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Evaluating the efficacy of bacterial-assisted phytoremediation using maize (*Zea mays* L.) to uptake heavy metals from fly ash

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ABSTRACT

The agricultural sector faces dual challenges of declining soil fertility and unsustainable waste accumulation. This study examines the synergistic effects of fly ash (FA) and plant growth-promoting bacteria (PGPB) on the growth and physiological performance of maize (*Zea mays* L.) under controlled (potted) conditions. FA, a coal combustion by-product rich in essential minerals, was applied at varying doses (1–4 t ha^{-1}) to assess its potential as a soil amendment with a bacterial strain (BSNK7) inoculated to enhance nutrient uptake and mitigate stress. Results showed a significant increase in fresh and dry biomass, leaf area, and chlorophyll content in treated plants. The combined application of 1 t ha^{-1} FA in conjunction with PGPB significantly increased fresh biomass by 1.57%, dry biomass by 0.94%, leaf area by 2.21%, and higher chlorophyll content compared to control (FA 0 t ha^{-1} and without bacteria). In contrast, FA 4 t ha^{-1}, when applied without bacterial inoculation, resulted in reduced fresh biomass by 19.94% and dry biomass by 17.39%, respectively, compared to the control (FA 0 t ha^{-1} and without bacteria), which indicates the creation of toxicity at elevated doses. These findings suggest that the integrated use of low-dose FA and PGPB can sustainably enhance maize growth while minimizing environmental risks. The Application of appropriate doses of FA with PGPB can increase crop productivity and soil health simultaneously. Further field-based studies are recommended to validate scalability, optimize application rates, and assess the long-term impacts on soil health.

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1. INTRODUCTION

In Saudi Arabia, heavy oil (HO) is used in water desalination stations and electric power generators releasing by-product known as fly ash (FA). Currently, they dispose of it by dumping it in large areas, including the Rabigh area and other locations. This leads to contamination and pollution of soil, water, and plants because FA contains toxic elements and heavy metals. Land and soil have been polluted in a hazardous way in the twenty-first century by FA (Ciupa et al., 2019). Soil metal contamination is essential in the developing countries, where many industries often discharge untreated waste into the open environments (Kinuthia et al., 2020). The power generation and desalination sector in Saudi Arabia is responsible for generating huge amounts of liquid and solid waste estimated at millions of tons per year. The most important of these pollutants is FA, which results from burning heavy oil and crude oil used as fuel in power plants, desalination plants, and cement plants that consume huge amounts of fuel (Permatasari et al., 2023). FA is composed of spherical particulate matter that ranges from 0.1 µm to >100 µm. It is predominantly composed of silica, aluminium, iron, calcium, and oxygen, but the particles may contain heavy metals such as arsenic and lead at trace levels. However, FA contains heavy oil elements as sulphur by 4-8%, and heavy metals such as vanadium, nickel, zinc, chromium and lead) (A-Qureshi et al., 2024). In contrast, researchers reported that FA has been used as a soil amendment for reclaiming wastelands or mine spoils (Adriano & Weber, 2001).

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Several methods are being used to remediate the environment from these polluting materials. Phytoremediation has become an effective and affordable technological solution for extracting metal pollutants from polluted soils. Phytoremediation involves the use of plant species as extractors and translocators of heavy metals and toxins from contaminated soil. The plant species absorb these metals and accumulate them in their different plant parts (roots, shoots, leaves, flowers, fruits, seeds, etc.). Many plant species succeeded in absorbing contaminating heavy metals from polluted soils (Asiminicesei et al., 2024). Selected plant species possess the genetic potential to remove, degrade or metabolize a wide range of contaminants. Plants use several natural biophysical and biochemical processes, such as adsorption, transport and translocation, hyperaccumulation and mineralization, to remediate pollutants (Yaashikaa et al., 2022). Given its sustainability and scalability, phytoremediation presents a promising alternative to energy-intensive cleanup techniques.

Application of PGPB to remediate polluted soils is an essential approach in regions facing severe pollution from industrial byproducts, such as Saudi Arabia. Saudi Arabia's growing demand for electricity and freshwater has created a widespread reliance on heavy oil combustion in power and desalination plants which produce large quantities of FA. Improper disposal of FA generated in landfills found in Rabigh, accumulates toxic substances in the environment. The persistent presence of vanadium, nickel, lead, chromium, and zinc raises serious concerns about soil degradation and water pollution. These metals pose considerable environmental and health risks, as they leach into groundwater, accumulate in crops, and eventually enter the food chain (Al-Solaimani et al., 2024). Traditional remediation methods including soil washing and chemical stabilization are comparatively costly, energy-intensive, and disruptive to natural ecosystems. To mitigate these challenges, phytoremediation has emerged as a sustainable and eco-friendly alternative (Yaashikaa et al., 2022). Plant-based remediation technique utilizes selected plant species capable of absorbing, stabilizing, or detoxifying pollutants in contaminated environments. Studies have shown that native plant species of Saudi Arabia possess the potential to remediate heavy metal-contaminated soils effectively, making phytoremediation a viable solution for managing FA pollution (Alghamdi & El-Zohri, 2024). By leveraging natural plant processes, this method aligns with global efforts to address pollution while minimizing environmental and economic trade-offs.

