Xplore Environment
An International Journal

OPEN ACCESS

ISSN 3117-6143
Original Research Article

Check for updates

Organic Carbon Sequestration and Air Pollutant Retention Efficiency of Maize (*Zea mays* L.) and Biofertilizers (Azotobacter and Mycorrhizal Fungi) in Industrial and Uncontaminated Areas

Jassim Wahab Mohammed AL-Yessary*[1], and Sanaa Khadem Abdul-Amir Al-Rubaie[2]

[1]*Ministry of Education / Directrate education of kerbala, Iraq*
[2]*Ministry of Education / Open Education College – Karbala Center in Iraq*

Corresponding author: **Jassim Wahab Mohammed AL-Yessary** | E-mail: **jassim.w@suokerbala.edu.iq**

Citation: Jassim Wahab Mohammed AL-Yessary, and Sanaa Khadem Abdul-Amir Al-Rubaie (2026). Organic Carbon Sequestration and Air Pollutant Retention Efficiency of Maize (*Zea mays* L.) and Biofertilizers (Azotobacter and Mycorrhizal Fungi) in Industrial and Uncontaminated Areas. *Xplore Environment: An International Journal.* **DOI: https://doi.org/10.51470/XE.2026.6.1.88**

Received 13 March 2026 | Revised 15 April 2026 | Accepted May 18 2026 | Available Online June 16 2026

Abstract

To investigate the effect of biofertilizers containing isolated Azotobacter fungi and mixed with other fungi on soil organic carbon sequestration, air pollutant retention, plant growth and yield improvement of maize (Zea mays L.), an experiment was conducted in two different fields (uncontaminated and industrial) in the summer of 2025. The results indicated that combined inoculation (B3) was significantly better than single inoculation and control in all traits studied. In the uncontaminated and contaminated plots, the content of soil organic carbon reached 13.2 g kg-1 and 11.9 g kg-1, respectively, which accounts for a higher increase of 57.0% and 56.6% compared to the content of soil organic carbon in the control treatment (8.4 g kg-1 and 7.6 g kg-1, respectively). The dust retention efficiency was also high for co-pollination, where the dust deposition rate was 2.68 mg cm^{-2} (uncontaminated plants) and 7.92 mg cm^{-2} (contaminated plants). Thus, the concentration of both lead and cadmium in plant tissues has significantly reduced. In contaminated areas, the lead decreased from 19.8 mg kg^{-1} to 8.7 mg kg^{-1} (a 56.1 percent decrease) and the cadmium decreased from 1.92 mg kg^{-1} to 0.79 mg kg^{-1} (decrease of 58.9 percent). Biochemical properties also showed improvement through co-pollination with total chlorophyll content rising to 2.68 and 2.29 mg g^{-1} in uncontaminated and contaminated areas, respectively, as compared to 15.6 and 13.8, respectively, in the control areas; and Air Pollution Tolerance Index (APTI) increasing to 20.6 and 18.7 in uncontaminated areas and contaminated areas, respectively, as compared with 15.6 and 13.8 in the control areas, respectively. This beneficial influence was also seen in growth and yield parameters such as plant height (183 mm) and yield (164 cm); ground dry weight in uncontaminated areas was 148 g plant-1 while it was 118 g plant-1 in contaminated areas. The yield of the grain was 168 g plant-^{1} in uncontaminated regions and 128 g plant-^{1} in polluted ones. The relative yield response was greater in the industrial pollution situation with a 121% increase compared to 75% in uncontaminated situation, suggesting that co-pollination efficiency is higher under increasing environmental stress. The keywords used are Maize, Azotobacter, Mycorrhizal fungi, Biofertilizer, Organic carbon sequestration, Air pollution tolerance index (APTI).

Keywords: *Organic carbon sequestration, Biofertilizers, Mycorrhizal fungi, Rhizosphere, Plant growth.*

1. Introduction

Maize (Zea mays L.) is a major cereal crop in the world and in the region, not only for its yield, but also its biomass productivity, as it is part of the C4 photosynthetic system, and because it is a key crop for food and feed security. But, industrial air pollution – especially dust and heavy metals like lead and cadmium – have a negative effect on the crop's photosynthetic efficiency. This is because the ions of these elements replace the magnesium in the chlorophyll molecules and this disrupts the photosynthetic system. A detailed study by [1]. revealed that in maize, exposure to lead and cadmium led to a significant reduction in the Robisco and D1 protein content and affected the chloroplast microstructure. Biofertilizers employing plant promoting microorganisms have become an important means of sustainable agriculture by meeting these challenges without excessive use of chemical fertilizers. These microorganisms include the free-living aerobic microorganisms of the genus Azotobacter spp. These microorganisms have the ability to biologically fix atmospheric nitrogen via nitrogen-fixing enzymes (nitrogenases) and convert it into a form that plants can absorb.

