Upregulate Soil Health and Wheat Yield: Conversion of Organic Waste into Bio-activated Zn-Enriched Compost for Deficient Soils

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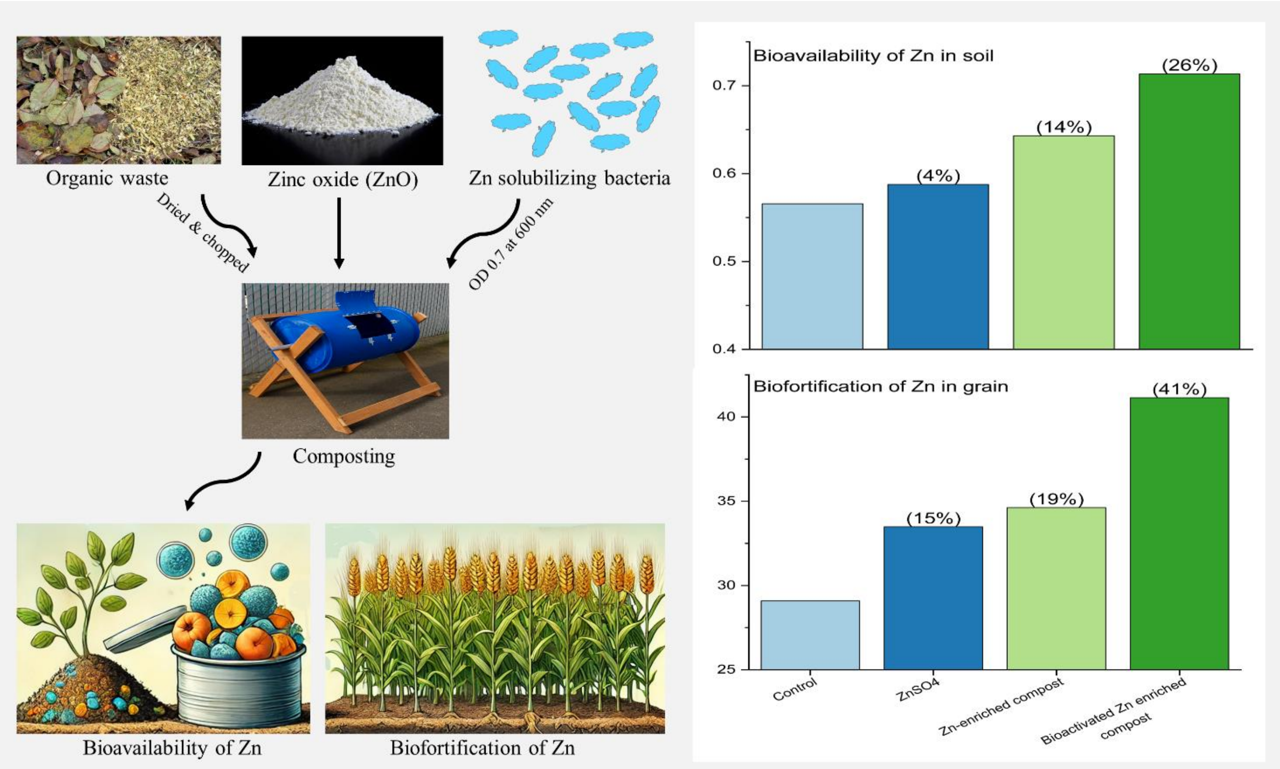
Graphical abstract



Abstract

Soil zinc (Zn) deficiency is a major constraint limiting wheat productivity and nutritional quality, especially in degraded agricultural lands. Sustainable organic waste management through composting is an environmentally friendly approach to improve soil health while reducing environmental pollution. This study investigates the development of zinc-enriched microbial compost (bioactivated Zn-enriched compost) from organic waste and its effects on soil fertility, microbial activity, and wheat yield in Zn-deficient soils. Domestic organic waste, including fruit and vegetable residues, was composted with Zn-enriched bio-inoculants to enhance nutrient bioavailability. A field experiment evaluated the effects of bioactivated Zn-enriched compost on soil biochemical properties, wheat growth, yield and antioxidant enzyme activity. Results showed significant improvements in soil organic matter, nutrient availability, and microbial biomass compared to conventional compost and control treatments. Application of bioactivated Zn-enriched compost increased soil microbial population (45%), microbial biomass carbon (48%), and nitrogen

(56%). Soil Zn and Fe availability improved by 26% and 12%, respectively. Wheat grain yield increased by 9%, with notable improvements in Zn content and antioxidant enzyme activity (CAT, 41% and POX, 32%). These results highlight the potential of bioactivated Zn-enriched compost to address soil nutrient deficiencies and promote sustainable agriculture through improved soil fertility and crop productivity.

Keywords: Compost, Organic matter, Organic waste management, Soil health, Zinc bioavailability

Introduction

Declining soil fertility is a major challenge in modern agriculture, particularly in nutrient-deficient soils where essential elements such as zinc (Zn) are often limited. Globally, nearly half of the agricultural soils are deficient in Zn, primarily due to high soil pH and calcareous parent material (Jian et al., 2020). This widespread deficiency adversely affects plant growth, metabolism, and yield, posing a significant threat to food security and human nutrition (Saleem et al., 2022; Akther et al., 2020). Zinc is a biocatalyst in critical processes such as carbohydrate production and plant defense mechanisms (Hacisalihoglu, 2020). Its deficiency impacts an estimated 2 billion people worldwide, highlighting the urgent need for sustainable agronomic interventions (Sindhu and Sharma, 2020).

Sustainable soil management practices, including organic amendments, have gained increasing attention as eco-friendly solutions to enhance soil fertility and crop performance (Rastogi et al., 2023). Compost, particularly when enriched with micronutrients like Zn, is a vital source of nutrients and promotes microbial activity, improving soil structure and fertility (Mustafa et al., 2019; Moharana et al., 2020).

In Pakistan, the issue of Zn deficiency is especially critical. Wheat (*Triticum aestivum* L.), the country's primary staple crop with a per capita consumption of 124 kg (Imran and Noureen, 2021), is sensitive to Zn deficiency. This sensitivity leads to stunted growth, lower grain yields, and diminished nutritional quality (Singh et al., 2023). The soil conditions in Pakistan, characterized by high pH and calcareous textures, further exacerbate Zn unavailability to crops, thus affecting food security and human health (Jian et al., 2020).

Given this pressing national context, the development of bioactivated zinc-enriched compost offers a promising solution. Enriching compost with Zn and beneficial microbes, such as zinc-solubilizing bacteria (ZnSB), can enhance Zn bioavailability, stimulate soil microbial activities, and promote better root-soil interactions (Joshi et al., 2020; Bhatt and Maheshwari, 2020). Such bio-inoculants also aid in the decomposition of organic matter and further improve soil biological properties (Kanwal et al., 2020; Wang et al., 2024).

The bioactivating compost with microbial inoculants, especially zinc-solubilizing bacteria (ZSB), is based on a synergistic interaction between organic matter decomposition and microbial-mediated nutrient transformation. Compost provides a nutrient-rich substrate and a favorable microenvironment that supports the growth and activity of beneficial microbes (Ahmed et al., 2023). When inoculated with ZSB, these microbes enhance the solubilization of otherwise unavailable forms of zinc in the soil, primarily through the secretion of organic acids and chelating agents (Sethi et al., 2025). This process increases the bioavailability of zinc, an essential micronutrient for plant growth, and contributes to overall soil fertility. Moreover, these microbes can stimulate microbial diversity and enzymatic activity in the rhizosphere, promoting improved nutrient cycling and plant nutrient uptake, thereby reinforcing the effectiveness of compost as a biofertilizer. The present study aims to evaluate the effects of bioactivated zinc-enriched compost on soil health and wheat productivity in nutrient-deficient soils under field conditions. By assessing key indicators of soil health, nutrient uptake efficiency, and crop yield, this research will provide insights into the potential of bioactivated Zn-enriched compost as a sustainable soil amendment to improve agricultural productivity.

Materials and Methods

Collection of Bacterial Strains

Five zinc solubilizing bacterial (ZnSB) strains IUB2 (*Bacillus subtilis*), IUB3 (*Bacillus velezensis*), IUB6 (*Bacillus subtilis*), IUB10 (*Bacillus vallismortis*) and IUB11 (*Bacillus megaterium*) with accession numbers MN696212, MN696213, MN696214, MN696215 and MN696216, respectively, were collected from the Department of Soil Science, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur (IUB), Pakistan. These strains previously demonstrated zinc solubilizing potential and plant growth promoting traits in maize under controlled conditions (Naseem et al., 2022).