Maize (*Zea mays* L.) is considered a promising candidate for phytoremediation due to its rapid growth rate, high biomass yield, and well-developed root system, which facilitate heavy metal uptake from contaminated soils (Rizwan et al., 2017), unlike hyperaccumulator species, which accumulate high metal concentrations but grow slowly. Maize can absorb and translocate significant amounts of metal while maintaining productivity, making it suitable for large-scale remediation applications. Previous studies reported that maize can uptake heavy metals such as lead, cadmium, and zinc, although soil conditions and metal bioavailability influence its phytoremediation efficiency (Atta et al., 2023; Tipu et al., 2021). High concentrations of heavy metals may adversely affect maize growth, limiting its remediation capacity. Susilowati et al. (2024) recommended combining inorganic fertilizers with other fertilizers to increase the yield of maize. To address this, researchers have employed plant growth-promoting bacteria (PGPB) assisted phytoremediation which improves heavy metal uptake from contaminated environments (Alves et al., 2022; Zhang et al., 2022).

PGPB, for example, *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Azospirillum brasilense* enhance phytoremediation by producing siderophores. It increases metal solubility, phosphates solubility and phytohormones synthesis that stimulates root development, and reduces ethylene-induced stress in plants caused by heavy metals (Chandwani et al., 2025; Singh et al., 2018). PGPB facilitates metal mobilization while promoting maize health, enabling higher accumulation of metals even in contaminated soils. Studies have reported that bacterial inoculation can increase metal uptake in maize by up to 40%, indicating its effectiveness as a remediation strategy. However, effectiveness depends on the dose of FA, soil conditions, and interactions between plants and microbes.

The application rate of FA has a critical impact on soil quality, plant performance, and metal uptake. Low to moderate FA levels (1–2 t ha^{-1}) can improve soil fertility through the addition of nutrients like calcium and magnesium, while also enhancing soil moisture retention. However, excessive FA (>4 t ha^{-1}) raises soil pH and electrical conductivity (EC), causing increased salinity and heavy metal toxicity, which suppresses maize growth and microbial activity (Jambhulkar et al., 2018; Usman et al., 2023; Varshney et al., 2022). Previous Research reported that higher FA doses reduce biomass and chlorophyll content due to pH-induced metal immobilization (Abhishek et al., 2024; Anbuganesan et al., 2024; Kaur et al., 2024). The present study introduces a novel solution by demonstrating how targeted PGPB (strain BSNK7) inoculation synergizes with optimal FA doses (1 t ha^{-1}) to enhance metal extraction efficiency in maize. Unlike generic PGPB applications, our approach identifies a specific bacterial strain and FA dose combination that maximizes phytoremediation while minimizing ecological risks. These findings provide strategies for developing low-cost, scalable methods to remediate FA-contaminated soils in industrialized areas, addressing both waste management and agricultural productivity gaps.

This study aims to evaluate the synergistic effects of FA and PGPB on maize growth and soil properties in the remediation of heavy metals from contaminated soils. Specific objectives included analysing changes in soil pH, EC across varying FA doses (0, 1, 2, and 4 t ha^{-1}); assessing growth and physiological responses of maize (biomass, chlorophyll content, and leaf area); and identifying the optimal FA dose for effective and sustainable phytoremediation.

Table 1. The initial soil properties of selected soil

P	K	N	CEC	$CaCO_3$	Clay	Silt	Sand	OM	pH	EC	Soil Texture
	mg kg^{-1}		(meq $100g^{-1}$)			%				dSm^{-1}	
2.37	117.12	298.33	12.84	2.29	19.83	15.80	64.37	1.03	6.33	3.18	Sandy Clay Loam

2. MATERIAL AND METHOD

2.1. Experiment location

This research was conducted from January to March 2025, in a screen house (length 3.9 m x width 2.8 m) located on the rooftop of Department of Agriculture, Faculty of Environmental Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. The experimental site is located at 21°29’45.769” N, 39°14’46.762” E, with an altitude of 41 m above sea level. The initial soil properties of each selected soil type are presented in Table 1.

2.2. Soil preparation, seeds and fertilizer application

Sandy loam soil was used in the study collected from the research station of King Abdulaziz University located at Hada Al Sham, Jeddah, Saudi Arabia. The collected soil was mixed with peat moss (in a 1:1 ratio) to improve water retention and aeration. FA samples were collected from a coal fired power plant and applied at four doses (0, 1, 2, and 4 t ha^{-1}) during soil preparation. Maize (*Zea mays* L.) seeds of a uniform hybrid variety were selected for planting in pots. Plastic pots (20 cm diameter × 30 cm height) were used as experimental units. A balanced NPK fertilizer (20-20-20) was applied at a rate 400 kg ha^{-1} to ensure adequate nutrient availability. Regular irrigation, weeding, and other intercultural operations are done as per the maize production manual. Standard tools (trowel, pH/EC meters, spectrophotometer) were employed for soil preparation and measurements (Miller & Kissel, 2010; Parry et al., 2014; Rinawati et al., 2020; Tagami & Uchida, 2025).

2.3. Isolation and identification of bacterial isolate

Bacteria were isolated from the collected soil of the rhizosphere of indigenous plants at *Al Mahd Ad Dhahab* using the serial dilution method. An Isolated inoculant (PGPB consortium) from *Al Mahd Ad Dhahab,* a gold mining company in Medina, Saudi Arabia was prepared for treatment. The bacterial isolate was used in this study were identified according to their morphological, cultural and physiological characteristic as a sated in Bergey’s Manual of Systematic Bacteriology (Belov et al., 2018; Masi et al., 2021).