© ***Authors:*** *Published in* ***Xplore Environment: An International Journal*** *under the CC BY-NC-ND 4.0 license (http://creativecommons.org/licenses/by-nc-nd/4.0/). No commercial use or modifications permitted.*

Plant growth regulators (PGRs) are secreted by these microorganisms, mostly indole-3-acetic acid (IAA), which improves root architecture and nutrient uptake, while these organisms also produce solubilizing compounds [2]. found that in this context, Azotobacter is a very important soil bio-enhancer and can greatly improve the nitrogen use efficiency (NUE) and stabilize the yield of nonleguminous crops. Moreover, the field trial experiment by [3]. on maize showed that inoculation with Azotobacter nigricans significantly improved the vegetative growth parameters and total yield of maize as compared to the uninoculated control, which was further increased by the application of balanced chemical fertilization.

At the same time, the arbuscular mycorrhizal fungi (AMF) colonize the roots of more than 80% of terrestrial plants. AMFs increase the efficiency of water and immobile elements uptake (especially phosphorus) by extensive extraradical hyphae networks and they immobilize toxic heavy metals, thus reducing their translocation to root tissues . Furthermore, [4] found that the use of AMFs and Bacillus subtilis (B. subtilis) as dual inoculants led to greater maize growth and nutrient uptake than the use of AMFs alone. Such synergism was put down to a multi-functional integration of biological nitrogen fixation, phosphorus mobilization and an increased root exploration volume.

In the meantime, the Governorate of Karbala has seen environmental air pollution to be a serious problem due to the increasing industrial activities. power plants and brick factories. Industrial areas are expanding along the two sides of the province to the south and east. Among the most effective, least expensive, and important biological methods of trapping dust particles from the atmosphere and the heavy metals associated with them is the plants' large leaf surface area. But the efficiency of the plants varies with the species and physiological status [5]. suggested the Air Pollution Tolerance Index (APTI) which is used to assess plant tolerance to air pollution and is a composite index of four selected biochemical parameters: total chlorophyll content, ascorbic acid concentration, leaf extract pH, and relative water content (RWC). Based on this index, [6]. evaluated the tolerance of air pollution and dust retention in some plant species grown in industrial and non-industrial reference areas, and found that the amount of foliar dust and heavy metal were significantly higher in industrial areas compared to non-industrial areas.

The effects of bioinoculation of individual biofertilizers such as Azotobacter spp. or arbuscular mycorrhizal fungi (AMF) on maize growth and abiotic stress tolerance have been well studied but few studies have focused on the relative or comparative effect of single bioinoculants versus dual bioinoculants under field conditions of industrial pollution, especially in Iraq. Furthermore, few studies have related these biofertilization approaches to the capacity of the crops to sequester soil organic carbon and to bind atmospheric pollutants (gases and particulate matter as well as heavy metals).

The present study was therefore designed to assess the interactive effects of the cultivation site location (industrially polluted vs. unpolluted control) and biofertilizer regime (Azotobacter, mycorrhizal fungi, and their co-inoculation) on the growth parameters, yield performance, carbon sequestration capacity, and foliar pollutant capture efficiency of maize (*Zea mays* L.). Besides this investigation, it was evaluated how soil organic carbon (SOC) dynamics changed, the reduction of heavy metal accumulation in plant tissue, and the consequent modulation of the Air Pollution Tolerance Index (APTI).

2. Materials and Methods

2.2 Test Site, Test Period, and Test Unit

This experiment was conducted in the summer of 2025 in the province of Saint Karbala. The soil used was 15 kg plastic pots filled with homogeneous silty clay loam soil collected from a single source. This ensured consistency of basic physical and chemical properties in all treatments and allowed for the isolation of sitespecific influences from the effects of soil type variations. Immediately before planting, representative samples of the soil used for filling were taken and their basic physical and chemical properties were analyzed (Table 1). This established that the soil was homogenous and suitable for normal crop growth and not contaminated with metals distribution to the test treatments. The pots were placed in two environmental different locations. The first (S1) was an uncontaminated area, which is isolated from industrial emissions and heavy traffic sources (orchard in the Al-Hussainiya district), and the second (S2) was an industrially contaminated area, close to the Karbala refinery, south of the city of Karbala. To ensure authentic exposure to pollutants or to clean air, the pots in both sites were left exposed to ambient air during the growing season. To ensure there is always enough water for the irrigation, a specified quantity of fresh water from the Euphrates River was poured into the pots to keep the moisture level close to the water holding capacity of the soil in the pots, and to avoid leaching or surface runoff of dust that may have been attached to the leaves before the measurement date.