Preparation of bioactivated Zn-enriched compost

Organic waste, including leaves from various trees and peels of fruits and vegetables, was collected from the State Care Department of the Islamia University of Bahawalpur. The collected material was air-dried in the shade for seven days, followed by oven drying at 65 ± 5 °C until a constant weight was achieved. The dried material was then ground using a grinding mill and placed into a composting drum with a capacity of 1000 kg. Moisture content was maintained at 40%. To accelerate the composting process, urea was added at 1% (w/w) and a consortium of zinc-solubilizing bacteria (ZSB) was added at 0.1% (v/w). The composting material was thoroughly mixed every fourth day by rotating the drum using an electric motor. After 24 days, the compost was enriched with Zn by adding ZnO @ 2% w/w and analyzed for chemical properties. Furthermore, the compost was bioactivated by adding a consortium of Zn-solubilizing bacteria @ 0.1 v/w. The chemical analysis of the bioactivated Zn-enriched compost compared the content of total nitrogen (N), potassium (K), phosphorus (P) and organic matter with that of simple compost (Naseem et al., 2022).

Field Trial

Soil samples were taken from the trial site at a 0-15 cm depth before sowing. The samples were then air dried, crushed and passed through a 2000 µm sieve to remove gravel and coarse particles. The particle size distribution, including sand, silt and clay fractions, was determined using the sieve method described by Moodie et al. (1959). The soil texture analysis classified the soil as sandy loam, with a composition of 55% sand, 30% silt, and 15% clay. A chemical analysis of the soil was carried out to assess its nutrient status. The results showed that the soil was deficient in essential nutrients, with the following characteristics: organic matter (0.28%), nitrogen (0.021%), Olsen P (4.8 mg kg^{-1}), extractable K (76 mg kg^{-1}), DTPA extractable Zn (0.56 mg kg^{-1}), and DTPA extractable Fe (0.50 mg kg^{-1}). The experiment was designed as a randomized complete block design (RCBD) with 10 treatments and three replications (Figure 1). The experimental site was laser leveled to a slope accuracy of 0.0625 inches per 100 feet to ensure uniform distribution of irrigation water across the field.

The treatments used were Control, $ZnSO_4$, ZnO, Compost, ZnSB, Zn-enriched compost, Bioactivated-$ZnSO_4$ (BZS), Bioactivated-ZnO (BZO), bioactivated compost (BC) and Bioactivated Zn-enriched compost (BZC). Seeds of the wheat variety Johar 2016 were purchased from the local market. The overnight grown bacterial broth culture was used to inoculate the seed. The consortium of the same bacterial strains was used to bioactivate the Zn-enriched compost. The seed was inoculated by coating with slurry, which is prepared by mixing peat, clay and consortium broth culture in a ratio of 5:4:1. Twenty percent (20%) sugar solution was used to provide initial carbon for bacterial growth. The sterilized broth was used to ensure the homogeneity of treatments in the uninoculated control. Full doses of recommended phosphorus (90 kg ha^{-1}) and potassium (60 kg ha^{-1}) were applied before sowing. Nitrogen (@ 120 kg ha^{-1}) was applied in three splits at 25-days intervals after germination. Other agronomic practices, including fertilization and irrigation, were applied to the plants accordingly.

At the maturity stage of wheat, plant height was measured by placing a 1-meter-long scale close to the plant. Plants were uprooted using a spatula; their roots were carefully removed from the soil and their length was measured. The number of productive tillers per square meter was counted and the crop was harvested to measure the number of grains per spike, 1000 grain weight and grain yield. Antioxidant enzyme activities and chlorophyll content were measured at the beginning of flowering, approximately 70-80 days after sowing.

Figure 1: Experimental layout consisting of ten treatments and three replications. T1: Control; T2: $ZnSO_4$; T3: ZnO; T4: Compost; T5: ZnSB; T6: Zn-enriched compost; T7: Bioactivated-$ZnSO_4$ (BZS); T8: Bioactivated-ZnO (BZO); T9: bioactivated compost (BC); T10: Bioactivated Zn-enriched compost (BZC)

Determination of antioxidant enzyme activities

Half a gram (0.5 g) of plant samples was placed in a pre-cooled mortar surrounded by ice and homogenized in 2-3 ml of phosphate buffer solution (pH 7.8). A further 5 ml of buffer solution was added and the supernatant was transferred to 15 ml centrifuge tubes and centrifuged at 10000 rpm for 20 minutes at 4ºC. The supernatant was used immediately for the determination of antioxidant activity. If storage was required, Eppendorf tubes (2 mL) were used to preserve the extract at -20 °C. Antioxidant enzyme activities were determined by measuring their absorbance on a spectrophotometer set at a different wavelength for each enzyme. Catalase activity (CAT) was measured at a wavelength of 240 nm (Chance and Machly, 1955). Ascorbate peroxidase (APX), peroxidase dismutase (POD), and peroxidase (POX) were determined at wavelengths of 290 nm (Schöner and Krause, 1990), 470 nm and 420 nm (Rao et al., 1996), respectively.

Determination of Chlorophyll "a" and "b", and Carotenoids

Fresh leaf samples were collected and analyzed using a standard spectrophotometric method to determine the concentrations of chlorophyll a, chlorophyll b, and total carotenoids. The samples were homogenized in 80% acetone using a mortar and pestle and then centrifuged at 4,000 rpm for 10 minutes to separate the supernatant. The absorbance of the extract was measured at wavelengths of 663 nm, 645 nm, and 470 nm using a UV-Vis spectrophotometer. The concentrations of chlorophyll **a**, **b**, and carotenoids were calculated using Arnon's equations (Arnon, 1949). All extractions and measurements were performed under dim light to avoid pigment degradation. The experiment was performed in triplicate to ensure accuracy and reproducibility.

Determination of Nutrient Concentrations in Grain

A representative sample of grain was collected, cleaned, and dried to remove impurities. The dried samples were ground to ensure uniformity before undergoing acid digestion, typically overnight digestion of a 0.5 g sample in 6 mL concentrated H_2SO_4. The next day, the samples were heated on a hot plate at 250 °C for 1 hour after adding 1 mL of H_2O_2. If the sample was digested, it will appear transparent or milky white; otherwise add 1 mL of H_2O_2 again and heat. Repeat this step until the sample is digested. The sample was then diluted with distilled water in a 50 ml volumetric flask and analyzed for N, P, K, Fe and Zn (Ryan et al., 2001). Nitrogen was determined by the Kjeldahl method, while phosphorus was quantified by spectrophotometry using the molybdenum blue method. Potassium concentration is measured by flame photometry. Atomic absorption spectrometry (AAS) is employed to accurately quantify micronutrients such as iron and zinc. Instrument calibration is performed using standard solutions, and quality control measures, including reagent blanks and sample replicates, are maintained to ensure the accuracy and reliability of results. The data obtained are statistically analyzed to assess variations in nutrient composition between treatments.

Rhizospheric soil analysis

Soil samples were collected from the study area, air dried, and sieved through a 2 mm mesh before analysis. Organic matter (OM) content was determined using the Walkley-Black method, which involves the oxidation of organic carbon using potassium dichromate and sulfuric acid, followed by titration with ferrous sulfate (Walkley and Black, 1934). Total nitrogen (N) was measured using the Kjeldahl digestion method, which involves acid digestion, distillation and titration to quantify nitrogen content (Bremner, 1965). Available phosphorus (P) was determined by the Olsen method, using sodium bicarbonate (0.5 M $NaHCO_3$) at pH 8.5 as the extractant and measuring phosphorus concentration with a spectrophotometer (Olsen et al., 1954). Extractable potassium (K) was analyzed by ammonium acetate extraction (1 M NH_4OAc, pH 7) followed by determination with a flame photometer (Hanway and Heidel, 1952). Micronutrients, including DTPA-extractable iron (Fe) and zinc (Zn), were assessed using diethylenetriaminepentaacetic acid (DTPA) as an extractant, and their concentrations were measured using atomic absorption spectrophotometry (Lindsay and Norvell, 1978).