2.4. Experimental design

A split-plot design with three replications was implemented, totalling 24 pots. The main plot factor was FA dose (0, 1, 2, and 4 t ha^{-1}), while the subplot factor was bacterial inoculation (with and without PGPB). The soil was homogenized with peat moss, and the FA was thoroughly mixed into the respective pots. Maize seeds were sown, and PGPB inoculant was applied according to the experimental layout. The experiment was conducted under controlled conditions to minimize external variability. PGPB isolation grows cultures on Luria Bertani media, then we take PGPB isolates from the Luria Bertani media, then grow them on liquid broth media, after 24 hours, applying them to the soil on the plants a week after planting.

2.5. Growth media management

To optimize drainage and moisture retention the sandy loam soil and peat moss mixture was carefully maintained at the ratio 1:1. FA was incorporated at predetermined rates, and NPK fertilizer was uniformly mixed into all pots. Maize seeds were treated against seed-borne pathogens before sowing. For PGPB-treated groups, a bacterial suspension was applied at the root zone during planting. Pots were arranged randomly to avoid positional bias and watered uniformly to maintain field capacity.

Soil pH and electrical conductivity (EC) were measured during pre and post-harvest to assess soil alkalinity and salinity changes. Plant health metrics including Leaf Area Index (LAI) were quantified using a leaf area meter; chlorophyll a, chlorophyll b, and carotene levels were analysed via spectrophotometry and fresh and dry biomass yield were measured during post-harvest to determine growth efficiency. The PGPB was cultured in nutrient broth, centrifuged, and resuspended in sterile saline. A 10^8 CFU mL^{-1} suspension was applied to maize rhizospheres at sowing and 15 days post-germination. Control groups received an equal volume of sterile water. Bacterial viability was confirmed through plate counts before application.

2.6. Data analysis

Data were analysed using two-way ANOVA to evaluate the effects of FA dose, bacterial treatment, and their interactions. Tukey’s HSD test ($p< 0.05$) was applied for mean separation. All analyses were performed in R software (v4.0.2), with assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene’s test) verified. Three biological replicates per treatment ensured statistical robustness. Instruments were calibrated prior to measurements, and blank samples were included in the spectrophotometric analyses. Harvested plant parts were dried in an oven at 60-70°C for 48-72 hours and weighed again to determine the dry biomass. Metal analysis was performed using EPA Method 3050B for acid digestion, with certified reference materials (NIST SRM 2711a) employed for quality assurance.

3. RESULTS

3.1. Identification of the bacterial isolate used in this study

Identification of isolated bacteria was carried out using the following characters: shape of cells, motility, Gram staining reaction, aerobiosis, spore forming, pigmentation, hydrolysis of casein, gelatin liquefaction, acetyl methyl carbinol production (VP test), reduction of methyl red (MR), nitrate reduction, starch hydrolysis, levan production, hydrogen sulfide production, indole production, utilization of carbon compounds (glucose, fructose, sucrose, lactose, galactose, arabinose, mannose and raffinose). Based on the morphological cultural physiological and pathological characteristics of the isolated bacteria and according to those reported by Belov et al. (2018) and Masi et al. (2021). It could be concluded that all the tested isolates could be identified as *Bacillus* sp.

3.2. Effect of FA and PGPB on fresh weight of root, stem, leaf and biomass in maize

Table 2 presents the treatment structure and analysis of variance for the field trial including fresh weights of root, stem, leaf and biomass in maize. FA and PGPB had a highly significant ($P \leq 0.01$) effect on the fresh weight of root, stem, leaf and total biomass. For 2-way interactions between FA and PGPB, a highly significant ($P \leq 0.01$) effect was also observed on the fresh weight of root, stem, leaf and total biomass. Table 3 presented that application of FA 1 t ha^{-1} significantly ($P \leq 0.05$) increased fresh root weight by 3.51%, stem weight by 3.49%, leaf weight by 3.50%, and total biomass by 3.51%, compared to control (0 t ha^{-1}). Besides that, application of FA 2 and 4 t ha^{-1} significantly ($P \leq 0.05$) reduced fresh root weight by 10.39% and 18.39%, stem weight by 10.40% and 18.38%, leaf weight by 10.41% and 18.36%, and total biomass weight by 10.39% and 18.41%, compared to control (FA 0 t ha^{-1}). However, applications of PGPB significantly ($P \leq 0.05$) increased fresh root weight by 11.62%, stem weight by 11.63%, leaf weight by 11.59%, and total biomass weight by 11.58%, compared to the control (without PGPB). The application of FA at 1 t ha^{-1} significantly ($P \leq 0.05$) improved the weights of fresh root, stem, leaf, and total biomass weights, indicating its positive effect at lower doses. However, higher rates of FA (2 and 4 t ha^{-1}) led to a notable reduction in biomass, suggesting potential toxicity or adverse effects at elevated levels. In contrast, the PGPB application consistently enhanced all growth parameters, highlighting its beneficial role in promoting plant biomass and overall growth performance.

