a indicating the toxic effects of lead as compared to control. The chlorophyll a content across different treatments. The control group (T0) shows a baseline value, while T0- (lead-only treatment) has a slightly reduced chlorophyll a concentration, reflecting lead's adverse effects. Treatments involving *Moringa* (T1 and T4), Zinc oxide (T2 and T5), and SCA (T3 and T6) in combination with lead reveal a progressive improvement in chlorophyll a content. Notably, higher concentrations of the agents (T4, T5, and T6) yield the

Table 8: Analysis of variance table for spike weight

Source	DF	SS	MS	F	P
Treatment	7	3.10912	0.44416	2.04	0.1214
Replicate	2	3.49163	1.74582		
Error	14	3.04863	0.21776		
Total	23	9.64938			

Table 9: Analysis of variance table for number of tillers

Source	DF	SS	MS	F	P
Replicate	2	4.083	2.0417		
Treatment	7	97.167	13.881	1.47	0.2566
Error	14	132.583	9.4702		
Total	23	233.833			

Table 10: Analysis of variance table for membrane stability index

Source	DF	SS	MS	F	P
Treatment	7	3150.1	450.014	7.54	0.0007
Replicate	2	473.57	236.783		
Error	14	835.78	59.699		
Total	23	4459.45			

Table 11: Analysis of variance table for chlorophyll a

Source	DF	SS	MS	F	P
Treatment	7	44.195	6.31357	0.86	0.5611
Replicate	2	1.536	0.76815		
Error	14	103.108	7.36485		
Total	23	148.839			

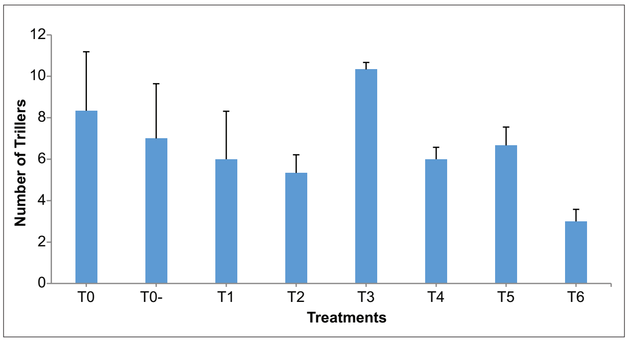

Figure 9: Effect of different growth parameters on number of tillers of wheat under heavy metal stress

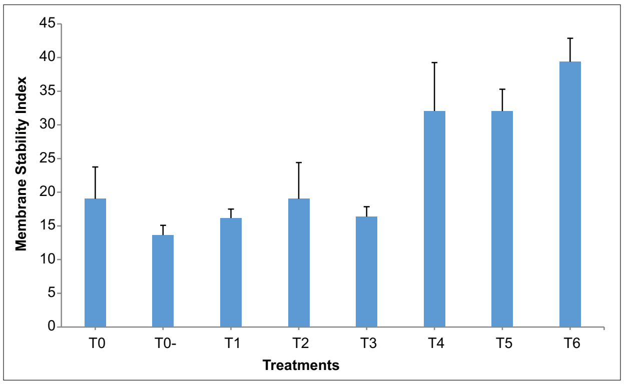

Figure 10: Effect of different growth parameters on membrane stability index of wheat under heavy metal stress

maximum chlorophyll a levels, indicating their effectiveness in mitigating lead toxicity (Figure 11).

Effect on chlorophyll b

Analysis of variance (ANOVA) presented in Table 12 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* Extract and their combined interaction on chlorophyll b. The heavy metal Pb treated plant showed decreased chlorophyll b indicating the toxic effects of lead as compared to control. The chlorophyll b content under similar conditions. The T1 treatment (*Moringa* + Pb) shows the highest chlorophyll b level, indicating that *Moringa* significantly enhances this pigment. Treatments T2 and T5 (ZnO + Pb) and T3 and T6 (SCA + Pb) also demonstrate improvements over the control with lead (T0-), though not as pronounced as T1. Higher concentrations of the agents (T4, T5, and T6) exhibit a general trend of better performance in boosting chlorophyll b than lower concentrations, reflecting dose-dependent efficacy (Figure 12).

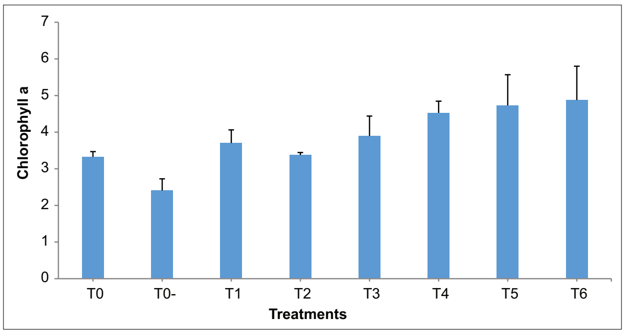

Figure 11: Effect of different growth parameters on chlorophyll of wheat under heavy metal stress

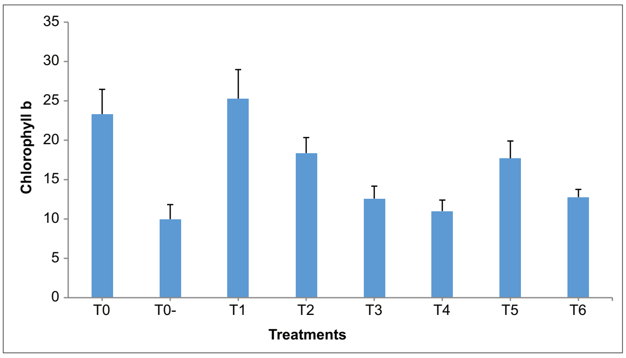

Figure 12: Effect of different growth parameters on chlorophyll b of wheat under heavy metal stress

Table 12: Analysis of variance table for chlorophyll b

Source	DF	SS	MS	F	P
Treatment	7	1222.18	174.597	3.41	0.0241
Replicate	2	210.61	105.305		
Error	14	716.2	51.157		
Total	23	2148.99			

Table 13: Analysis of variance table for total chlorophyll content

Source	DF	SS	MS	F	P
Treatment	7	384.94	54.992	1.18	0.3737
Replicate	2	9.93	4.9654		
Error	14	652.64	46.6169		
Total	23	1047.51			

Effect on total chlorophyll content

Values of analysis of variance (ANOVA) presented in Table 13 are significant among wheat treatments with Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their interaction effect on total chlorophyll content (P≤0.05). Treatment with the heavy metal Pb showed a lower total chlorophyll content which suggests toxicity due to lead compared with control. The total chlorophyll content comes out to be in different treatments of lead (Pb) and some mitigation agents. The control is set by T0 while T0-(Pb alone) evidenced a declining trend for total chlorophyll content compared to control, demonstrating the adverse impact of lead. Similar was the case in treatments with lead along with *Moringa* (T1 and T4), Zinc Oxide (T2 and T5), and SCA (T3 and T6); higher than T0-. Among them, T4, T5, and T6 are usually higher in chlorophyll content as the concentration of mitigants increases, showing a dose-dependent ameliorative effect. Interestingly, T3 (SCA at 100 ppm) stands out with a very high chlorophyll content. All this underlines the anti-lead toxicity effects of this treatment (Figure 13).