3.2 Plant Materials and Test Treatment

CADZ hybrid corn was used. Five seeds were planted in each pot on 20th August 2025 at a depth of 5cm. Thinning was done after germination to allow one healthy seedling per pot.

Two main factors were considered in this study. The first was the location of the pots, which were classified into two stages (S1: uncontaminated, S2: artificially contaminated). The second method involved the application of biological fertilizers, divided into four stages (B0: control group with no biological fertilizer, B1: inoculated with Azotobacter bacteria only, B2: inoculated with mycorrhizal fungal sporangia only, B3: inoculated with both Azotobacter bacteria and mycorrhizal fungi simultaneously).

Furthermore, this study considered two main factors. The first was the location where the pots were placed, which was classified into two stages (S1: uncontaminated, S2: artificially contaminated).

4-2 Acquilanti Isolates and Preparation

Soil samples were collected from the rhizosphere (root zone) of uncontaminated farmland in Karbala province at a depth of 0–15 cm and transported to the laboratory for isolation of Azotobacter using the serial dilution method. The dilutions were cultured on Ashby nitrogen-free mannitol agar, which inhibits the growth of bacteria that cannot fix atmospheric nitrogen, and incubated at 28°C for 5–7 days. The culture method followed the standard method described by [7]. Large, regularly bordered, myxoid colonies characteristic of the Azotobacter genus were selected and purified by repeated streaking onto the same culture medium. Initial characteristics were then examined by microscopic observation (oval to spherical Gram-negative cells) and identification biochemical tests (ammonia production, catalase activity, nitrate reduction, indole-acetic acid production). Selected isolates were grown in liquid Ashby medium at 28°C for 48 hours using a rotary shaker (150 rpm) until a cell density of approximately 10^8 cells/ml^1 was reached, after which they were used as biological infectants [2] ; [7].

5-2 Acquisition and Growth of Mycorrhizal Inoculum

Inoculum of shrub-borne vesicular mycorrhizal fungi was prepared using the trap culture method. Soil and root samples were collected from uncontaminated rhizospheres with protomycorrhizae and cultured together with trap host plants (the same maize and Sudanese sorghum) in containers with a mixture of sand and sterile soil under conditions intentionally lowered by phosphate concentration to promote fungal colonization. The growth cycle lasted approximately 10–12 weeks. Fungal spores were extracted from the soil using wet sieving and decantation. The extracted spores were first characterized by microscopic observation based on morphological characteristics (size, color, wall thickness). The final inoculum consisted of a mixture of spore-containing soil, root fragments in the soil, and hyphae, and was a natural mixture of several common genera, including Glomus and Rhizophagus. This method is used for the preparation of mycorrhizal inoculum for research and field applications, as described by [4].

6–2 Seed Treatment Method by Bioinoculation (Seed Coating)

Seeds were treated immediately before sowing according to the seed coating method described by [8] To inoculate corn with mycorrhizal fungi and growth-promoting bacteria, the seeds were mixed with an adhesive (acacia gum solution or carboxymethylcellulose CMC at a concentration of 5%) to ensure the inoculation adhered to the seed surface. Next, the seeds were coated with mycorrhizal fungal inoculation powder (for treatments B2 and B3) or immersed in a 10^8 cell mL^{-1} density Azotobacter bacterial suspension (for treatments B1 and B3). The treated seeds were then dried in the shade for 30 minutes immediately before planting to ensure microbial viability.

In the co-inoculation treatment (B3), the bacterial inoculation was first immersed in the seeds, followed immediately by the application of fungal pollen to ensure both groups adhered to the same adhesive layer on the seed surface [4]; [8].