The interactive effect of FA and PGPB showed significant ($P \leq 0.05$) improvement in fresh biomass. Figure 1 showed that FA 1 t ha^{-1} in conjunction with bacteria significantly ($P \leq 0.05$) increased fresh biomass by 1.57% compared to control (FA 0 t ha^{-1}), while FA 2 and 4 t ha^{-1} reduced fresh biomass by 15.64% and 19.94% respectively, compared to 0 t ha^{-1}. Similarly, FA 1 t ha^{-1} in the absence of bacteria significantly ($P \leq 0.05$) increased fresh biomass by 7.74% compared to 0 t ha^{-1}, while FA 2 and 4 t ha^{-1} reduced fresh biomass by 4.17% and 17.14 % respectively, compared to 0 t ha^{-1}.

3.3. Effect of FA and PGPB on dry weight of root, stem, leaf and biomass in maize

Table 4 presents the treatment structure and analysis of variance for the field trial examining the dry weights of root, stem, leaf, and biomass in maize. Treatments FA, PGPB, and the interaction between FA and PGPB applications had highly significant ($P \leq 0.01$) effects on the dry weight of root, stem, leaf, and biomass in maize. The interaction between FA and PGPB had a strong impact, suggesting that their combined use may have a synergistic or additive effect on plant growth.

Table 2. Analysis of variance of fresh weight of root, stem, leaf and total biomass (gm) of maize under the effects of fly ash and PGPB

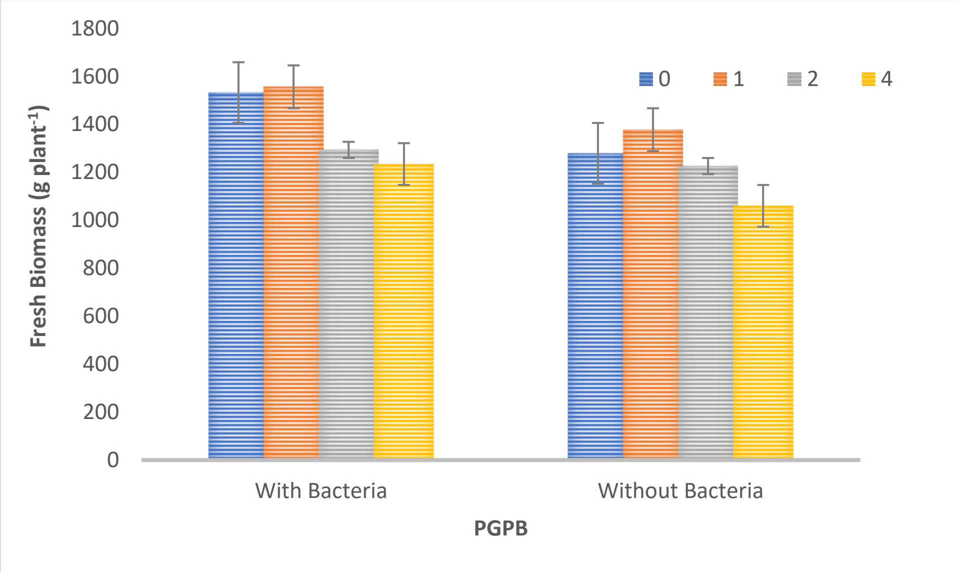
Source of Variation	DF	Mean of Square			
		Root	Stem	Leaf	Biomass
Rep	2	18.37	331.89	186.68	1308.11
Fly Ash	3	1660.81**	29998.55**	16874.81**	118237.73**
Error (a)	6	8.98	162.31	91.26	639.63
PGPB	1	2223.37**	40159.71**	22590.91**	158288.03**
Fly Ash * PGPB	3	122.48**	2212.40**	1244.53**	8720.11**
Total Error	8	7.83	141.49	79.59	557.68

Remarks: **: Significant at $p \leq 0.01$; *: Significant at $p \leq 0.05$.

Table 3. Effects of fly ash and PGPB on fresh weight of root, stem, leaf and biomass of maize

Treatments	Fresh Weight (g plant^{-1})			
	Root	Stem	Leaf	Biomass
Fly Ash (t ha^{-1})				
0	166.66 b	708.33 b	531.25 b	1406.25 b
1	172.50 a	733.12 a	549.84 a	1455.47 a
2	149.33 c	634.66 c	476.00 c	1260.00 c
4	136.00 d	578.00 d	433.50 d	1147.50 d
$LSD_{0.05}$	4.23	17.99	13.49	35.72
PGPB				
Without PGPB	146.50 b	622.62 b	466.97 b	1236.09 b
With PGPB	165.75 a	704.43 a	528.33 a	1398.51 a
$LSD_{0.05}$	2.63	11.19	8.39	22.23

Remarks: Means having similar letter(s) are not significantly ($p \leq 0.05$) different from each other using Fisher's LSD.



Notes: Bars indicate ± standard error, and error bars that are not overlapping each other indicate that the means are significantly ($p \leq 0.05$) different from each other.