Biochemical parameters

Effect on proline content

Analysis of variance (ANOVA) presented in Table 14 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on proline content. The heavy metal Pb treated plant showed decreased proline content indicating the toxic effects of lead as compared to control. The proline concentration under different treatments involving lead (Pb) exposure and its mitigation through *Moringa*, zinc oxide (ZnO), and supercritical antioxidants (SCA). The control group (T0) displayed the highest proline levels, suggesting no stress-induced inhibition. Treatment T0-, which involved lead alone (100 ppm), exhibited the lowest proline concentration, highlighting stress-related suppression of proline accumulation. *Moringa* extract combined with lead at 100 ppm (T1) showed slight proline recovery compared to T0-. ZnO (T2) and SCA (T3) at the same concentrations had minimal impact on proline levels. However, *Moringa* at a higher concentration (200 ppm, T4) significantly improved proline content, outperforming ZnO (T5) and SCA (T6) at the same elevated concentration. The data suggest that *Moringa* extract at higher concentrations is more effective in mitigating Pb-induced stress by promoting proline biosynthesis (Figure 14).

Effect on carbohydrates content

Analysis of variance (ANOVA) presented in Table 15 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on carbohydrate content. The heavy metal Pb treated plant showed decreased carbohydrate indicating the toxic effects of lead as compared to control.The carbohydrate content across various treatments. Treatment T0 (control) shows a moderate carbohydrate level, which slightly decreases in T0- (Pb at 100 ppm). In T1 (*Moringa* 100 ppm + Pb 100 ppm) and T2 (ZnO 100 ppm + Pb 100 ppm), carbohydrate levels remain comparable to the control. A significant increase is observed in T3 (SCA 100 ppm + Pb 100 ppm), indicating its effectiveness in maintaining or enhancing carbohydrate levels under stress conditions. However, in T4 (*Moringa* 200 ppm + Pb 100 ppm) and T5 (ZnO 200 ppm + Pb 100 ppm), the carbohydrate content drops compared to T3, though it stays above T0-. T6 (SCA 200 ppm + Pb 100 ppm) reflects a slight improvement compared to T4 and T5. Error bars suggest variability across replicates, particularly noticeable in T3, emphasizing the treatment's pronounced effect (Figure 15).

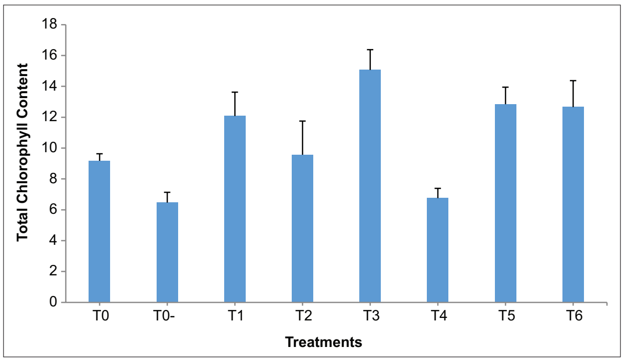

Figure 13: Effect of different growth parameters on total chlorophyll content of wheat under heavy metal stress

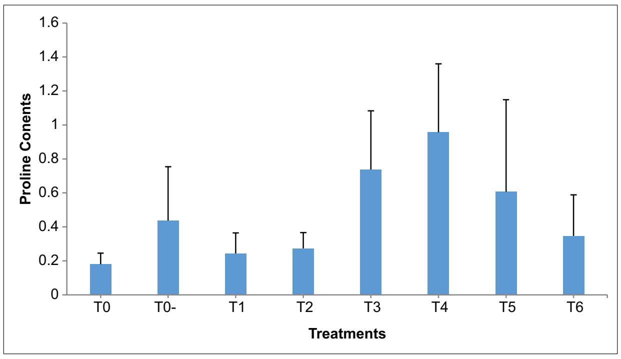

Figure 14: Effect of different growth parameters on proline contents of wheat under heavy metal stress

Effect on free amino acid

Analysis of variance (ANOVA) presented in Table 16 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on free amino acid. The heavy metal Pb treated plant showed decreased free amino acid indicating the toxic effects of lead as compared to control. Free amino acid under various treatments showed negative effect by lead.T0 represents the control group with an average of 0.4658, while T0- involves the addition of 100 ppm lead (Pb) with a reduced free amino acid of 0.3675, indicating lead toxicity effects. T1 (*Moringa* 100 ppm + Pb 100 ppm) shows partial mitigation with in concentration. T2 (ZnO 100 ppm + Pb 100 ppm) further positive effect on free amino acid the average to 0.4671, nearly matching the control. T3 (SCA 100 ppm + Pb 100 ppm) results in a moderate recovery. Higher concentrations of treatments show more pronounced effects: T4 (*Moringa* 200 ppm + Pb 100 ppm) achieves an average of 0.5704, T5 (ZnO 200 ppm + Pb 100 ppm) exhibits the highest concentration rate than others and followed by T4

Table 14: Analysis of variance table for proline content

Source	DF	SS	MS	F	P
Treatment	7	1.55264	0.22181	0.82	0.5846
Replicate	2	0.80125	0.40062		
Error	14	3.77424	0.26959		
Total	23	6.12813			

Table 15: Analysis of variance table for carbohydrates content

Source	DF	SS	MS	F	P
Treatment	7	2.23413	0.31916	1.05	0.4391
Replicate	2	0.70138	0.35069		
Error	14	4.2366	0.30261		
Total	23	7.17211			

Table 16: Analysis of variance table for free amino acid

Source	DF	SS	MS	F	P
Treatment	7	0.28833	0.04119	109.49	0
Replicate	2	0.0196	0.0098		
Error	14	0.00527	0.00038		
Total	23	0.3132			

and T6 These results suggest that increasing treatment concentrations of *Moringa*, ZnO, and SCA can effectively counteract lead-induced stress, with ZnO 200 ppm showing the most significant improvement (Figure 16).