Soil health indicators and rationale for selection

To evaluate the biological responses of soil to compost and microbial treatments, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and bacterial population were selected as key soil health indicators. These indicators are well-established metrics for assessing soil biological activity, nutrient cycling potential, and soil fertility. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using the chloroform fumigation-extraction method. Soil samples were collected from the study site, sieved to remove debris, and divided into fumigated and non-fumigated portions (Brookes et al., 1985). The fumigated samples (30 g) were exposed to ethanol-free chloroform vapor in a desiccator for 24 hours to lyse microbial cells, while the non-fumigated samples (30 g) were left untreated. Both sets of samples were extracted with 0.5 M K_2SO_4 solution. The resulting extracts were then analyzed for carbon content with TOC analyzer and nitrogen content using the Kjeldahl digestion method. The carbon and nitrogen of the microbial biomass were calculated as the difference between fumigated and non-fumigated extracts using appropriate conversion factors.

The bacterial population was assessed using the colony forming unit (CFU) method by serial dilution and spread plating (Alexander, 1982). Soil suspensions were prepared by diluting 1 g of soil up to 10^7 dilutions in sterile distilled water and vortexing to homogenize. Serial dilutions were plated onto nutrient agar and incubated for 48 hours at 30 ± 2 °C for bacterial growth. After incubation, bacterial colonies were counted and expressed as CFU per gram of soil. All analyses were performed in triplicate to ensure accuracy, and standard laboratory procedures were followed to maintain aseptic conditions throughout the experiment.

Statistical Analysis

The study used statistical analysis techniques, including analysis of variance (ANOVA), Pearson correlation analysis, and Principal Component Analysis (PCA), to examine relationships and patterns within the dataset. One-way ANOVA was used to determine whether there were statistically significant differences between the means of the treatments, allowing for the identification of variations between categorical independent variables. Pearson correlation analysis assesses the strength and direction of linear relationships between continuous variables, providing insight into associations within the dataset. PCA was used to reduce the dimensionality of the data while retaining important variance, thus helping to identify underlying patterns and key contributing factors. All analyses were performed using statistical software (Origin pro 2021b), and results are interpreted based on significance levels and eigenvalues to draw meaningful conclusions.

Results and Discussion

Effect of bioactivated Zn-enriched compost on soil biological properties

The effect of Zinc-solubilizing bacteria, zinc-enriched compost and zinc sources on soil microbial properties is prominent (Figure 2). The microbial biomass carbon and nitrogen were observed with maximum values provided by the bioactivated Zn-enriched compost treatment. The application of compost increased the microbial biomass carbon by 48% and the microbial biomass nitrogen by 56%. The bioactivated compost treatment resulted in a 42% increase in microbial biomass carbon and nitrogen. Statistical analysis revealed that the sole application of ZnO and ZnSb showed a non-significant improvement in microbial biomass nitrogen compared to the control. Furthermore, all applied treatments showed significant improvement in bacterial population (in terms of CFU) except the sole application of $ZnSO_4$ and ZnO, which showed a non-significant increase. The maximum increase was observed with the application of bioactivated Zn-enriched compost, which showed 45 % increase compared to the control. The next treatment that performed better was the bioactivated compost (compost + ZnSB), which showed an increase of 36 %. The effect of bioactivated Zn-enriched compost in improving soil biological properties, i.e., microbial population and microbial biomass carbon and nitrogen, was evident from the literature (Ghorbanzadeh et al., 2020). These amendments release various organic acids that alter the biochemical properties of the soil and make essential nutrients available to plants (Bhatt and Maheshwari, 2020; Ait et al., 2020). The application of indigenous soil biota helped improve soil organic content and soil microbial biomass through their exponential growth (Ullah et al., 2020). The findings are consistent with the study conducted by Ijaz et al, (2019), which indicated that solubilizing bacteria along with enriched compost drastically improved the biological properties of the soil. The co-application of compost with beneficial microbial strains enhances the decomposition of organic matter and thus promotes microbial metabolic activities (Ditta et al., 2018). In the present study, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and bacterial population were selected as key soil health indicators to assess the effectiveness of compost and microbial treatments. These indicators are widely recognized for their sensitivity to soil management practices and ability to reflect changes in soil biological activity and nutrient cycling. MBC and MBN are considered reliable proxies for the size and activity of the soil microbial community, which plays a central role in organic matter decomposition and nutrient transformation (Kooch et al., 2019). An increase in MBC and MBN indicates enhanced microbial-driven processes and improved soil fertility status. Similarly, bacterial population dynamics provide insights into the abundance of beneficial microorganisms and the overall biological health of the soil ecosystem (Ambrosini et al., 2016).

The results showed a significant positive relationship between the bacterial population and microbial biomass carbon and nitrogen, as indicated by the Pearson's correlation coefficients (R = 0.71 and R = 0.56, respectively). These results are consistent with previous research highlighting the role of microbial communities in organic matter decomposition and nutrient cycling in soil ecosystems (Raza et al., 2023). The strong correlation between bacterial population and microbial biomass carbon suggests that an increase in bacterial abundance enhances microbial biomass accumulation, likely due to greater organic matter decomposition and assimilation (Chen et al., 2020). Similarly, the moderate correlation with microbial biomass nitrogen suggests that bacterial activity contributes to nitrogen mineralization and retention in soil, although to a slightly lesser extent than carbon (Ibrahim et al., 2020). Organic fertilizers stimulate microbial growth by providing essential nutrients and energy sources, which improve microbial biomass carbon and nitrogen levels (Gao et al., 2020). This reinforces the importance of organic amendments in promoting soil microbial health and nutrient availability, ultimately improving soil fertility and sustainability.

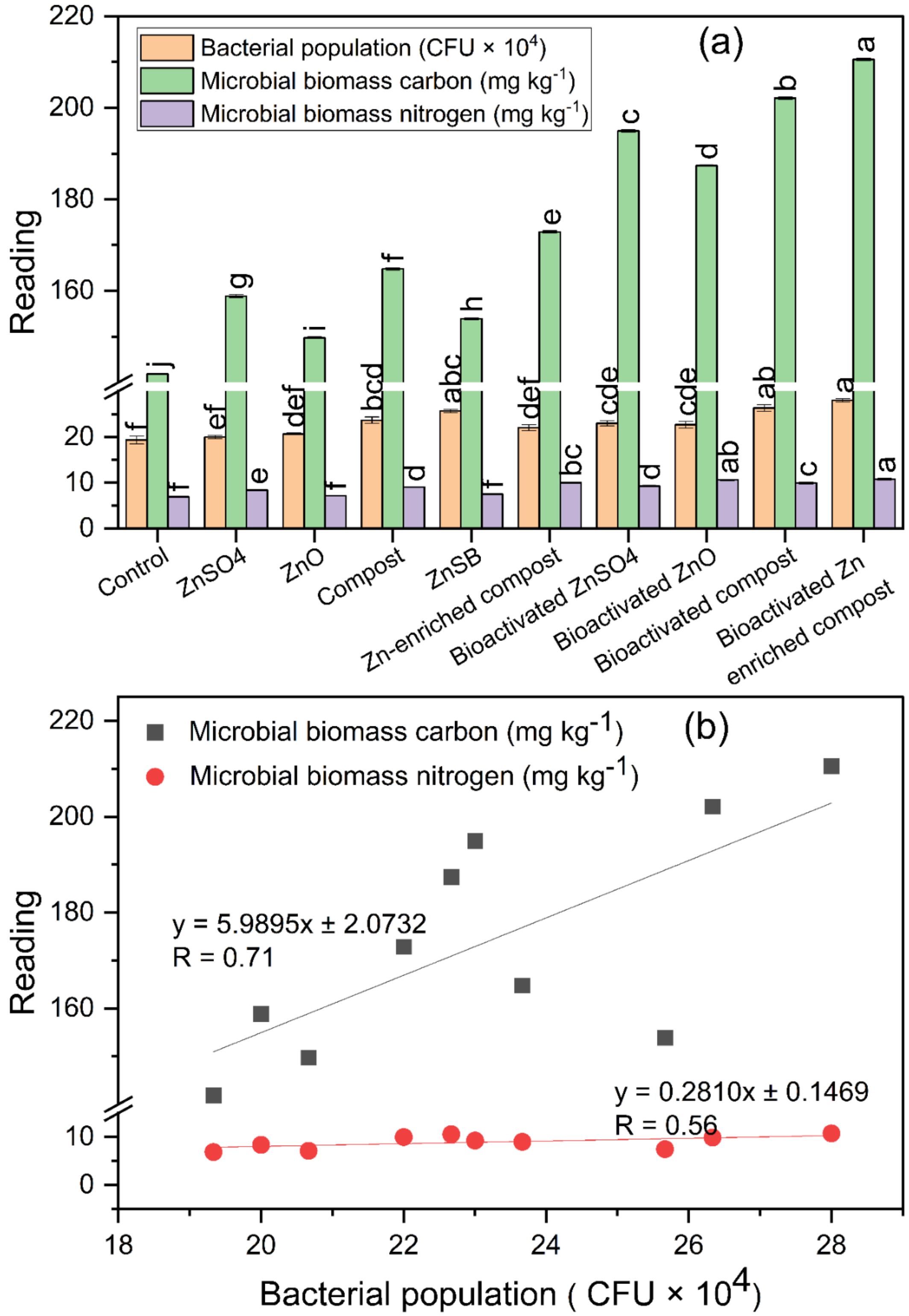


Figure 2: (a) Effect of integrated application of inorganic, organic and bioactivated Zn-enriched compost on soil biochemical properties. Bars with the same letter(s) are not different at the 5 % probability level (n = 3). **(b)** Linear correlation of bacterial population with microbial biomass carbon