Figure 1. Interaction effects of fly ash and PGPB on fresh biomass of maize

Table 4. Analysis of variance of dry weight of root, stem, leaf and total biomass of maize under the effects of fly ash and PGPB

Source of Variation	DF	Mean of Square			
		Root	Stem	Leaf	Biomass
Rep	2	1.77	20.76	6.21	70.23
Fly Ash	3	160.14**	1877.81**	562.04**	6351.16**
Error (a)	6	0.86	10.16	3.03	34.36
PGPB	1	214.32**	2514.33	752.41**	8501.30**
Fly Ash * PGPB	3	11.76**	138.57*	41.46**	468.35**
Total Error	8	0.75	8.85	2.64	29.95

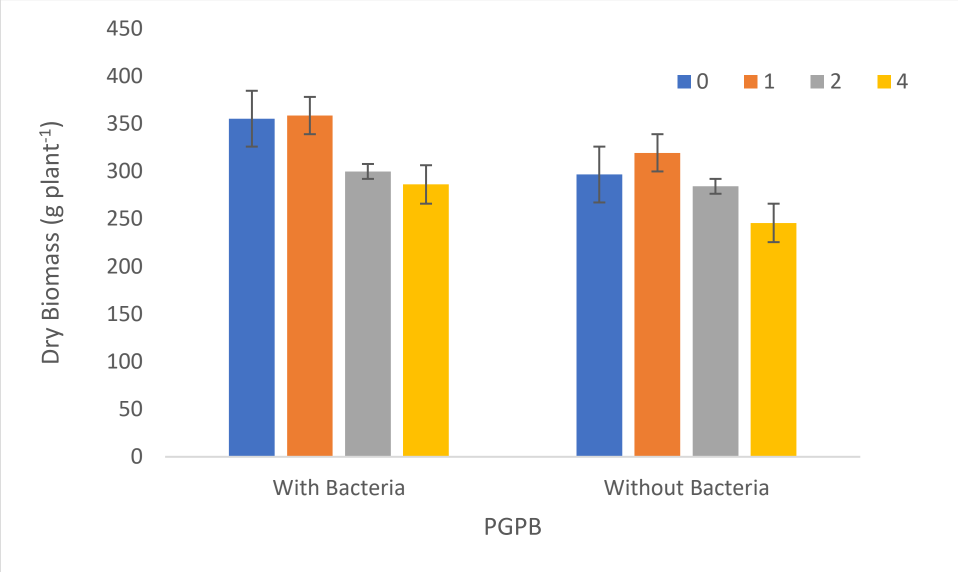
Remarks: **: Significant at $p \leq 0.01$; *: Significant at $p \leq 0.05$.

Table 5. Effects of fly ash and PGPB on dry weight of root, stem, leaf and biomass of maize

Treatments	Dry Weight (g plant^{-1})			
	Root	Stem	Leaf	Biomass
		Fly Ash (t ha-^{1})		
0	49.75 b	171.22 b	96.95 b	322.92 b
1	53.56 a	183.42 a	100.34 a	337.33 a
2	46.36 c	158.79 c	86.87 c	292.03 c
4	42.22 d	144.61 d	79.11 d	265.95 d
$LSD_{0.05}$	1.31	4.51	2.46	8.28
		PGPB		
Without PGPB	44.42 b	155.78 b	85.22 b	285.49 b
With PGPB	51.46 a	176.25 a	99.42 a	324.13 a
$LSD_{0.05}$	0.81	2.80	1.53	5.15

Remarks: Means having similar letter(s) are not significantly ($p \leq 0.05$) different from each other using Fisher's LSD.

Table 5 presented that application of FA 1 t ha^{-1} significantly ($P \leq 0.05$) increased dry root weight by 7.65%, stem weight by 7.12%, leaf weight by 3.49%, and total biomass weight by 4.46%, compared to control (FA 0 t ha^{-1}). Besides that, the application of FA 2 and 4 t ha^{-1} significantly ($P \leq 0.05$) reduced dry root weight by 6.81% and 15.13%, stem weight by 7.25% and 15.54%, leaf weight by 10.39% and 18.41%, and total biomass by 9.56% and 17.64%, compared to control (FA 0 t ha^{-1}). However, the application of PGPB significantly ($P \leq 0.05$) increased dry root weight by 15.84%, stem weight by 13.14%, leaf weight by 16.66%, and total biomass by 13.53%, compared to the control (without PGPB). Application of FA 1 t ha^{-1} positively influenced dry biomass accumulation, especially in roots and stems, suggesting improved plant growth at this level. However, higher FA doses (2 and 4 t ha^{-1}) negatively impacted dry weights across all plant parts, indicating potential stress or toxicity. In contrast, the PGPB application markedly enhanced dry biomass in all components, confirming its role in promoting plant health and growth under the tested conditions.



Notes: Bars indicate ± standard error, and error bars that are not overlapping each other indicate that the means are significantly ($p \leq 0.05$) different from each other.

Figure 2. Interaction effects of fly ash and PGPB on dry weight of biomass of maize.

The interactive effect of FA and PGPB resulted in a significant improvement in the dry biomass of maize. Figure 2 showed that FA 1 t ha^{-1} in conjunction with bacteria significantly ($P \leq 0.05$) increased dry biomass by 0.94% compared to 0 t ha^{-1}, while FA 2 and 4 t ha^{-1} reduced dry biomass by 15.59% and 19.45% respectively compared to 0 t ha^{-1}. Similarly, FA 1 t ha^{-1} in the absence of bacteria significantly ($P \leq 0.05$) increased dry biomass by 7.74% compared to 0 t ha^{-1}, while FA 2 and 4 t ha^{-1} reduced dry biomass by 4.24% and 17.39 % respectively compared to 0 t ha^{-1}.