Effect on anthocyanin

Analysis of variance (ANOVA) presented in Table 17 depicts the significant variation ($P \leq 0.05$) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on anthocyanin. The heavy metal Pb treated plant showed decreased anthocyanin indicating the toxic effects of lead as compared to control. The concentration of anthocyanin under different treatments involving lead (Pb) exposure and various additives. The T0 treatment (control) exhibits the highest anthocyanin concentration, indicating a baseline without Pb exposure. In contrast, T0- (Pb alone) leads to a significant reduction in anthocyanin concentration, highlighting the adverse effect of Pb. Treatments T1, T2, T3, T4, T5, and T6 combine Pb with different concentrations of *Moringa* extract, Zinc Oxide (ZnO), and Sodium Copper Alginate (SCA). Among these, T2 (ZnO + Pb) shows a relatively higher anthocyanin level compared to other combinations, while T5 (higher ZnO concentration + Pb) results in the lowest anthocyanin concentration. The error bars indicate variability in the data, with some treatments showing considerable variation. Overall, the graph suggests that the type and concentration of additives influence the mitigation of Pb's negative effects on anthocyanin levels (Figure 17).

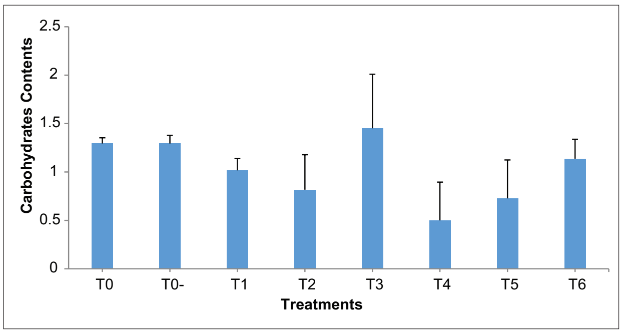

Figure 15: Effect of different growth parameters on carbohydrates of wheat under heavy metal stress

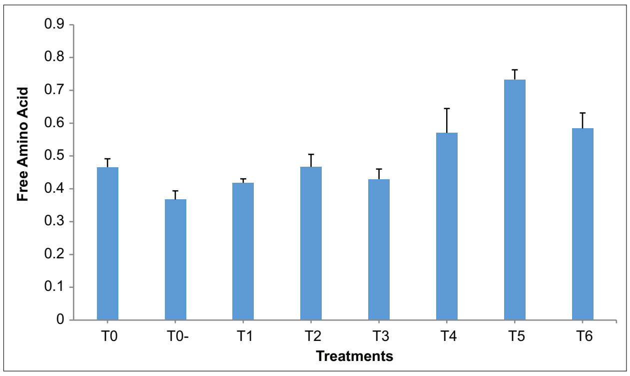

Figure 16: Effect of different growth parameters on free amino acid of wheat under heavy metal stress

Effect on total soluble sugar content

Analysis of variance (ANOVA) presented in Table 18 depicts the significant variation ($P \leq 0.05$) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on total soluble sugar content. The heavy metal Pb treated plant showed decreased total soluble content indicating the toxic effects of lead as compared to control. The total soluble sugar content under various treatments (T0 to T6). The control group (T0) shows the highest sugar content, while Pb stress alone (T0-) leads to a significant decline. Treatments involving 100 ppm *Moringa*, ZnO, and SCA (T1–T3) result in a moderate recovery of sugar content, but values remain lower compared to the control. Interestingly, 200 ppm treatments (T4–T6) show varied responses: T5 (ZnO at 200 ppm + Pb) records the highest sugar content among all Pb-stressed treatments, while T6 (SCA at 200 ppm) shows moderate recovery, and T4 (*Moringa* at 200 ppm) stabilizes at a similar level to T3. The graph highlights that higher concentrations of treatments, particularly ZnO, help improve sugar content under Pb-induced stress conditions (Figure 18).

Effect on hydrogen peroxide

Analysis of variance (ANOVA) presented in Table 19 depicts the significant variation ($P \leq 0.05$) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* Extract and their combined interaction on Hydrogen per oxide. The heavy metal Pb treated plant showed decreased hydrogen per oxide indicating the toxic effects of lead as compared to control. The effects of different treatments on hydrogen peroxide (H_2O_2) levels under various concentrations. The treatments include a control group (T0), lead exposure alone (T0-, 100 ppm Pb), and combinations of lead (100 ppm) with *Moringa oleifera* extract, zinc oxide nanoparticles (ZnO), or sulfocyclic acid (SCA) at 100 ppm (T1, T2, T3) and 200 ppm (T4, T5, T6).

Table 17: Analysis of variance table for anthocynin

Source	DF	SS	MS	F	P
Treatment	7	0.36118	0.0516	0.72	0.6541
Replicate	2	0.07761	0.0388		
Error	14	0.99639	0.07117		
Total	23	1.43518			

Table 18: Analysis of variance table for total soluble sugar content

Source	DF	SS	MS	F	P
Treatment	7	15959.3	2279.9	118.04	0
Replicate	2	36.4	18.21		
Error	14	270.4	19.31		
Total	23	16266.1			

Table 19: Analysis of variance table for hydrogen peroxide

Source	DF	SS	MS	F	P
Treatment	7	2.19743	0.31392	1.69	0.1907
Replicate	2	3.04406	1.52203		
Error	14	2.59901	0.18564		
Total	23	7.8405			

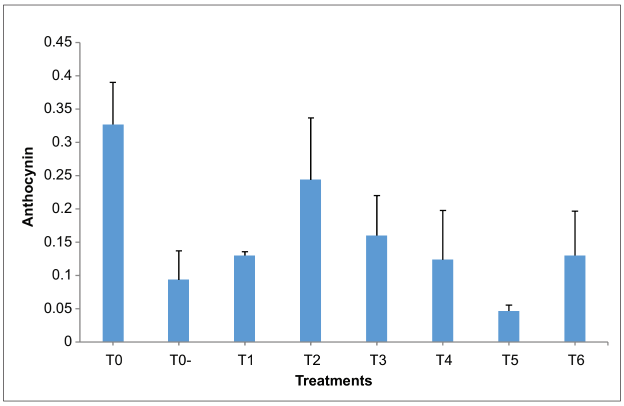

Figure 17: Effect of different growth parameters on anthocyanin of wheat under heavy metal stress

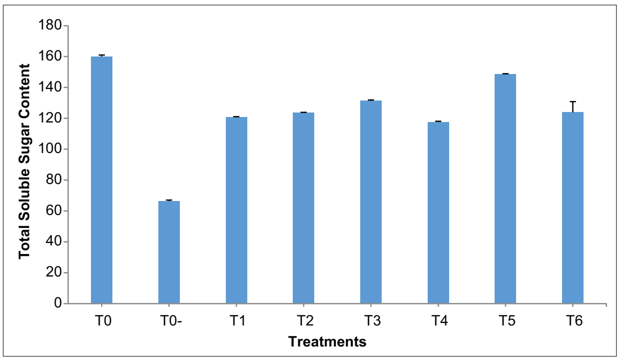

Figure 18: Effect of different growth parameters on total soluble sugar content of wheat under heavy metal stress

The results suggest that treatment with *Moringa*, ZnO, or SCA reduces H_2O_2 levels compared to lead alone, with varying efficacy based on concentration. Higher doses (200 ppm) tend to exhibit stronger effects in mitigating oxidative stress caused by lead exposure. Error bars indicate variability in the measurements (Figure 19).