and nitrogen.

Effect of bioactivated Zn-enriched compost on soil chemical properties

ZnSB, Zn-enriched compost, ZnO, and $ZnSO_4$ significantly improved soil chemical properties by increasing organic matter content and availability of essential macro- and micronutrients (Figure 3). The incorporation of compost, ZnO, $ZnSO_4$, and ZnSB into the soil contributed to improved soil fertility, as organic matter plays a crucial role in nutrient retention, microbial activity, and soil structure (Hussain et al., 2023). Among the treatments, bioactivated Zn-enriched compost was the most effective, resulting in a 34% increase in organic matter content, which is consistent with previous studies highlighting the role of organic material in improving soil organic carbon and nutrient dynamics (Xu et al., 2024; Wang et al., 2025). Compost with ZnO and ZnSB also resulted in notable increases of 26% and 25%, respectively, indicating the synergistic effects of organic amendments and Zn sources in improving soil quality.

Furthermore, the application of bioactivated Zn-enriched compost resulted in a significant increase in the availability of essential nutrients, with the greatest improvements recorded in available phosphorus (22%), extractable potassium (23%), and total nitrogen (10%). These results support existing literature showing that compost improves nutrient solubility and availability by promoting microbial decomposition and mineralization (Lela et al., 2024). The increased availability of micronutrients, particularly Fe and Zn, further validates the efficacy of bioactivated Zn-enriched compost in improving soil fertility. The 30% and 41% increase in Fe and Zn availability, respectively, highlights the ability of organic amendments to chelate and retain essential micronutrients in the rhizosphere (Madhupriyaa et al., 2024). In addition, the combined application of $ZnSO_4$ + ZnSB and ZnO + ZnSB also improved Fe and Zn availability, showing increases of 24% and 32%, respectively, with $ZnSO_4$ + ZnSB being more effective than ZnO + ZnSB. This suggests that ZnSO, as a readily soluble source of Zn, improves Zn bioavailability, as reported in previous studies (Xu et al., 2021).

The results indicate that bioactivated Zn-enriched compost is the most effective treatment for improving soil chemical properties by increasing organic matter content and macro- and micronutrient availability. The combination of Zn sources, especially $ZnSO_4$ and ZnSB, also showed beneficial effects, emphasizing the importance of integrated nutrient management strategies for maintaining soil fertility and crop productivity. These results are consistent with previous research advocating for organic and inorganic Zn sources to maximize nutrient availability and soil health (Imran, 2024; Yang et al., 2025). The superior performance of bioactivated Zn-enriched compost compared to individual Zn sources can be attributed to several key factors, including differences in solubility, mobility in the soil, and plant uptake efficiency. Although zinc sulfate is highly soluble, it is quickly leached or fixed in alkaline soils, reducing its long-term availability to plants (Riaz et al., 2020). In contrast, Zn from compost-based sources is often gradually released due to its complexation with organic matter, improving its persistence and availability in the rhizosphere (Ahmed et al., 2023). Furthermore, the bioactivation process enhances microbial activity, facilitates Zn solubilization and root uptake. This integrated mechanism likely explains the enhanced agronomic performance observed with bioactivated Zn-enriched compost, controlled Zn release with improved soil health and microbial interactions.

Pearson's correlation heatmap analysis revealed a strong relationship between organic matter content and major soil nutrients, including total nitrogen, Olsen P, extractable K, and DTPA-extracted Fe and Zn. This result aligns with earlier research emphasizing the essential role of soil organic matter in enhancing nutrient availability and retention. The positive correlation between organic matter and total nitrogen suggests that organic matter acts as a reservoir for nitrogen, influencing its mineralization and subsequent availability to plants (Jilling et al., 2018). Similarly, the association between organic matter and Olsen P supports the concept that organic matter increases phosphorus availability by reducing its fixation in the soil (Vermeiren et al., 2022; Gong et al., 2025). The observed relationship with extractable K may be attributed to the cation exchange capacity (CEC) of organic matter, which helps to retain potassium in a plant-available form (Gurav et al., 2024). In addition, the correlation with DTPA-extracted Fe and Zn suggests that organic matter contributes to the chelation and solubilization of micronutrients, thereby improving their bioavailability (Madhupriyaa et al., 2024). The thickness of the ellipses described the Pearson's correlation coefficient (R) values. The thinner the ellipses, the higher the correlation coefficient and the thicker ellipses, the lower the correlation coefficient.