7.2 Experimental Design and Statistical Analysis

The research was carried out in a Randomized Complete Block Design (RCBD) with six replications and one pot per experimental unit. The treatment was based on two study sites (S1, S2) and four biofertilizer regimes (B_0, B_1, B_2, B_3) which resulted in eight different treatments: S1B0, S1B1, S1B2, S1B3, S2B0, S2B1, S2B_2 and S2B3. A total of 48 pots were used, 24 pots per experimental site. To avoid destructive sampling interference:

Three replications were allocated to destructive measurements for dry biomass accumulation of plants. The remaining three replications were used for non-destructive spatial monitoring of plant height followed by final yield components.The data collected were analysed using two way analysis of variance (way ANOVA) based on factorial randomized complete block design (RCBD) to determine the main effects and interactions among the various treatments. For significant F-tests, the treatment means were compared with each other using Duncan's Multiple Range Test (DMRT) at 5% level of significance. Data was analysed by means of the standard statistical software.

Table 1: Physical and Chemical Properties of Soil Before Planting

Property	Unit	Value
Sand	g kg^{-1}	251
Silt	g kg^{-1}	379
Clay	g kg^{-1}	370
Soil texture	–	Silty clay loam
Soil pH (1:1)	–	7.6
Electrical conductivity (EC 1:1)	dS m^{-1}	2.354
Organic matter	g kg^{-1}	11.20
Organic carbon (before planting)	g kg^{-1}	6.5
Available nitrogen	mg kg^{-1}	35.64
Available phosphorus	mg kg^{-1}	7.8
Available potassium	mg kg^{-1}	149
Total calcium carbonate	g kg^{-1}	210
Bulk density	Mg m^{-3}	1.32
Available lead (Pb)	mg kg^{-1}	3.987
Available cadmium (Cd)	mg kg^{-1}	0.131

8-2 Measurement and Estimation Methods

- **Soil Fixed Organic Carbon:** The state of organic carbon (SOC) in post-harvest potting soil samples was estimated using the Walkley-Black method and expressed in g/kg soil units.
- Heavy metals were quantified and foliar dust deposition was measured. The foliar dust deposition and heavy metal quantification were performed.

The dust deposition rate on the leaves was calculated as the quantity of dust (mg) per unit area of leaf tissue (cm^2) with the collected leaves being washed in deionized water. Suspension was filtered into Whatman No. 42 filter paper, dried to a constant weight and gravimetrically quantified. The dried dust residue was subjected to digestion and the concentrations of lead (Pb) and cadmium (Cd) were determined by Atomic Absorption Spectrophotometry (AAS).

Screening for heavy metals in plant tissues. Heavy metal screening in plant tissues.

Dried leaf samples were finely powdered and digested by acid digestion with a 3:1 HNO_3:$HClO_4$ mixture (3:1, v/v). The real tissue level of Pb and Cd measured by AAS and reported in mg kg^{-1} DW. The evaluation of systemic metal bioaccumulation was done separating surface physical adsorption from internal physiological adsorption following the methodological approach of [1].

- **Air Pollution Tolerance Index (APTI):** The Air Pollution Tolerance Index (APTI) was determined by the biochemical approach suggested by [5] on the basis of four-leaf parameters:
- **Total Chlorophyll Content:** Measured spectrophotometrically after extraction with 80% acetone.
- **Ascorbic Acid Content:** Measuring the amount of ascorbic acid in the leaf extracts by titration using 2,6-dichlorophenolindophenol (DCPIP).
- **Relative Water Content (RWC%):** A standard fresh weight, turgid weight and dry weight measurement.
- **Leaf Extract pH:** pH was measured using a calibrated digital pH meter. The respective values were then added to the typical APTI equation for each experimental treatment.
- The plant growth and yield parameters are agronomic. The plant growth and yield parameters are agronomic.
- **Plant Dry Biomass (g cdot\plant^{-1}):** Shoots were harvested from soil surface, washed with deionized water, put in perforated paper bags and were oven dried at 70°C for 72 hours until constant weight was reached.
- **Plant Height (cm):** Height measured from the soil line to the apex of the growing point of three plants in each experimental unit and the average height taken.
- **Grain Yield (g cdo text plant $^{-1}$):** The grain of three plants per replicate was harvested and the grain yield was normalized to obtain means of individual plant grain yield.