Effect on superoxide dismutase activity

Analysis of variance (ANOVA) presented in Table 20 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on superoxide dismutase activity. The heavy metal Pb treated plant showed decreased super dismutase activity indicating the toxic effects of lead as compared to control. The impact of various treatments on superoxide dismutase (SOD) activity under lead (Pb) stress conditions. The control (T0) exhibits the highest SOD activity, while Pb stress alone (T0-) significantly reduces SOD levels, indicating oxidative damage. Treatments combining Pb with *Moringa* extract (T1, T4), zinc oxide nanoparticles (ZnO, T2, T5), or a chelating agent (SCA, T3, T6) improve SOD activity, with higher concentrations (T4, T5, T6) showing greater efficacy. This indicates that antioxidant agents like *Moringa* extract, ZnO, and SCA mitigate Pb-induced oxidative stress, with higher doses being more effective at restoring SOD activity and reducing oxidative damage (Figure 20).

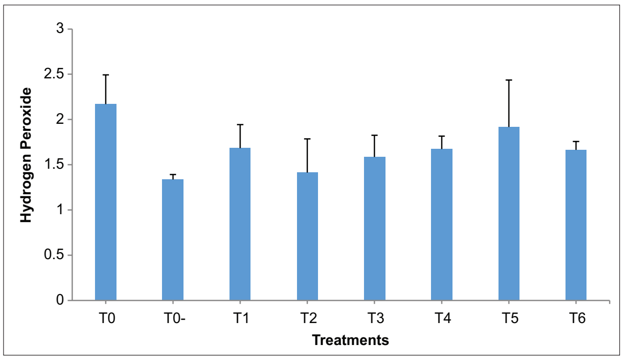

Figure 19: Effect of different growth parameters on hydrogen peroxide of wheat under heavy metal stress

Table 20: Analysis of variance table for superoxide dismutase activity

Source	DF	SS	MS	F	P
Treatment	7	11763.1	1680.44	2.9	0.0427
Replicate	2	3037	1518.51		
Error	14	8114.6	579.62		
Total	23	22914.7			

Effect on catalase activity

Analysis of variance (ANOVA) presented in Table 21 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on catalase activity. The heavy metal Pb treated plant showed decreased catalase activity indicating the toxic effects of lead as compared to control. The catalase activity across various treatments involving lead (Pb) exposure and combinations of different supplements at two concentrations (100 ppm and 200 ppm). T0 (control) exhibits significant catalase activity, while T0- (Pb alone) shows a marked reduction, indicating stress caused by Pb exposure. Treatments T1, T2, and T3, which combine *Moringa*, ZnO, and SCA (100 ppm) with Pb, show partial recovery of catalase activity, though the levels remain lower compared to the control. Notably, higher concentrations (200 ppm) of the same supplements (T4, T5, T6) result in a greater increase in catalase activity, with T4 (*Moringa* 200 ppm + Pb) displaying the highest level, followed by T6 (SCA 200 ppm + Pb). This suggests that the 200 ppm concentration of supplements, particularly *Moringa* and SCA, effectively mitigates Pb-induced oxidative stress by enhancing catalase activity. Error bars indicate variability, but the overall trend highlights the protective role of higher supplement concentrations (Figure 21).

Effect on phenolic content

Analysis of variance (ANOVA) presented in Table 22 depicts the significant variation (P≤0.05) between wheat due to impact of Sulfocyclic acid, ZnO nanoparticle and *Moringa* extract and their combined interaction on phenolic content. The heavy metal Pb treated plant showed decreased phenolic content indicating the toxic effects of lead as

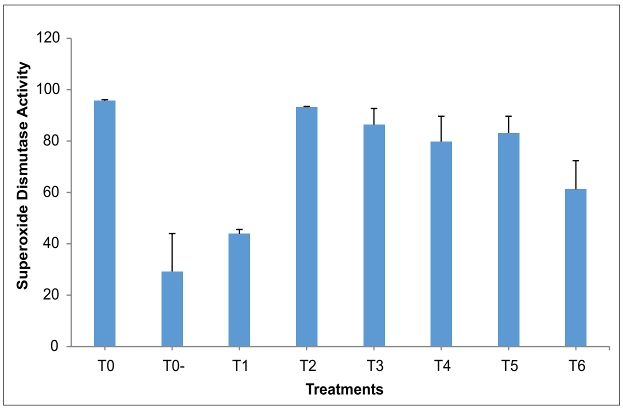

Figure 20: Effect of different growth parameters on superoxide dismutase activity of wheat under heavy metal stress

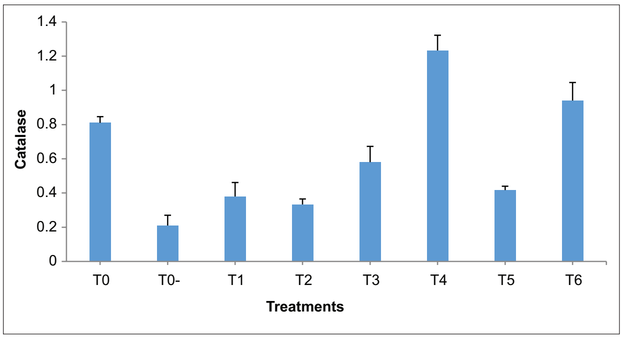

Figure 21: Effect of different growth parameters on catalase of wheat under heavy metal stress

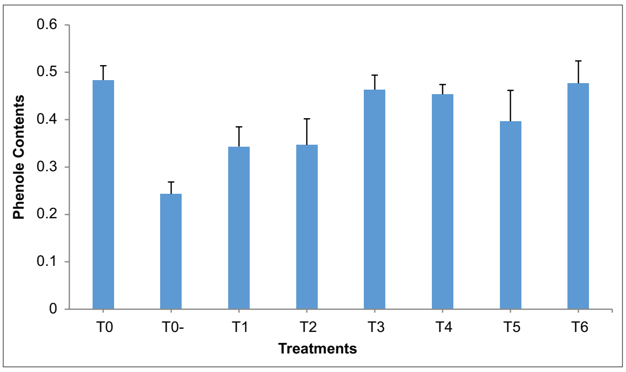

Figure 22: Effect of different growth parameters on phenolic content of wheat under heavy metal stress