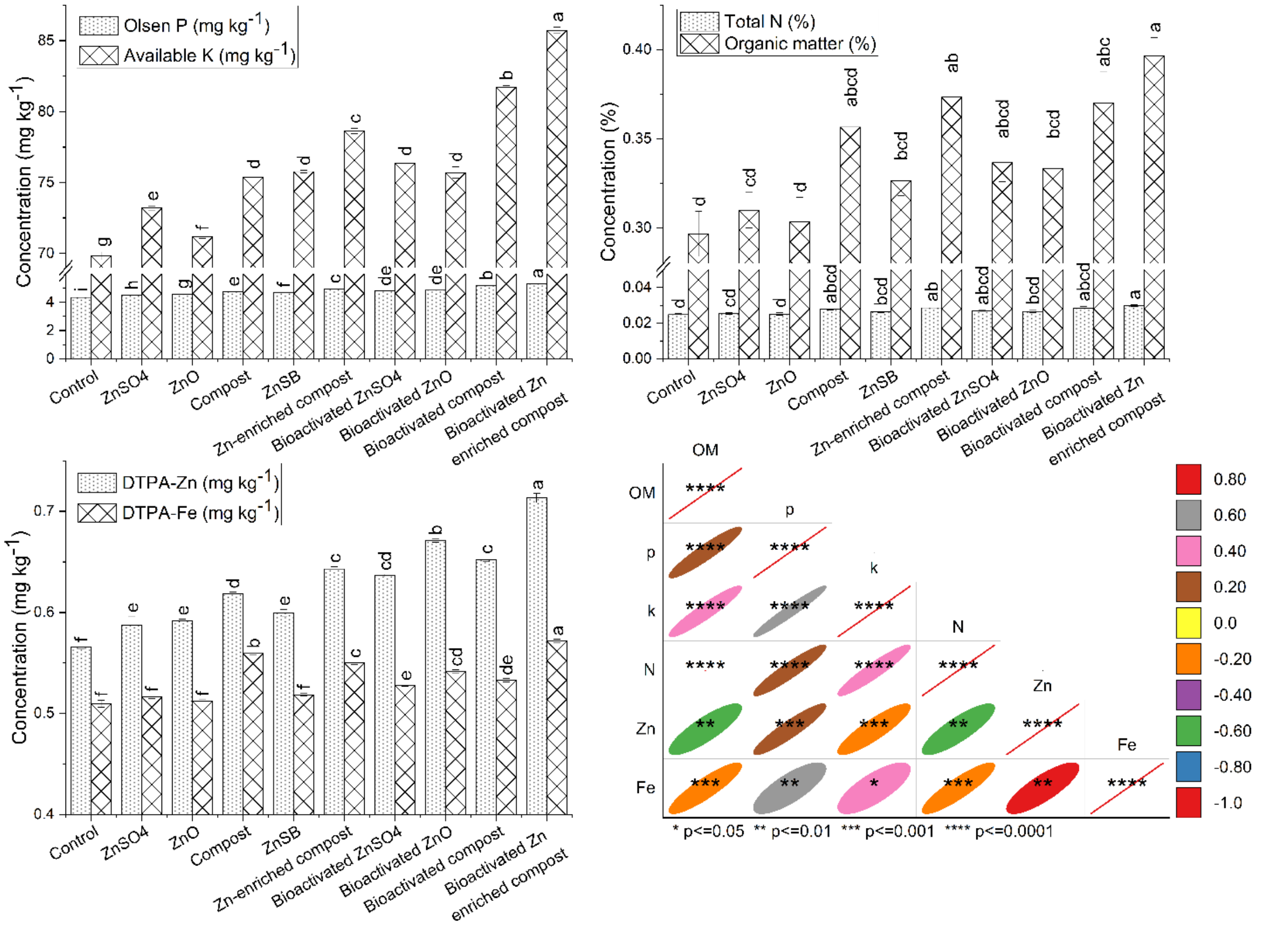


Figure 3: Effect of integrated application of inorganic, organic and bioactivated Zn-enriched compost on soil chemical properties. Bars with the same letter(s) are not different at 5 % probability level (n = 3). Pearson's correlation heatmap shows the relationship between the studied parameters

Effect of bioactivated Zn-enriched compost on plant growth

The effect of ZnSB, compost, bioactivated Zn-enriched compost and Zn sources on plant growth was remarkable (Table 1). The maximum root length (10.5 cm) and shoot length (85.0 cm) were recorded by bioactivated Zn-enriched compost, which were 27 and 14 % more than the control, respectively. The combined application of ZnO + ZSB and compost + ZnO improved shoot length by 9 %, similar to the application of ZnSB. Bioactivated compost showed 23% increase in root length, next to bioactivated Zn-enriched compost. Commercial zinc sources and bioactivated zinc-enriched compost significantly enhanced root and shoot dry biomass. The maximum increase of 27 and 17 % in root and shoot dry biomass, respectively, was observed by bioactivated Zn-enriched compost. The indigenous soil biota has also improved growth and morphological traits (Naveed *et al*, 2020). Furthermore, organic amendments have been found to release nutrients required for plant structure (Naseem et al, 2022). This enhances plant growth, i.e., root and shoot biomass and their length, for plant morphological characteristics and yield (Piccinin et al, 2013). The results align with the findings of Mustafa et al. (2019) and Beura et al. (2020), which demonstrate that nutrient-solubilizing bacteria combined with enriched compost significantly enhance yield characteristics. Thus, the bioavailability and assimilation of nutrients enabled the plant to grow vigorously and thrive under poor fertility (Mumtaz et al, 2018). Due to the bioavailability of essential nutrients, the plant increased its photosynthetic activities to achieve optimal growth and development (Bashir et al, 2020).

Table 1: Effect of inorganic, organic and bioactivated Zn-enriched compost on plant growth in nutrient-deficient soil.

Treatment / **Parameters**	**Root length**	**Root dry biomass**	**Shoot length**	**Shoot dry biomass**
	(cm)	**(g plant^{-1})**	**(cm)**	**(g plant^{-1})**
Control	8.3 ± 0.27 d	0.59 ± 0.025 d	74.0 ± 0.58 d	5.06 ± 0.05 c
$ZnSO_4$	8.6 ± 0.23 cd	0.61 ± 0.015 cd	77.7 ± 0.51 bcd	5.43 ± 0.15 abc
ZnO	9.2 ± 0.33 bcd	0.61 ± 0.015 cd	75.7 ± 1.39 cd	5.38 ± 0.16 abc
Compost	10.2 ± 0.30 ab	0.63 ± 0.005 bcd	78.3 ± 0.69 bcd	5.21 ± 0.05 bc
ZnSB	9.2 ± 0.22 bcd	0.63 ± 0.007 bcd	80.7 ± 1.71 abc	5.61 ± 0.10 abc

Zn-enriched compost	9.9 ± 0.13 ab	0.63 ± 0.018 bcd	81.0 ± 0.88 abc	5.56 ± 0.16 abc
Bioactivated $ZnSO_4$	9.9 ± 0.22 ab	0.65 ± 0.013 bcd	81.3 ± 0.69 ab	5.73 ± 0.11 ab
Bioactivated ZnO	9.8 ± 0.23 abc	0.67 ± 0.010 abc	80.3 ± 1.17 abc	5.75 ± 0.10 ab
Bioactivated compost	10.2 ± 0.31 ab	0.69 ± 0.013 ab	82.7 ± 0.51 ab	5.61 ± 0.10 abc
Bioactivated Zn enriched compost	10.5 ± 0.33 a	0.74 ± 0.014 a	85.0 ± 1.67 a	5.92 ± 0.04 a
LSD ($p \leq 0.05$)	**1.2897**	**0.077**	**5.5526**	**0.5866**

Data are presented as the mean of three replicates ± standard error. Columns with the same letter(s) do not differ at the 5 % probability level.

Bioactivated Zn-enriched compost upregulates antioxidant enzyme activities

The effect of bioactivated Zn-enriched compost and different commercial sources of Zn (i.e., $ZnSO_4$, ZnO) on the antioxidant properties of the plant is remarkably shown in Figure 4. The individual application of treatments, i.e., $ZnSO_4$, ZnO, compost and ZnSB, increased the catalase (CAT) activity by 1%, 11%, 15% and 8%, respectively. However, the bioactivated compost and zinc oxide increased the catalase activity by 35% and 20%, respectively. A similar trend of applied treatments was observed for the improvement of peroxidase (POX), peroxidase dismutase (POD) and ascorbate peroxidase (APX) activities. The bioactivated Zn-enriched compost showed maximum increases of 42, 32, 29 and 24 % in POX, POD and APX, respectively. Moreover, Principal Component Analysis (PCA) revealed that the treatments positively affected antioxidant enzyme activity. The data were distributed on two principal components (PC), 14.72% for PC1 and 80.71% for PC2. Among the applied treatments, bioactivated ZnO (ZnO +ZnSB) was positively distributed on PC1, while the sole application of $ZnSO_4$ and ZnSB was positively distributed on PC2. The PCA biplot included 4 quadrants, where the top right quadrant represents the positive distribution of data on both principal components. All the treatments where compost was applied (i.e., compost, Zn-enriched compost, bioactivated compost and bioactivated Zn-enriched compost) were positively distributed on both principal components (PC1 and PC2). Bioactivated Zn-enriched compost showed maximum eigenvalues of 3.31% for PC1 and 0.06% for PC2. The exogenous addition of beneficial microbes has also been found to improve the antioxidant properties of plants (Naveed et al, 2020). These antioxidant properties of plants responded to the signaling and activation of their defense mechanism by beneficial microbes (Khalid et al, 2022). Compost is a primary carbon source and its application increases organic carbon and improves soil physicochemical processes (Omara et al, 2022). Composts are quite productive for beneficial microbes (Sarwar et al., 2023). Meanwhile, their combined application has a more pronounced effect on improving plant antioxidant properties, including catalase and ascorbate (Benidire et al, 2021).