3- Results and Discussion

1-3 Effects of Field and Biofertilizer on Soil Organic Carbon

The results in Table (2) show that mixed fertilization (B3) had a significant effect on the organic carbon content of the culture soil in both fields. The carbon content reached 13.2 g kg^{-1} in the treated area S1B3, a 57% increase compared to 8.4 g kg^{-1} in the uninoculated control area S1B0. On the other hand, in the contaminated field, the carbon content reached 11.9 g kg^{-1} in S2B3, a 56.6% increase compared to 7.6 g kg^{-1} in S2B0. These very similar rates of increase at both sites indicate that the efficiency of co-fertilization, which promotes organic carbon sequestration, is not significantly affected by the degree of contamination at the site, unlike other parameters such as yield. Compared to the baseline level of organic carbon in the soil before planting (6.5 g kg^{-1}, Table 1), recent studies have shown that inoculation with beneficial microorganisms, particularly rhizomatous fungi, increases soil organic carbon storage by increasing plant biomass and stabilizing organic matter [9]. All biofertilizer treatments clearly achieved increases above the baseline level, even in the control treatment in uncontaminated sites (S1B0: 8.4), due to the addition of root biomass and plant residues during the growing season. In contrast, the control treatment in contaminated sites (S2B0: 7.6) exceeded this baseline level by only a very small amount (1.1 g kg^{-1}), reflecting the effect of industrial pollution on inhibiting the activity of native microorganisms responsible for the carbon cycle in uninoculated soil. Furthermore, it is noteworthy that single mycorrhizal fungal treatment (B2) was more effective in increasing organic carbon at both sites than single azotobacter treatment (B1) (11.8 vs. 9.6 g/kg at S1, and 10.5 vs. 8.7 g/kg at S2). This is consistent with the direct role of the hyphae network extending beyond the roots in promoting the formation of stable soil aggregates, increasing organic carbon storage, protecting it from rapid decomposition, and improving the transfer of plant-derived carbon to the soil [10]. On the other hand, the role of is mainly limited to improving root biomass productivity through bionitrogen fixation, which is an indirect and relatively inefficient pathway for increasing organic carbon throughout the soil. The superiority of the interaction treatment (B3) over both individual treatments reflects a true functional integration between the two pathways: on the one hand, increased root biomass due to nitrogen nutrition, and on the other hand, increased density of the fungal carbon-fixing hyphae network, which is consistent with the general principle of integrating plant growth-promoting organisms to enhance the ecological function of the soil.

Table 2. Effect of Site and Biofertilization Treatments on Soil Organic Carbon Content

Treatment	Soil Organic Carbon (g kg^{-1})
S1B0	8.4 f
S1B1	9.6 d
S1B2	11.8 b
S1B3	13.2 a
S2B0	7.6 g
S2B1	8.7 e
S2B2	10.5 c
S2B3	11.9 b

Note: *Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at P ≤ 0.05.*

2-3 Effects of Location and Biofertilizer on Dust Deposition and Foliar Heavy Metal Concentrations

The results in Table 3) show that, in all biofertilizer treatments, plants in the contaminated industrial site (S2) exhibited a significant advantage in dust deposition rates and foliar lead and cadmium concentrations compared to the uncontaminated site (S1). Dust deposition rates reached 7.92 mg cm^{-2} in treatment S2B3, compared to only 2.68 mg cm^{-2} in the corresponding treatment S1B3. This indicates that deposition rates were approximately three times higher in the contaminated site despite identical biofertilizer treatment. This clearly reflects the true difference in air pollutant loads between the two locations.

A similar trend was observed for lead and cadmium concentrations in the deposited dust. In the contaminated site, under the same treatment conditions, lead concentrations increased by approximately 2.6–2.7 times, and cadmium concentrations increased by approximately three times. Within the same site, a significant stepwise increase in dust deposition rates and the concentrations of two heavy elements was observed as treatment progressed from control to Azotobacter treatment, mycorrhizal fungal treatment, and finally to combined treatment. For example, the dust deposition rate in the contaminated site increased from 5.42 mg/cm^2 of S2B0 to 7.92 mg/cm^2 of S2B3 (a 46% increase). This reflects the fact that improved nitrogen and phosphorus nutrition increases leaf area and vegetation density, playing a role in expanding the surface area for the deposition of suspended dust particles and the associated heavy elements, as shown in the reviews by [11] and [12]. This result supports the idea that the efficiency of surface dust capture by plants is more closely related to vegetation volume and leaf area size than to specific types of fertilizing organisms. In other words, treatments that promote plant growth inevitably increase surface dust capture, regardless of their specific biological mechanisms. However, it should be noted that, as the results in Table 3 below show, an increase in surface deposition of heavy elements does not necessarily mean a parallel increase in actual internal concentrations within plant tissues. This is because the dust deposited on the leaf surface represents external physical capture that is mechanically separated from internal physiological absorption through roots and stomata.