3.4. Effect of FA and PGPB on leaf chlorophyll a, chlorophyll b, and carotene and leaf area of maize

Table 6 shows the treatment structure and analysis of variance of the findings from leaf chlorophyll a, chlorophyll b, carotene and leaf area of maize plants. Treatment FA had significant ($P \leq 0.01$) effects on chlorophyll a, chlorophyll b, carotene and leaf area of maize. Treatment with PGPB had significant effects on chlorophyll b, carotene and leaf area of maize but it had no significant effect on chlorophyll a. The interaction between FA and PGPB also had significant impact on chlorophyll a, carotene and leaf area while it had no significant effect on chlorophyll b. Results showed that the combined application of FA and PGPB showed a synergistic effect on chlorophyll a, carotene, and leaf area, though it did not significantly influence chlorophyll b. These results suggest that integrating of FA and PGPB can be an effective strategy to enhance chlorophyll concentrations and leaf area in maize.

Table 7 presented that the application of FA 1 t ha^{-1} significantly ($P \leq 0.05$) increased chlorophyll a by 3.91%, chlorophyll b by 20.13%, carotene by 6.12%, and leaf area by 5.81%, compared to control (FA 0 t ha^{-1}). Besides that, the applications of FA 2 and 4 t ha^{-1} significantly ($P \leq 0.05$) reduced chlorophyll a by 10.24% and 18.53%, chlorophyll b by 3.87% and 9.03%, carotene by 8.16% and 16.32%, and leaf area by 8.39% and 16.57%, compared to control (FA 0 t ha^{-1}). However, application of PGPB significantly ($P \leq 0.05$) increased chlorophyll a by 13.33%, chlorophyll b by 13.15%, carotene by 11.36%, and leaf area by 13.13%, compared to the control (without PGPB). These results suggest that moderate levels of FA and bacterial inoculation can improve physiological parameters in maize, but excessive FA may hinder leaf area development. These results indicate that moderate FA application combined with bacterial inoculation can enhance chlorophyll synthesis and improve plant physiological performance.

Table 6. Analysis of variance of chlorophyll a, chlorophyll b and carotene (mg g^{-1} FW) and leaf area of maize under the effects of fly ash and PGPB

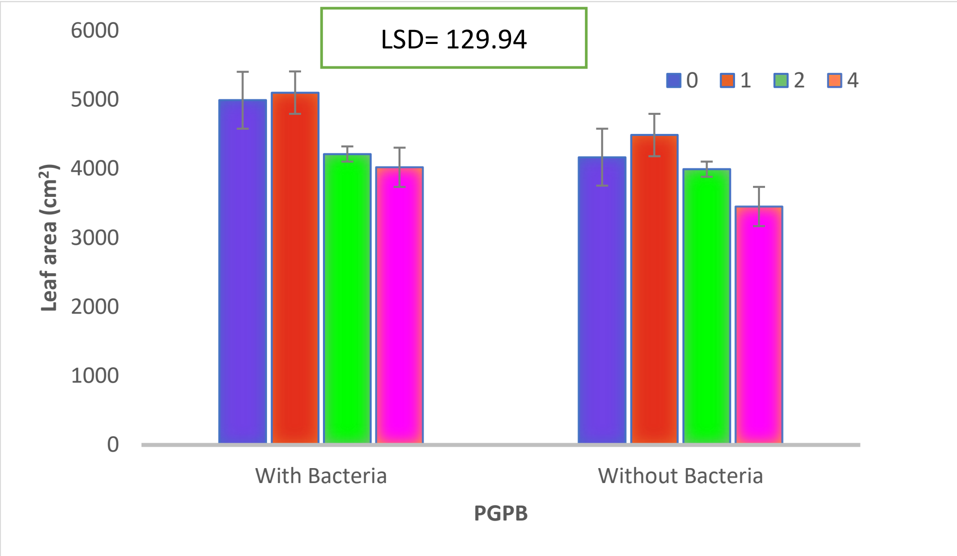
Source of variation	DF	Mean of Square			
		Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Carotene (mg g^{-1})	Leaf area (cm^2)
Rep	2	0.01	0.01	0.00	13861.84
Fly Ash	3	0.25*	0.18**	0.02**	1252950.84**
Error (a)	6	0.01	0.01	6.250E-5	6779.34
PGPB	1	0.34	0.24**	0.02**	1677348.33**
Fly Ash * PGPB	3	0.02**	0.01	0.01**	92406.87**
Total Error	8	0.01	0.01	0.00	5909.48

Remarks: **: Significant at $p \leq 0.01$; *: Significant at $p \leq 0.05$.

Table 7. Effects of fly ash and bacteria on chlorophyll a (mg g^{-1}), chlorophyll b (mg g^{-1}) and carotene (mg g^{-1}) and leaf area of maize

Treatments	Chlorophyll A (mg g^{-1})	Chlorophyll B (mg g^{-1})	Carotene (mg g^{-1})	Leaf area (cm^2)
Fly Ash (t ha^{-1})				
0	2.05 b	1.49 b	0.49 b	4477.77 b
1	2.13 a	1.79 a	0.52 a	4738.00 a
2	1.84 c	1.55 c	0.45 c	4101.68 c
4	1.67 d	1.41 d	0.41 d	3735.46 d
$LSD_{0.05}$	0.05	0.04	0.01	116.32
PGPB				
Without PGPB	1.80 b	1.52 b	0.44 b	4023.86 b
With PGPB	2.04 a	1.72 a	0.49 a	4552.59 a
$LSD_{0.05}$	0.03	0.02	0.01	72.37

Remarks: Each value is a mean of 3 replicates. Means within the same column and having similar letter(s) are not significantly ($p \leq 0.05$) different from each other using Fisher's LSD.