Table 21: Analysis of variance table for catalase activity

Source	DF	SS	MS	F	P
Treatment	7	7.00506	1.00072	19.74	0
Replicate	2	0.05356	0.02678		
Error	14	0.70958	0.05068		
Total	23	7.7682			

Table 22: Analysis of variance table for phenolic content

Source	DF	SS	MS	F	P
Treatment	7	0.08867	0.01267	0.36	0.9126
Replicate	2	0.00756	0.00378		
Error	14	0.49711	0.03551		
Total	23	0.59333			

compared to control. The phenol content across various treatments involving lead (Pb) exposure and supplements at two concentrations (100 ppm and 200 ppm). T0 (control) shows the highest phenol content, while T0- (Pb alone) exhibits a significant reduction, indicating the negative impact of Pb stress. Treatments T1, T2, and T3 (100 ppm of *Moringa*, ZnO, and SCA combined with Pb) show a gradual increase in phenol content compared to T0-, but still lower than the control. Higher concentrations (T4, T5, T6) of the same supplements (200 ppm) result in a further increase in phenol levels, with T3 and T6 achieving levels close to the control. This suggests that supplement treatments, particularly at 200 ppm concentrations, effectively enhance phenol content, indicating a protective response against Pb-induced stress. Error bars show slight variability, but the overall trend highlights the beneficial impact of higher supplement concentrations in restoring phenol levels (Figure 22).

Discussion

Lead is a well-known toxic heavy metal that can interfere with various biological processes in plants. When plants absorb lead, it can disrupt essential metabolic activities, affecting growth and development. The analysis of variance (ANOVA) (4.1) suggest that there are significant differences in spike weight of wheat plants treated with different substances (Sulfocyclic acid, ZnO nanoparticles, *Moringa* extract, and their interactions), with the P-value being less than 0.05, indicating statistical significance. The observation that plants treated with the heavy metal Lead (Pb) showed a decreased spike weight, indicating toxic effects of lead. Lead stress alone (T0-) causes a significant reduction in root dry weight due to its toxic effects. Combination Treatments (T1, T2, T3) show slight improvements in root dry weight, suggesting that *Moringa*, ZnO, and SCA have some protective effects when used in combination with lead stress. Higher concentrations of *Moringa* and ZnO (T4 and T5) lead to notable improvements in root dry weight, likely due to more effective mitigation of oxidative stress and enhanced nutrient uptake and growth promotion. A study by Hussein *et al.* (2026) investigated the role of *M. oleifera* extract in reducing lead toxicity in *Vigna unguiculata* (cowpea) plants. *Moringa* extract significantly improved root and shoot growth under lead stress, likely due to its antioxidant properties, which helped mitigate oxidative damage. The treatment led to a reduction in reactive oxygen species (ROS) and improved photosynthetic efficiency, suggesting that *Moringa*'s high levels of polyphenols and flavonoids helped neutralize the oxidative stress induced by lead. The study found that *Moringa* treatments increased root biomass, which aligns with your findings of enhanced root dry weight under lead stress when *Moringa* was used. In terms of nutrient uptake, *Moringa* also improved macronutrient levels like nitrogen and potassium, potentially enhancing growth and mitigating the toxicity of lead. Results of our study perfectly align with the study of Hussein *et al.* (2026) found that *Moringa* extracts improved root biomass and protected against oxidative stress. However, the 200 ppm concentration in your study may be pushing the limits of *Moringa*'s effectiveness, as higher doses can sometimes lead to phytotoxicity if not properly managed, though your results suggest a positive response at higher concentrations.

A study by Mahmood *et al.* (2020) explored how *Moringa* extract affected wheat under various stress conditions, including heavy metals. The results indicated that *Moringa* significantly reduced malondialdehyde (MDA) levels (a marker of lipid peroxidation) in plants exposed to lead. Root dry weight and overall plant growth were significantly enhanced by *Moringa* treatment, corroborating the antioxidant and nutrient-enhancing roles of the extract. *Moringa*'s beneficial effects were linked to its role in activating defense enzymes like superoxide dismutase (SOD) and catalase (CAT), which help mitigate oxidative damage. Mahmood *et al.* (2020) findings support the idea that *Moringa* can mitigate oxidative stress and enhance root growth under lead stress. Their results seem consistent with your study, where higher concentrations of *Moringa* (T4) led to notable improvements in root biomass, likely due to its antioxidant properties.

The mechanism of action involved enhanced nutrient uptake (especially zinc, phosphorus, and potassium), which promoted better growth and reduced the toxic effects of lead. ZnO nanoparticles also helped in the reduction of oxidative stress, as indicated by decreased levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), while increasing antioxidant enzyme activity (such as SOD and CAT).This study confirmed that ZnO nanoparticles at higher concentrations (200 ppm) had a synergistic effect in improving plant tolerance to heavy metal stress by enhancing nutrient use efficiency and oxidative stress management.

Singh *et al.* (2024) investigated the impact of ZnO nanoparticles on wheat plants under lead exposure. ZnO nanoparticles improved root dry weight, shoot height, and chlorophyll content, even in the presence of lead stress. The study found that ZnO facilitated better zinc uptake, improving enzyme activity and photosynthesis, which in turn helped plants cope with lead-induced oxidative stress. ZnO nanoparticles were also linked to enhanced root development, possibly due to their role in phosphorus and nitrogen uptake, key nutrients that are often limited in plants stressed by heavy metals. Singh *et al.* (2024) findings corroborate your study's results on ZnO nanoparticles. In both studies, ZnO at higher concentrations improves root dry weight, likely through improved nutrient uptake and reduced oxidative damage, further validating the idea that ZnO nanoparticles are beneficial for mitigating lead toxicity in plants. In our study, higher concentrations of *Moringa* (T4) notably improve root dry weight, likely due to its antioxidant effects and promotion of nutrient uptake. This is consistent with other studies that show *Moringa*'s ability to reduce oxidative stress and enhance root growth under heavy metal stress, particularly in wheat and other plants.

Current study also highlights that ZnO nanoparticles at 200 ppm (T5) improve root dry weight, likely through enhanced nutrient uptake and oxidative stress reduction. This aligns with other studies, including those on wheat and lettuce, which show that ZnO at higher concentrations can mitigate the negative impacts of lead stress by improving nutrient efficiency and plant growth. Biochemical parameters such as proline content, carbohydrates content, free amino acid, anthocynin, membrane stability index, total soluble sugar sontent, hydrogen peroxide, superoxide dismutase activity, catalase, phenolic content, chlorophyll a, chlorophyll b, and total chlorophyll content showed improvement when plants are treated with ZnO nanoparticles. ZnO nanoparticles have been shown to activate key antioxidant enzymes in plants, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX). These enzymes play critical roles in scavenging reactive oxygen species (ROS), which are generated under stress conditions like heavy metal toxicity (e.g., Pb or Cd). Zn (zinc) is an essential micronutrient involved in the functioning of various enzymes, and ZnO nanoparticles may facilitate the availability of zinc at the cellular level, thus enhancing the plant's ability to neutralize oxidative stress. Studies have demonstrated that ZnO nanoparticles increase SOD, CAT, and glutathione reductase activities, thereby improving the plant's capacity to cope with oxidative damage caused by stress.