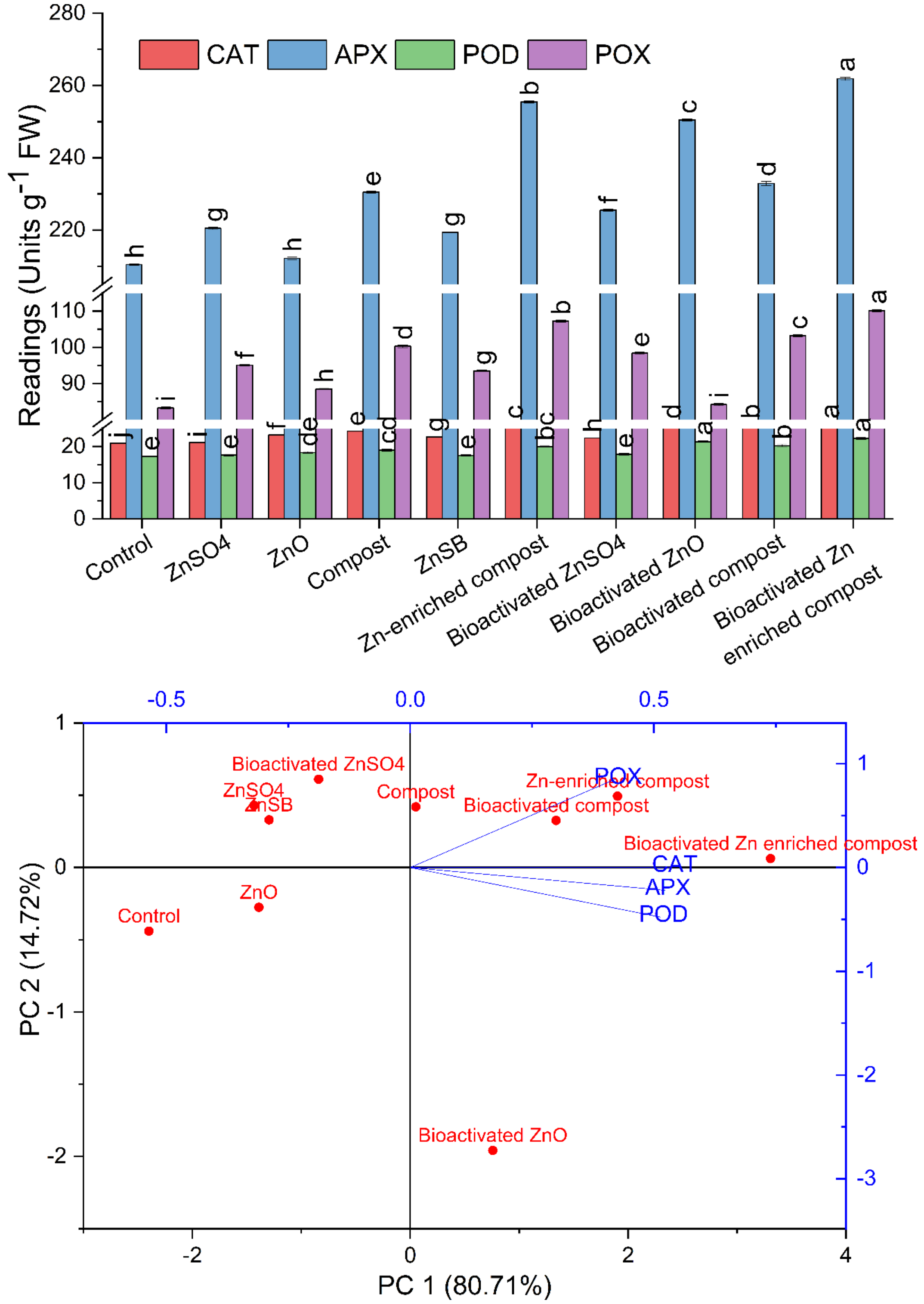


Figure 4: Effect of integrated application of inorganic, organic and bioactivated Zn-enriched compost on soil chemical properties. Bars with the same letter(s) are not different at the 5 % probability level (n = 3). Principal component analysis (PCA) showed the effectiveness of applied treatments

Plant photosynthetic pigments improved by bioactivated Zn-enriched compost

The photosynthetic pigments, i.e., chlorophyll “a”, “b”, and carotenoids, were significantly increased by bioactivated Zn-enriched compost and Zn sources (Figure 5). Bioactivated Zn-enriched compost increased chlorophyll a and b by 25 % while carotenoids improved by 31%. The next treatment that performed better was the bioactivated compost (compost + ZnSB), which showed 20, 21 and 22 % in chlorophyll a, b and carotenoid, respectively. The combined application of ZnSB + $ZnSO_4$ and ZnSB + ZnO showed up to 16 % improvement in photosynthetic pigments. The studies showed that compost and ZnSB application released nutrients required for the photosynthetic pigments and other metabolic activities (Mustafa et al., 2019). Their exogenous application increased the microbial activities that released nutrients bound to soil particles (Fidel et al., 2017; Hussain et al, 2019). These nutrients improved the concentration of photosynthetic pigments, i.e., chlorophyll “a”, “b” and carotenoids (Elfadil et al, 2019). Similar results were found in studies where compost and solubilizing bacteria were applied to improve the concentration of photosynthetic pigments (Basak et al, 2020; Khalid et al, 2022).

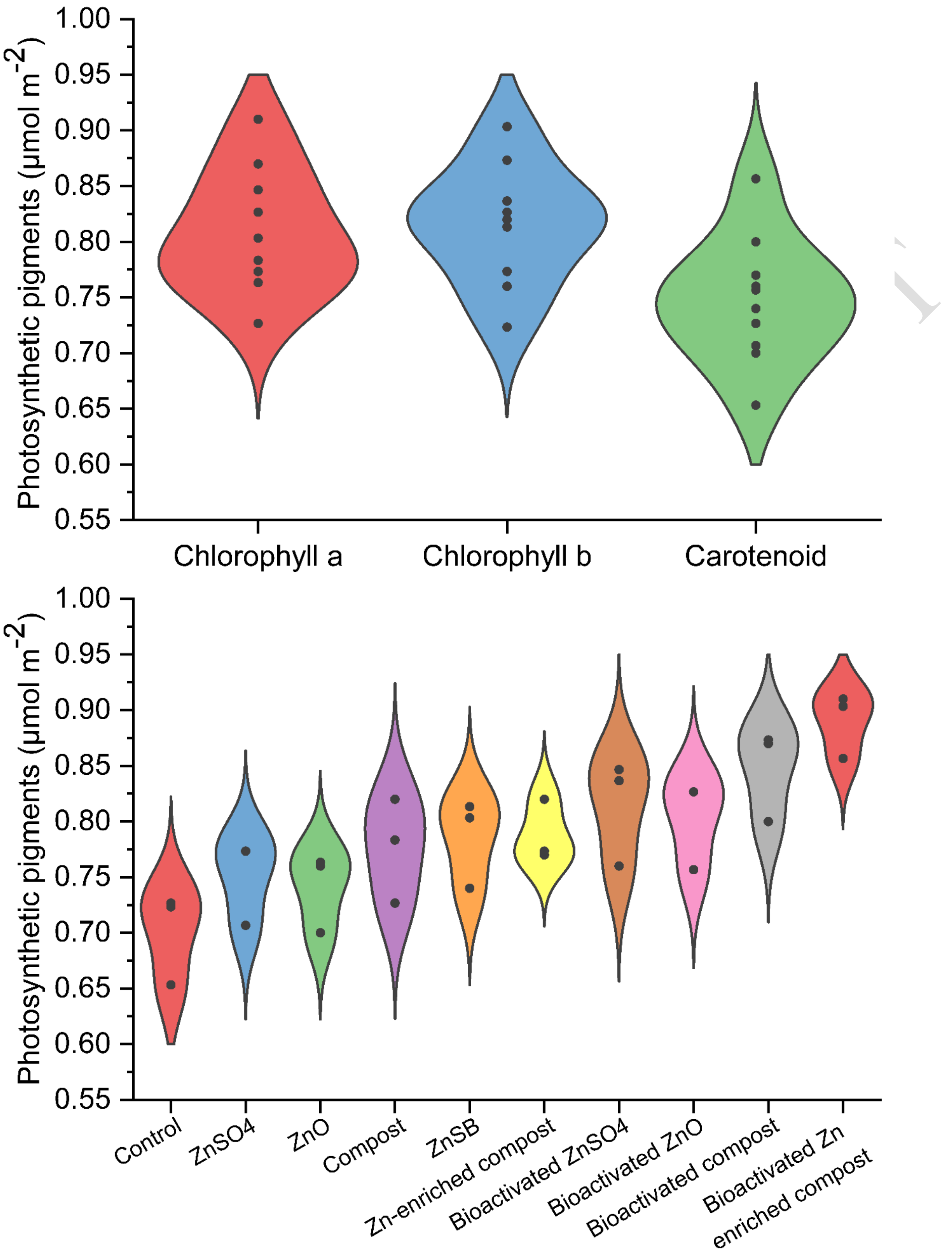


Figure 5: Violin diagram analysis showed the effectiveness of organic, inorganic and bioactivated Zn-enriched compost in improving photosynthetic pigments in the wheat crop

Effect of bioactivated Zn-enriched compost on yield related parameters and grain yield