Table 3: Effect of Site and Biofertilization Treatments on Dust Deposition Rate and Heavy Metal Concentrations on the Leaf Surface

Treatment	Dust Deposition (mg cm^{-2})	Lead (Pb) (mg kg^{-1} dust)	Cadmium (Cd) (mg kg^{-1} dust)
S1B0	1.78 h	31.8 h	0.89 h
S1B1	2.15 g	37.1 g	1.03 g
S1B2	2.35 f	40.5 f	1.14 f
S1B3	2.68 e	46.2 e	1.32 e
S2B0	5.42 d	85.4 d	2.81 d
S2B1	6.68 c	98.7 c	3.24 c
S2B2	7.15 b	108.3 b	3.58 b
S2B3	7.92 a	118.6 a	3.92 a

Note: *Means followed by the same letter within each column are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).*

3-3 Effects of Site and Biological Fertilization on Heavy Metal Concentrations in Plant Tissue

The results in Table (4 show that all biological fertilization treatments significantly reduced the actual concentrations of lead and cadmium in plant tissue at both sites. Mycorrhizal fungal treatment (B2) was clearly superior to azotobacter treatment (B1) in this reduction, and interference treatment (B3) was even superior to the combined total. Internal lead concentrations decreased from 19.8 mg kg^{-1} in treated S2B0 to 15.6 mg kg^{-1} in S2B1, 11.2 mg kg^{-1} in S2B2, and to just 8.7 mg kg^{-1} in treated S2B3. This represents an overall reduction of 56% compared to the control treatment within the same contaminated site.

On the other hand, the reduction rate of internal cadmium concentration at the same site was 58.9% (1.92 to 0.79 mg kg^{-1}). In uncontaminated site S1, when comparing treatments B3 and B0, the reduction rate of lead was approximately 47% (3.4 to 1.8 mg kg^{-1}) and the reduction rate of cadmium was 51.7% (0.29 to 0.14 mg kg^{-1}). It is noteworthy that in contaminated sites, the reduction rates of both elements were slightly higher compared to uncontaminated sites. This indicates that the relative protective effect of biofertilizers that reduce internal absorption of heavy elements increases as the pollution load around plants increases. This is a logical consequence because the immobilization and biochelation mechanisms act on more free ions available in a more contaminated environment. This gradual change is explained by the fact that although the two protective mechanisms of the organism are different in nature, their effects are complementary. Mycorrhizal fungi, as confirmed by [13], directly sequester metals within mycelia and exohyphae. Azotobacter, on the other hand, contributes to reducing the likelihood of absorption through a different mechanism, including the secretion of side ophores and chelating compounds that bind to heavy metal ions in the periroot soil solution. This is further complicated by the dilution effect resulting from accelerated plant biomass growth by nitrogen fixation. This reduces the relative concentration per unit weight because the same amount of heavy metal is absorbed and dispersed into larger tissue masses. This finding is consistent with the study by [14], which showed that the reduction in actual internal concentrations of heavy metals directly affects the stability of photosynthetic pigments and the efficiency of the photosynthetic system. In B3 treatment, the combination of these two effects can explain why the bioprotective effect on plants is doubled, resulting in the lowest concentrations within the plant at both locations.

Table 4: Effect of Site and Biofertilization Treatments on Lead and Cadmium Concentrations in Shoot Tissues (mg kg^{-1} Dry Weight)

Treatment	Lead (Pb) in Shoot Tissues (mg kg^{-1} DW)	Cadmium (Cd) in Shoot Tissues (mg kg^{-1} DW)
S1B0	3.4 e	0.29 e
S1B1	2.9 f	0.24 f
S1B2	2.3 g	0.18 g
S1B3	1.8 h	0.14 h
S2B0	19.8 a	1.92 a
S2B1	15.6 b	1.51 b
S2B2	11.2 c	1.05 c
S2B3	8.7 d	0.79 d

Note: *Means followed by the same letter within each column are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).*

4-2 Effects of habitat and biofertilizer on biochemical components and air pollution tolerance index (APTI)

The results in Table (5) show that the total chlorophyll content and relative water content improved significantly with the transition from the control plot to the biofertilizer treatment plot, and the intervention treatment (B3) showed superior results at both sites.