ZnO nanoparticles may improve photosynthetic pigment concentration (e.g., chlorophyll a, chlorophyll b, and carotenoids), leading to enhanced photosynthesis. This, in turn, boosts overall plant growth and productivity. Zn plays a key role in the structure and function of chloroplasts, including the regulation of enzymes involved in the photosynthetic process. Additionally, ZnO nanoparticles can help stabilize the photosynthetic machinery under stress conditions, such as heavy metal contamination, thereby reducing the degradation of chlorophyll and increasing photosynthetic efficiency. Several studies show that ZnO nanoparticles increase chlorophyll content and photosynthetic rate, resulting in improved energy production and plant growth.

ZnO nanoparticles have been shown to improve the uptake of essential nutrients, such as nitrogen (N), phosphorus (P), and potassium (K), especially under stress conditions. ZnO nanoparticles may facilitate the mobilization of nutrients in the soil, increase the surface area for nutrient absorption by plant roots, and enhance nutrient transport and assimilation within the plant. Increased availability of zinc itself, an essential micronutrient, may further promote the proper functioning of enzyme systems that are involved in metabolism, growth, and resistance to stress. Studies have shown that ZnO nanoparticles increase the uptake of nitrogen, phosphorus, and potassium, as well as zinc itself, contributing to improved plant health and biochemical parameters. Under environmental stress, particularly from heavy metals, plants accumulate reactive oxygen species (ROS) that can damage cellular components like proteins, lipids, and DNA. ZnO nanoparticles can help reduce ROS levels and mitigate oxidative stress in plants. ZnO nanoparticles can act as a reservoir of zinc, which is involved in stabilizing the cell membrane and regulating antioxidant systems. By decreasing ROS levels, ZnO nanoparticles protect plants from lipid peroxidation, protein degradation, and other oxidative damages. Studies show that ZnO nanoparticles reduce malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels, which are markers of oxidative damage, thereby improving the plant's biochemical balance and tolerance to environmental stresses.

ZnO nanoparticles have been shown to promote root elongation, root biomass, and overall shoot growth, which indirectly influence biochemical parameters such as total soluble sugars, proteins, and amino acids. ZnO nanoparticles may help stimulate root growth by enhancing the availability of nutrients and water uptake. This promotes better overall plant health, leading to increased biosynthesis of organic molecules involved in cellular structure and metabolism. Increased root and shoot growth due to ZnO treatment leads to a higher accumulation of organic compounds such as sugars and proteins, improving overall plant vitality. ZnO nanoparticles enhance protein synthesis and promote the metabolism of amino acids, which are fundamental building blocks for plant growth and stress tolerance. Zinc is involved in various biochemical processes such as nucleic acid synthesis, protein formation, and enzyme activation. ZnO nanoparticles help increase the bioavailability of zinc in plants, which can enhance the efficiency of these processes. Increased protein synthesis supports growth and the production of structural proteins, enzymes, and stress-related proteins, all of which contribute to the improved biochemical parameters. Research has shown that ZnO nanoparticles improve protein content in various plants under stress, supporting both growth and resilience. ZnO nanoparticles may also influence the production of plant hormones like indole acetic acid (IAA), cytokinins, and gibberellins, which regulate cell division, elongation, and overall growth. Zinc is a cofactor for many enzymes involved in the biosynthesis of plant hormones, and ZnO nanoparticles could boost these hormone levels, improving cell growth and stress tolerance. Studies indicate that ZnO nanoparticles can influence auxin and cytokinin levels, which promotes root and shoot growth and can improve the biochemical profile of plants exposed to stress. ZnO nanoparticles not only directly improve biochemical parameters but can also interact with other environmental factors like soil pH, nutrient availability, and water retention.

ZnO nanoparticles may improve soil conditions, leading to better root growth and nutrient uptake, which indirectly boosts biochemical markers such as protein content, sugars, and enzymatic activity. Studies suggest that ZnO nanoparticles have a synergistic effect when combined with other treatments like humic acids or plant growth-promoting bacteria (PGPB), further enhancing the plant's biochemical parameters. The significant increase in biochemical parameters following the application of ZnO nanoparticles can be attributed to several interrelated mechanisms, Activation of antioxidant defense systems (e.g., SOD, CAT), Improved photosynthetic efficiency and pigment content, Enhanced nutrient uptake, especially zinc and other essential nutrients, Reduction in oxidative stress and ROS levels, Promotion of root and shoot growth due to better nutrient and water uptake, Improvement in protein synthesis and plant metabolism, Regulation of growth hormones, leading to better overall growth and biochemical composition.

These combined effects of ZnO nanoparticles contribute to enhanced biochemical parameters in plants, improving their growth, stress tolerance, and overall health under challenging environmental conditions, such as heavy metal exposure.

Recommendation and conclusion

The study investigated the impact of different growth stimulants on the growth and biochemical parameters of wheat cultivated in a lead (Pb)-contaminated environment. The findings indicate that Pb toxicity significantly impairs wheat growth, reducing biomass, chlorophyll content, and essential biochemical markers. However, the application of various growth stimulants, such as plant growth regulators, *Moringa* leaf extract, and sulfosalic acid, effectively mitigated the toxic effects of Pb. Among the tested stimulants, ZnO nanoparticles exhibited the most significant improvement in plant growth, photosynthetic efficiency, and stress tolerance mechanisms. These results highlight the potential of growth stimulants in enhancing wheat resilience under heavy metal stress conditions. Future studies should explore the molecular mechanisms behind growth stimulant-induced stress tolerance to develop targeted strategies for improving crop resilience.

Author contributions

Syda Zahra Haider: Conceptualization, Data curation, Investigation, Formal analysis, Writing – original draft. Ayesha Batool: Investigation, Methodology Formal analysis, Validation, Visualization. Rehan Jameel: Writing – review & editing. All authors approved the final manuscript.