A significant effect of applied treatments was observed on yield parameters (Table 2). The effects of applied treatments on yield parameters highlight the importance of zinc (Zn) enrichment and bioactivation in improving crop productivity. The number of tillers per square meter was maximally increased by 13% with the application of bioactivated Zn-enriched compost, followed by an 8% increase with $ZnSO_4$ + Zn-solubilizing bacteria (ZnSB). These results are consistent with previous studies that emphasized the role of bioactivated compost in improving soil fertility and microbial activity, which, in turn, enhances root proliferation and tillering (Sande et al., 2024). Similarly, the application of $ZnSO_4$ and ZnO resulted in a 5% and 6% increase in the number of grains per spike, respectively, which is consistent with reports indicating the role of Zn in pollen viability, fertilization, and grain formation (Kandil et al., 2022). Notably, bioactivated compost (compost + ZnSB) and bioactivated zinc oxide (ZnO + ZnSB) increased the 1000-grain weight by 7% and 6%, respectively, supporting the hypothesis that Zn-enriched treatments promote seed development and grain filling (Luís et al., 2021). The highest improvements in the number of spikelets per spike (16%), number of grains per spike (10%), and 1000-grain weight (8%) were observed with bioactivated Zn-enriched compost, reinforcing the effectiveness of combining organic matter with microbial inoculants to enhance Zn bioavailability and plant uptake (Khatoon et al. 2022). In addition, grain yield showed an overall improvement with the Zn treatments; the sole applications of ZnO and compost resulted in non-significant differences from the control, probably due to limited Zn solubility and availability in these forms. The highest increase in grain yield (9%) was achieved with bioactivated Zn-enriched compost, highlighting the synergistic effects of organic amendments and microbial inoculation in improving Zn uptake and grain production. These results underline the need to integrate bioactivated Zn-enriched compost as an effective strategy to improve crop yield and nutrient use efficiency in Zn-deficient soils.

Table 2: Effect of inorganic, organic and bioactivated Zn-enriched compost on yield-related parameters and grain yield in nutrient-deficient soil.

Treatment / Parameters	Number of tiller m^2	Number of spikelets $spike^{-1}$	Number of grains $spike^{-1}$	1000-grain weight (g)	Grain yield (t ha^{-1})
Control	278 ± 1.5 f	13.1 ± 0.35 b	35.0 ± 0.33 c	39.0 ± 0.10 d	4.55 ± 0.02 g
$ZnSO_4$	291 ± 1.3 cd	13.7 ± 0.34 ab	36.7 ± 0.51 bc	39.9 ± 0.05 cd	4.71 ± 0.04 def
ZnO	289 ± 1.5 de	13.8 ± 0.12 ab	37.0 ± 0.33 bc	40.0 ± 0.16 cd	4.62 ± 0.01 fg
Compost	302 ± 1.3 b	13.4 ± 0.54 ab	38.0 ± 0.33 ab	40.2 ± 0.11 c	4.67 ± 0.03 efg
ZnSB	282 ± 1.2 ef	14.0 ± 0.40 ab	38.3 ± 0.51 ab	40.5 ± 0.28 bc	4.76 ± 0.01 cde
Zn-enriched compost	298 ± 1.8 bc	14.3 ± 0.44 ab	39.7 ± 0.69 a	41.3 ± 0.11 ab	4.78 ± 0.01 bcde
Bioactivated $ZnSO_4$	301 ± 0.9 b	14.7 ± 0.33 ab	38.3 ± 0.51 ab	41.8 ± 0.31 a	4.81 ± 0.04 bcd
Bioactivated ZnO	302 ± 1.7 b	14.3 ± 0.38 ab	37.7 ± 0.38 ab	41.7 ± 0.19 a	4.84 ± 0.03 abc
Bioactivated compost	304 ± 1.4 b	14.3 ± 0.19 ab	37.7 ± 0.69 ab	41.6 ± 0.36 ab	4.90 ± 0.03 ab
Bioactivated Zn enriched compost	314 ± 1.7 a	15.2 ± 0.33 a	38.7 ± 0.51 ab	42.2 ± 0.44 a	4.96 ± 0.01 a
LSD (*$p \leq 0.05$*)	**7.6016**	**1.9181**	**2.332**	**1.08**	**0.133**

Data are presented as the mean of three replicates ± standard error. Columns with the same letter(s) do not differ at the 5 % probability level.

Effect of bioactivated Zn-enriched compost on mineral nutrient contents in grain

Bioactivated Zn-enriched compost and commercial sources of Zn significantly improved the mineral nutrient content (N, P, K, Fe, and Zn) in wheat grain (Figure 6). This improvement is especially significant because it enhances both plant nutrition and the nutritional quality of the human diet. Statistical analysis showed that the sole application of $ZnSO_4$ and ZnO did not increase macronutrient (N, P, and K) content. However, when these Zn sources were combined with zinc-solubilizing bacteria (ZnSB), the measured parameters showed a significant improvement. The finding is consistent with previous studies suggesting that microbial inoculants improve nutrient availability and uptake in crops (Li et al., 2022). The bioactivated Zn-enriched compost showed the most significant effect, with a 10% increase in N and P content and an 8% increase in K content compared to the control. These improvements are likely due to increased microbial activity, which facilitates nutrient solubilization and uptake (Rawat et al., 2021). In addition, biofortification of wheat grains was achieved through this treatment, with Fe and Zn concentrations reaching 40.68 mg kg^{-1} and 41.15 mg kg^{-1}, respectively. This represents an increase of 30% and 40%, respectively, compared to the control, indicating the effectiveness of bioactivated Zn-enriched compost in improving the nutritional quality of wheat grains. These results support previous research highlighting the role of Zn-enriched organic amendments in improving the micronutrient content of staple crops (Nayak et al., 2022). Consequently, the integration of bioactivated Zn-enriched compost into agricultural practices could serve as a sustainable approach to improve crop nutrition and address micronutrient deficiencies in human diets.