Notes: Bars indicate ± standard error, and error bars that are not overlapping each other indicate that the means are significantly ($p \leq 0.05$) different from each other

Figure 3. Interaction effects of fly ash and PGPB on leaf area of maize

The interactive effect of FA and PGPB showed significant improvement in the leaf area of maize. Figure 3 showed that FA 1 t ha^{-1} in conjunction with PGPB significantly ($P \leq 0.05$) increased leaf area by 2.21% compared to 0 t ha^{-1}, while FA 2 and 4 t ha^{-1} reduced leaf area by 19.99% and 30.82% respectively compared to 0 t ha^{-1}. Similarly, FA 1 t ha^{-1} in the absence of bacteria significantly ($P \leq 0.05$) increased leaf area by 7.69% compared to 0 t ha^{-1}, while FA 2 and 4 t ha^{-1} reduced leaf area by 4.17% and 17.14% respectively compared to 0 t ha^{-1}. Findings revealed that low-dose FA (1 t ha^{-1}), especially with PGPB, significantly ($P \leq 0.05$) improved maize leaf area, indicating a synergistic effect. In contrast, higher FA levels (2 and 4 t ha^{-1}) reduced leaf area, suggesting potential negative impacts at excessive doses.

3.5. Effect of FA and PGPB on soil PH and EC

Table 8 shows the treatment structure and analysis of variance of the soil PH and EC after application of treatments. Treatment FA had significant impact on the soil PH and EC of the soil of maize cultivation. Treatment PGPB had significant effects on soil EC, but it had no significant effect on soil PH. The interaction between FA and PGPB also had significant effects on soil EC and significant effects on soil PH. The combined application of FA and PGPB significantly influenced soil properties, showing a considerable reduction in soil electrical conductivity (EC) and soil pH. This indicates that their interaction can effectively improve soil chemical conditions, potentially enhancing nutrient availability and soil health for maize cultivation. Table 9 presents that the application of FA at 4 t ha^{-1} significantly ($P \leq 0.05$) increased soil PH by 19.03%, 6.18% and 6.03% compared to 0, 1 and 4 t ha^{-1} respectively. Similarly, FA 4 t ha^{-1} significantly ($P \leq 0.05$) increased soil EC by 34.60%, 22.19% and 7.61% compared to 0, 1 and 2 t ha^{-1} respectively. However, application of PGPB increased soil PH by 1.76% and soil EC by 5.55% respectively compared to the control (without PGPB). These results suggest that moderate levels of FA and bacterial inoculation can improve soil properties, but excessive FA may hinder soil health.

Table 8. Analysis of variance of soil PH and EC of maize under the effects of fly ash and PGPB

Source of variation	DF	Mean of Square	
		pH	EC
Rep	2	0.07	0.01
Fly Ash	3	1.46**	1.40**
Error (a)	6	0.03	0.01
PGPB	1	0.08	0.23**
Fly Ash * PGPB	3	0.42*	0.11**
Total Error	8	0.08	0.01

Remarks: **: Significant at $p \leq 0.01$; *: Significant at $p \leq 0.05$.

Table 9. Effects of fly ash and bacteria on soil PH and EC of maize

Treatments	pH	EC
	Fly Ash (t ha^{-1})	
0	6.20 c	3.15 d
1	6.95 b	3.47 c
2	6.96 b	3.94 b
4	7.38 a	4.24 a
LSD0.05	0.26	0.06
	PGPB	
Without PGPB	6.81 a	3.60 b
With PGPB	6.93 a	3.80 a
LSD0.05	0.26	0.096

Remarks: Means having similar letter(s) are not significantly ($p \leq 0.05$) different from each other using Fisher's LSD

4. DISCUSSION

Our study investigated the synergistic effects of integrating FA and PGPB in maize (*Zea mays* L.) cultivation to enhance plant productivity while mitigating the toxic effects of heavy metals. The findings revealed a positive interaction between FA and PGPB activity in plant growth and soil chemistry. Although FA, a residue from coal combustion is viewed as challenging waste due to its high pH, salinity, and heavy metal content, it can be converted into organic matter for plants. It was observed that, during individual application of FA at low doses (1 t ha^{-1}), it can serve as a valuable soil amendment. The results revealed that, FA with 1 t ha^{-1} significantly improved maize growth parameters including increased fresh and dry biomass as well as chlorophyll content. The findings have similarity with (Hussain et al., 2024) where they stated that, lower rates (2 t ha^{-1}) of FA showed an increase in plant height, photosynthetic pigment and dry biomass in maize. Panda et al. (2015) also observed that, plant growth mainly was enhanced in the treatments with 20–40% FA. Besides that, the alkaline nature of FA helps to neutralize the acidic soils, while its mineral content such as calcium and magnesium improves nutrient availability. Ram and Masto (2010) also concluded that FA enhanced nutrient availability in the applied soil. These benefits were most distinct when FA was combined with PGPB, creating a positive relationship.