References

Ahmad, M. S. A., Ashraf, M., Tabassam, Q., Hussain, M., & Firdous, H. (2011). Lead (Pb)-induced regulation of growth, photosynthesis, and mineral nutrition in maize (*Zea mays* L.) plants at early growth stages. *Biological Trace Element Research, 144*, 1229-1239. https://doi.org/10.1007/s12011-011-9099-5

Ali, M., Bari, A., Ibrahim, M., Ahmed, I., Ahmed, U., Zafar, R., Ahmad, W., Saeed, M., & Khalid, M. (2025). Impact of irrigation schedule and integrated nutrient management on wheat yield and nutrients uptake under semi-arid regions. *Irrigation Science, 43*, 519-532. https://doi.org/10.1007/s00271-024-00986-8

Alloway, B. J. (2012). Sources of heavy metals and metalloids in soils. In B. J. Alloway (Eds.), *Heavy metals in soils: trace metals and metalloids in soils and their bioavailability* (Vol. 22, pp. 11-50). Springer. https://doi.org/10.1007/978-94-007-4470-7_2

Asad, N. I., Wang, X.-B., Dozois, J., Azarbad, H., Constant, P., & Yergeau, E. (2023). Early season soil microbiome best predicts wheat grain quality. *FEMS Microbiology Ecology, 99*(1), fiac144. https://doi.org/10.1093/femsec/

fiac144

Babula, P., Adam V., Opatrilova R., Zehnalek J., Have, L., & Kizek R. (2008). Uncommon heavy metals, metalloids and their plant toxicity: a review. *Environmental Chemistry Letters,* 6, 189-213. https://doi.org/10.1007/s10311-008-0159-9

Cakmak, I., & Marschner, H. (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascrobate peroxidase, and glutathione reductase in bean leaves. *Plant Physiology, 98*, 1222-1227

Chapman, A. R. O., & Craigie, J. S. (1977). Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Marine Biology, 40*(3), 197-205. https://doi.org/10.1007/BF00390875

Chopra, A. K., Pathak, C., & Prasad, G. (2009). Scenario of heavy metal contamination in agricultural soil and its management. *Journal of Applied and Natural Science, 1*(1), 99-108.

Elbudony, K. A. M. (2017). *Genes controlling coleoptile length and seedling*. Doctoral dissertation, Washington State University.

Godbold, D. L., & Kettner, C. (1991). Lead influences root growth and mineral nutrition of *Picea abies* seedlings. *Journal of Plant Physiology, 139*(1), 95-99. https://doi.org/10.1016/S0176-1617(11)80172-0

Hamilton, P. B., & Van Slyke, D. D. (1943). The gasometric determination of free amino acids in blood filtrates by the ninhydrin-carbon dioxide method. *Journal of Biological Chemistry, 150*(1), 231-250. https://doi.org/10.1016/S0021-9258(18)51268-0

Hossain, M. Z., Teixeira da Silva J. A., & Fujita M. (2006). Differential roles of glutathione S-transferase in oxidative stress modulation. In J. A. Teixeira da Silva (Eds.), *Floriculture, ornamental and plant biotechnology: advances and topical issues* (Vol. 3, pp. 108-116). Global Science Books.

Hussain, D., Asrar, M., Khalid, B., Hafeez, F., Saleem, M., Akhter, M., Ahmed, M., Ali, I., & Hanif, K. (2022). Insect pests of economic importance attacking wheat crop (*Triticum aestivum* L.) in Punjab, Pakistan*. International Journal of Tropical Insect Science, 42*, 9-20. https://doi.org/10.1007/s42690-021-00574-9

Hussain, J., Khaliq, T., ur Rahman, M. H., Ullah, A., Ahmed, I., Srivastava, A. K., Gaiser, T., & Ahmad, A. (2021). Effect of temperature on sowing dates of wheat under arid and semi-arid climatic regions and impact quantification of climate change through mechanistic modeling with evidence from field. *Atmosphere, 12*(7), 927. https://doi.org/10.3390/atmos12070927

Hussein, J. B., Workneh, T. S., Ilesanmi, J. O. Y., Allison, S. D., Sanusi, M. S., & Oke, M. O. (2026). Exploring the commercialization of nutrient-dense Moimoi and Akara made from cowpea (*Vigna unguiculata*) and *Moringa oleifera* seed flour blends. In T. S. Workneh, D. O. Mbuge & G. Bultosa (Eds.), *Processing, nutrition and value addition principles of neglected and underutilized African foods* (pp. 111-126). Academic Press. https://doi.org/10.1016/B978-0-443-33433-7.00008-3

Islam, M. R. (2017). *Effect of sowing dates on phenology, growth and yield of three wheat (Triticum aestivum L.) varieties*. Doctoral Dissertation, Hajee Mohammad Danesh Science and Technology University.

Jagaba, A. H., Lawal, I. M., Birniwa, A. H., Affam, A. C., Usman, A. K., Soja, U. B., Saleh, D., Hussaini, A., Noor, A., & Yaro, N. S. A. (2024). Sources of water contamination by heavy metals. In J. Jaafar, A. A. Zaidi & M. N. Naseer (Eds.), *Membrane technologies for heavy metal removal from water* (pp. 3-27). CRC Press.

Jones, C. G., Hare, J. D., & Compton, S. J. (1989). Measuring plant protein with the Bradford assay: 1. Evaluation and standard method. *Journal of Chemical Ecology, 15*(3), 979-992. https://doi.org/10.1007/BF01015193

Kiran, Bharti, R., & Sharma, R. (2022). Effect of heavy metals: An overview. *Materials Today: Proceedings*, *51* (Part 1), 880-885. https://doi.org/10.1016/j.matpr.2021.06.278

Kumar, M., Gogoi, A., Kumari, D., Borah, R., Das, P., Mazumder, P., & Tyagi, V. K. (2017). Review of perspective, problems, challenges, and future scenario of metal contamination in the urban environment. *Journal of Hazardous, Toxic, and Radioactive Waste, 21*(4), 04017007. https://doi.org/10.1061/(ASCE)HZ.2153-5515.0000351

Lamhamdi, M., El Galiou, O., Bakrim, A., Novoa-Munoz, J. C., Arias-Estevez, M., Aarab, A., & Lafont, R. (2013). Effect of lead stress on mineral content and growth of wheat (*Triticum aestivum*) and spinach (*Spinacia oleracea*) seedlings. *Saudi Journal of Biological Sciences, 20*(1), 29-36. https://doi.org/10.1016/j.sjbs.2012.09.001

Li, J. X., Yang, X. E., He, Z. L., Jilani, G., Sun, C. Y., & Chen, S. M. (2007). Fractionation of lead in paddy soils and its bioavailability to rice plants. *Geoderma, 141*(3-4), 174-180. https://doi.org/10.1016/j.geoderma.2007.05.006

Liu, H. S., & Li, F. M. (2005). Root respiration, photosynthesis and grain yield of two spring wheat in response to soil drying. *Plant Growth Regulation, 46*, 233-240. https://doi.org/10.1007/s10725-005-8806-7

Lopushinsky, W., & Max, T. A. (1990). Effect of soil temperature on root and shoot growth and on budburst timing in conifer seedling transplants. *New Forests, 4*, 107-124. https://doi.org/10.1007/BF00119004