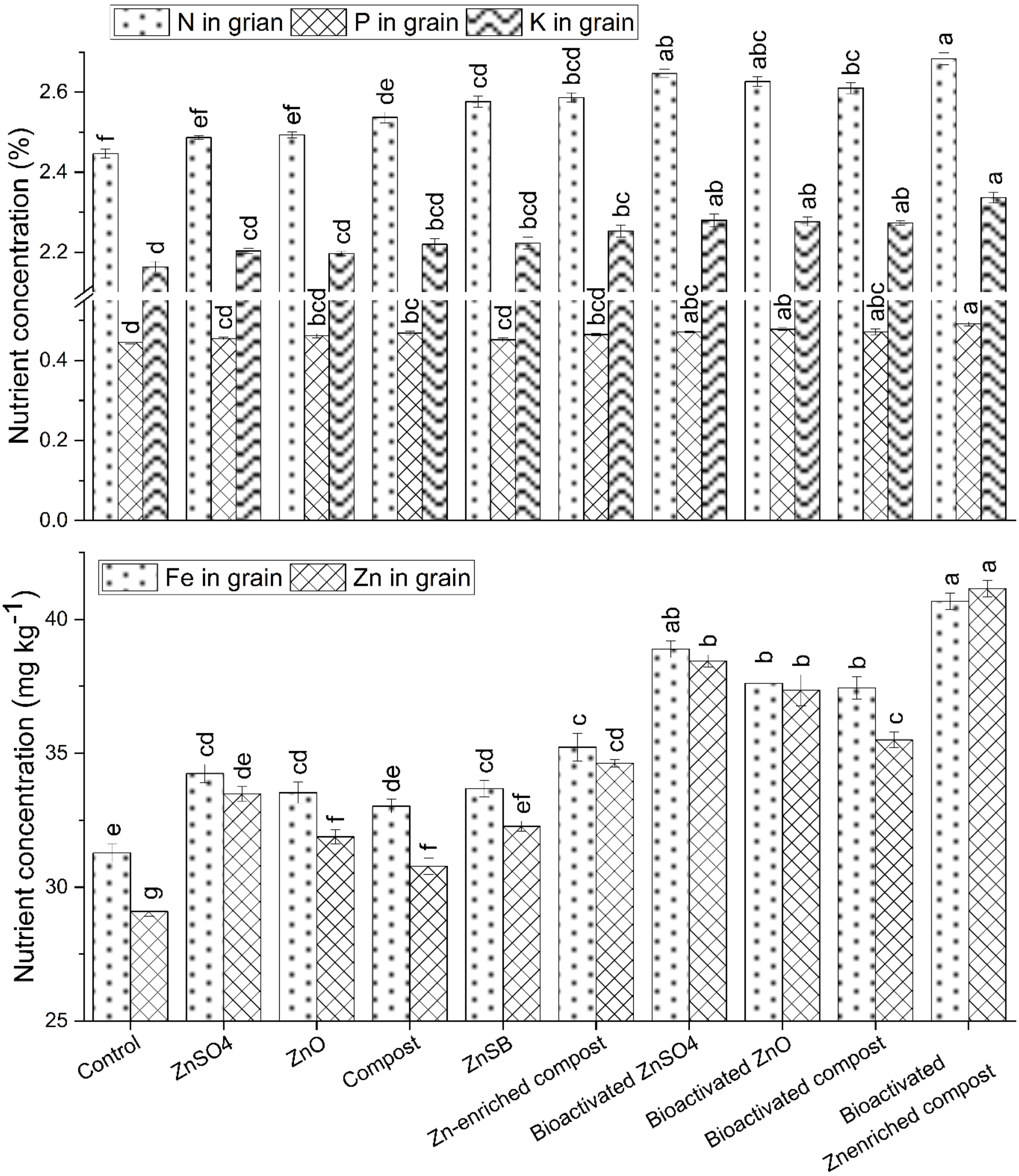


Figure 6: Effect of integrated application of inorganic, organic and bioactivated Zn-enriched compost on mineral nutrient content in wheat grain under nutrient-deficient soil. Bars with the same letter(s) are not different at the 5 % probability level (n = 3)

Correlation analysis

Pearson's correlation analysis revealed a positive relationship between the parameters studied, indicating an association between bacterial population (CFU) and grain mineral content (N (R =

0.79), P (R = 0.65), K (R = 0.77), and Fe (R = 0.65)), photosynthetic pigments (chlorophyll a (R = 0.86) & b (R = 0.90) and carotenoids (R = 0.86)) and grain yield (R = 0.81) under nutrient-deficient soil conditions (Figure 7). The thickness of ellipses indicated the strength of the correlation coefficient (R). Thinner ellipses represent strong correlation and thicker ellipses show weak correlation. The strong correlation between bacterial population and mineral content suggests that beneficial bacteria may enhance nutrient availability and uptake, ultimately improving plant growth and productivity. This is consistent with previous studies highlighting the role of plant growth promoting rhizobacteria (PGPR) in improving nutrient solubilization and uptake, thereby increasing crop yield (Etesami and Adl, 2020). Furthermore, the strong correlation between photosynthetic pigments and grain yield suggests that increased chlorophyll and carotenoid concentrations contribute to improved photosynthetic efficiency, biomass accumulation and productivity (Simkin et al., 2022). Visualization of the correlation through ellipses further supports these findings, with thinner ellipses indicating stronger correlations, highlighting the significant impact of bacterial population, nutrient availability, and photosynthetic efficiency on yield. These results highlight the importance of microbial inoculation and nutrient management strategies in sustaining crop production under nutrient-deficient conditions. Although zinc (Zn) is an essential micronutrient for plant growth, excessive accumulation can lead to phytotoxicity and soil contamination over time. In the present study, Zn levels in soil remained within acceptable limits; however, long-term application may result in a buildup beyond safe thresholds. According to Alloway (2008), the critical toxicity level of Zn in soil for wheat is generally considered to be around 300 mg kg^{-1}, while phytotoxic effects in plants may occur at shoot concentrations exceeding 200–400 mg kg^{-1}. To mitigate risks, it is important to monitor soil Zn concentrations regularly and tailor application rates based on soil testing. Future studies should also assess the cumulative impact of repeated Zn additions, including potential interactions with other heavy metals and effects on soil microbial activity and crop health.

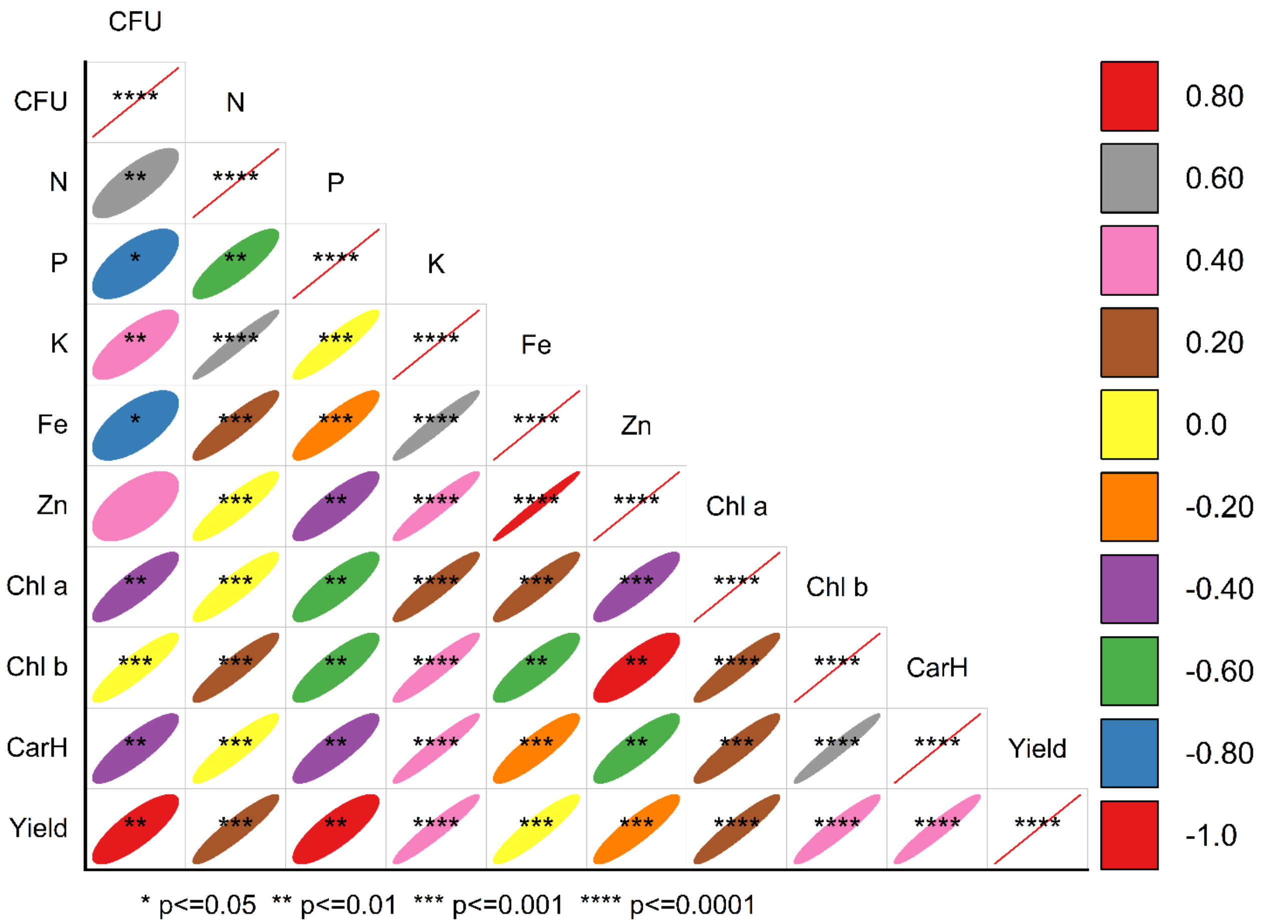


Figure 7: Correlation analysis of bacterial population (CFU), grain nutrient content, photosynthetic pigments (chlorophyll a, b and carotenoids) and grain yield

Conclusion

The results of this study highlight the potential of bioactivated zinc-enriched compost as a sustainable soil amendment for improving soil fertility, microbial activity, and wheat productivity in Zn-deficient soils. The significant increases in soil organic matter, nutrient availability, and microbial biomass demonstrate the efficacy of composting with Zn-enriched bio-inoculants in revitalizing degraded agricultural soils. Furthermore, the observed increases in wheat growth, grain yield and grain Zn content highlight agronomic and nutritional benefits of this approach. The improved antioxidant enzyme activities also improve plant stress tolerance. These results support the wider adoption of bioactivated Zn-enriched compost as an environmentally friendly strategy to improve soil health, reduce reliance on chemical fertilizers, and promote sustainable wheat production. Future research should optimize compost formulations and assess long-term effects in different agroecosystems to maximize its benefits.

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