Our findings highlighted the importance of combining the application of FA and PGPB in enhancing maize growth and soil productivity. At the best FA dose of 1 t ha^{-1}, PGPB-inoculated maize exhibited up to 18.41% increase in fresh biomass and 17.64% increase in dry biomass compared to controls. Similar findings were observed by Chandrakar et al. (2015); Kumar and Patra (2013), and Nayak et al. (2015). Likewise, chlorophyll A, chlorophyll B, and carotenoids, also increased by 10-13%, reflecting improved photosynthetic efficiency. Results showed similarity with those of Haris et al. (2021). Treatments also contributed to increasing the leaf area of plants. Research findings indicated that PGPB not only improved nutrient uptake but also strengthened the plant's performance.

We also observed that the benefits of FA are not linear in trend. As the application rate increased more than 2 t ha^{-1}, phytotoxicity begins to emerge in this situation. The findings may be because higher EC and pH levels with increased concentrations of heavy metals created an unfavorable environment for plant growth. Similar results observed by Panda et al. (2015) concluded that with higher concentrations of FA, led to a reduction in the measured parameters in maize. Likewise, biomass and chlorophyll content declined by 15–22% at 4 t ha^{-1} FA, indicating that the stress due to excessive FA compensated for its nutritional benefits. Dinssa and Elias (2021) reported that soil nutrient fixation also affected by the pH of the soil. These findings highlighted the existence of a toxicity threshold, beyond which FA becomes detrimental rather than beneficial. Wang et al. (2020) reported that higher doses of FA cause harmful effects in plants. The presented results showed that PGPB continued to exert a protective effect even under these high-stress conditions, while PGPB could not fully reverse the damage caused by excessive FA, they did partially repair in key growth parameters. Gupta and Pandey (2023) observed that PGPB promotes plant growth and alleviates stress from heavy metals and nutrient deficiency. We presented that root biomass remained higher in PGPB inoculated plants, along with a higher leaf area. It indicates that PGPB can buffer plants against environmental stressors by reducing the toxic effects of metals through siderophore production or by altering metal uptake pathways. These findings are supported by Huo et al. (2021).

The results revealed that the interaction between FA and PGPB had a significant effect on improving soil health. FA applications increased soil pH and EC, particularly at higher doses, which could negatively affect microbial diversity and nutrient availability. However, the presence of PGPB appeared to moderate these changes, which helps maintain balanced soil conditions. This microbial modulation of soil properties is essential for long-term sustainability, as it helps prevent the degradation of soil structure and function. Usmani et al. (2019) reported that a gradual increase in FA improved the physico-chemical properties, enzymatic activities, microbial biomass, carbon and microbial populations in soil. In contrast, these findings align with previous studies that have documented the dual role of PGPB in promoting plant growth while mitigating environmental stress. For instance, Azeem et al. (2022) reported similar microbial resilience in metal-contaminated soils, whereas Ram and Masto (2010) identified a range of 1–2 t ha^{-1} of FA as optimal for crop productivity. The current study concludes that the integration of FA and PGPB is effective, and scalable, with appropriate treatments.

Despite these promising results, several limitations should be taken into consideration during the application of FA. The composition of FA can vary significantly depending on its source which affects its chemical and physical properties as described by Alterary and Marei (2021). Additionally, the efficacy of PGPB may be influenced by environmental factors such as temperature, moisture, and soil type, which can limit its performance under field conditions. Additionally, the results concluded that PGPB enhanced the positive effects of FA while mitigating its negative effects. These beneficial microbes are known for their ability to solubilize phosphates and produce phytohormones through ACC deaminase activity. PGPB likely enhanced metal tolerance via siderophore-mediated Fe acquisition and ACC deaminase activity, reducing ethylene stress. Usman et al. (2023); Pattnaik et al. (2021); and Li et al. (2022) reported similar findings to our results.

We concluded that the combined use of FA and PGPB in maize cultivation would be an effective way to increase the availability of organic matter, which could bring sustainability in agriculture and environmental remediation. Our results demonstrated that low-dose FA, when paired with microbial inoculants, could improve plant growth, increase soil fertility and reduce the toxic effects of heavy metals. However, success depends on accurate dosing and careful monitoring of the plant conditions. With the right safety measures and inventions, FA can be transformed from an environmental burden into a valuable agricultural resource, and PGPB can serve as the biological bridge that makes this transformation possible.

5. CONCLUSION

This pot study confirms that the integrated application of low-dose FA (1 t ha^{-1}) in conjunction with PGPB significantly enhances maize growth, improves photosynthetic capacity, and mitigates heavy metal stress through microbial mechanisms. While higher doses of FA (≥2 t ha^{-1}) resulted in phytotoxic effects due to elevated pH, salinity, and heavy metal concentrations, the 1 t ha^{-1} treatment emerged as the optimal dose for balancing soil fertility enhancement and metal immobilization. These findings highlight the potential of FA-PGPB integration as a sustainable and circular approach to repurpose industrial waste for agricultural benefit. This strategy is particulary relevant for FA-contaminated regions such as Rabigh, Saudi Arabia, where disposal challenges persist. However, to ensure safe and scalable applications, further research might focus on long-term field trials, economic viability assessments, optimization of bacterial consortia, and the development of regulatory frameworks. FA and PGPB offer a promising pathway for enhancing crop resilience and advancing sustainable agriculture in polluted environments.

Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear to influence the work reported in this paper.

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