Maccaferri, M., Sanguineti, M. C., Giuliani, S., & Tuberosa, R. (2009). Genomics of tolerance to abiotic stress in the Triticeae. In G. J. Muehlbauer & C. Feuillet (Eds.), *Genetics and Genomics of the Triticeae* (Vol. 7, pp. 481-558). Springer. https://doi.org/10.1007/978-0-387-77489-3_18

Mahmood, S., Ahmad, W., Ali, Z., Eed, E. M., Khalifa, A. S., Naeem, M., Bibi, A., Tahir, A., Waqas, K., & Wahid, A. (2022). Exploring the potential of *Moringa* leaf extract for mitigation of cadmium stress in *Triticum aestivum* L. *Applied Sciences, 12*(16), 8199. https://doi.org/10.3390/app12168199

Naveed, M., Hussain, M. B., Zahir, Z. A., Mitter, B., & Sessitsch, A. (2014). Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. *Plant Growth Regulation, 73*, 121-131. https://doi.org/10.1007/s10725-013-9874-8

Nazir, M. A., Hasan, M., Mustafa, G., Tariq, T., Ahmed, M. M., Golzari Dehno, R., & Ghorbanpour, M. (2024). Zinc oxide nano-fertilizer differentially effect on morphological and physiological identity of redox-enzymes and biochemical attributes in wheat (*Triticum aestivum* L.). *Scientific Reports, 14*, 13091. https://doi.org/10.1038/s41598-024-63987-9

Premachandra, G. S., Saneoka, H., & Ogata, S. (1990). Cell membrane stability, an indicator of drought tolerance, as affected by applied nitrogen in soybean. *The Journal of Agricultural Science, 115*(1), 63-66. https://doi.org/10.1017/S0021859600073925

Qin, J., Jiang, X., Qin, J., Zhao, H., Dai, M., Liu, H., & Chen, X. (2023). Effects of lead pollution on photosynthetic characteristics and chlorophyll fluorescence parameters of different populations of *Miscanthus floridulus*. *Processes, 11*(5), 1562. https://doi.org/10.3390/pr11051562

Rahman, S. U., Qin, A., Zain, M., Mushtaq, Z., Mehmood, F., Riaz, L., Naveed, S., Ansari, M. J., Saeed, M., Ahmad, I.,

& Shehzad, M. (2024). Pb uptake, accumulation, and translocation in plants: Plant physiological, biochemical, and molecular response: A review. *Heliyon, 10*(6), e27724. https://doi.org/10.1016/j.heliyon.2024.e27724

Ramzan, M., Ayub, F., Shah, A. A., Naz, G., Shah, A. N., Malik, A., Sardar, R., Telesiński, A., Kalaji, H. M., Dessoky, E. S., & Elgawad, H. A. (2022). Synergistic effect of zinc oxide nanoparticles and *Moringa oleifera* Leaf extract alleviates cadmium toxicity in *Linum usitatissimum*: Antioxidants and physiochemical studies. *Frontiers in Plant Science, 13*, 900347. https://doi.org/10.3389/fpls.2022.900347

Riaz, M. W., Yousaf, M. I., Hussain, Q., Yasir, M., Sajjad, M., & Shah, L. (2023). Role of lignin in wheat plant for the enhancement of resistance against lodging and biotic and abiotic stresses. *Stresses*, *3*(2), 434-453. https://doi.org/10.3390/stresses3020032

Rout, G. R., & Das, P. (2003). Protected article Effect of Metal Toxicity on Plant Growth and Metabolism: I. Zinc. *Agronomie, 23*, 3-11.

Sairam, R. K. (1994). Effect of moisture stress on physiological activities of two contrasting wheat genotypes. *Indian Journal of Experimental Biology, 32*, 594-597.

Sarker, B., Keya, K. N., Mahir, F. I., Nahiun, K. M., Shahida, S., & Khan, R. A. (2021). Surface and ground water pollution: causes and effects of urbanization and industrialization in South Asia. *Scientific Review, 7*(3), 32-41. https://doi.org/10.32861/sr.73.32.41

Shah, S. A. A., Wu, H., Farid, M. F., Tareen, W.-U.-H., & Badar, I. H. (2024). Climate trends and wheat yield in Punjab, Pakistan: Assessing the change and impact. *Sustainability, 16*(11), 4443. https://doi.org/10.3390/su16114443

Sharma, P., & Dubey, R. S. (2005). Lead toxicity in plants. *Brazilian Journal of Plant Physiology, 17*(1), 35-52.

Sidhu, G. P. S., Singh, H. P., Batish, D. R., & Kohli, R. K. (2017). Alterations in photosynthetic pigments, protein, and carbohydrate metabolism in a wild plant *Coronopus didymus* L. (Brassicaceae) under lead stress. *Acta Physiologiae Plantarum, 39*, 176. https://doi.org/10.1007/s11738-017-2476-8

Singh, D., Sharma, N. L., Singh, D., Siddiqui, M. H., Sarkar, S. K., Rathore, A., Prasad, S. K., Gaafar, A.-R. Z., & Hussain, S. (2024). Zinc oxide nanoparticles alleviate chromium-induced oxidative stress by modulating physio-biochemical aspects and organic acids in chickpea (*Cicer arietinum* L.). *Plant Physiology and Biochemistry, 206*, 108166. https://doi.org/10.1016/j.plaphy.2023.108166

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Science, 151*(1), 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1

Wardlaw, I. F., & Willenbrink, J. (1994). Carbohydrate storage and mobilisation by the culm of wheat between heading and grain maturity: the relation to sucrose synthase and sucrose-phosphate synthase. *Australian Journal of Plant Physiology, 21*(3), 255-271.

Yang, D.-H., Webster, J., Adam, Z., Lindahl, M., & Andersson, B. (1998). Induction of acclimative proteolysis of the light-harvesting chlorophyll a/b protein of photosystem II in response to elevated light intensities. *Plant Physiology, 118*(3), 827-834. https://doi.org/10.1104/pp.118.3.827

Yemm, E. W., & Willis, A. J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal, 57*(3), 508.

Zahra, N., Wahid, A., Hafeez, M. B., Ullah, A., Siddique, K. H. M., & Farooq, M. (2021). Grain development in wheat under combined heat and drought stress: Plant responses and management. *Environmental and Experimental Botany, 188*, 104517. https://doi.org/10.1016/j.envexpbot.2021.104517

Zulfiqar, U., Farooq, M., Hussain, S., Maqsood, M., Hussain, M., Ishfaq, M., Ahmad, M., & Anjum, M. Z. (2019). Lead toxicity in plants: Impacts and remediation. *Journal of Environmental Management, 250*, 109557. https://doi.org/10.1016/j.jenvman.2019.109557