Comparing treatments B3 and B0, total chlorophyll content increased by 40% (from 1.92 mg g^{-1} to 2.68 mg g^{-1}) at site S1 and by 48.7% (from 1.54 mg g^{-1} to 2.29 mg g^{-1}) at contaminated site S2, indicating that the relative response to biofertilizer was higher at the environmentally stressed site. On the other hand, ascorbic acid showed the opposite trend. The highest concentration of 10.8 mg g^{-1} was recorded in treatment S2B0, which gradually decreased with single and combined biofertilizers, reaching 7.2 mg g^{-1} in S2B3 (33.3% reduction). This reflects the actual reduction in oxidative stress reaching the plant cells as a result of the reduction in the internal concentration of heavy elements (Table 4) and the reduction in the production of free radicals by reaction with cellular components. This is a non-enzymatic defense response [15]. Despite this decrease in ascorbic acid, the biofertilizer significantly increased the overall APTI value, reaching the highest value of 20.6 in the S1B3 treatment (32% increase over S1B0), followed by 18.7 in S2B3 (35.5% increase over S2B0). This value was 19.9% higher than the unfertilized S1B0 treatment (15.6) despite being grown in a contaminated site. This confirms that co-fertilization with biofertilizer increases the plant's tolerance to air pollution to a level that exceeds that of plants without biofertilizer in a clean environment. This contrasting pattern between the four components of the APTI index (three components increased and ascorbic acid decreased) reflects the integrative nature of the index, which considers the multiple and sometimes conflicting contributions of its subcomponents. It also confirms that relying on a single component (e.g. ascorbic acid alone) can lead to conclusions that are quite different from the actual pollution tolerance of plants.

Table 5. Effect of Site and Biofertilization Treatments on Biochemical Traits and Air Pollution Tolerance Index (APTI)

Treatment	Total Chlorophyll (mg g^{-1})	Ascorbic Acid (mg g^{-1})	Relative Water Content (RWC) (%)	Leaf pH	APTI
S1B0	1.92 f	6.8 e	78.4 d	6.3 b	15.6 f
S1B1	2.15 d	6.3 f	81.2 c	6.4 ab	17.8 d
S1B2	2.51 b	5.9 g	83.6 b	6.5 a	19.3 b
S1B3	2.68 a	5.4 h	86.2 a	6.6 a	20.6 a
S2B0	1.54 h	10.8 a	67.1 h	5.6 d	13.8 g
S2B1	1.78 g	9.4 b	70.5 g	5.9 c	15.9 f
S2B2	2.02 e	8.1 c	73.2 f	6.0 c	17.1 e
S2B3	2.29 c	7.2 d	76.8 e	6.2 b	18.7 c

Note: *Means followed by the same letter within each column are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).*

5-3 Effects of Fields and Biofertilizers on Growth and Yield Characteristics

The results in Table (6) show that in both fields, the interaction treatment (B3) was significantly superior in all growth and yield characteristics investigated. In the uncontaminated field (S1), comparing treatments B0 and B3, plant height increased from 142 cm to 183 cm (28.9% increase), above-ground dry weight increased from 88 g to 148 g (68.2% increase), and grain yield increased from 96 g to 168 g (75% increase). In the contaminated field (S2), plant height increased from 118 cm to 164 cm (39% increase), dry weight increased from 64 g to 118 g (84.4% increase), and grain yield increased from 58 g to 128 g (121% increase). It is noteworthy that the relative response to co-fertilization was higher in contaminated areas compared to uncontaminated areas. This is consistent with recent research findings that plant growthpromoting microorganisms exert their greatest effects under environmental stress conditions by improving nutrient absorption, enhancing plant physiological activity, and mitigating the toxic effects of heavy metals [16]; [17]. Furthermore, in a comparison of treatments B1 and B2, mycorrhizal fungi were more effective than Azotobacter in improving grain yield in contaminated areas, with a yield of 98 g^{-1} for treatment S2B2 compared to 76 g^{-1} for treatment S2B1, a difference of approximately 29%. This is because rhizophytes have a positive impact on growth and productivity by reducing the transfer of heavy metals to plant tissues, in addition to their ability to improve the absorption efficiency of phosphorus and less mobile elements [18].The relative similarity between the results of treatment S2B3 (128 g $plant^{-1}$) and treatment S1B1 (118 g $plant^{-1}$) supports the idea that co-pollination can offset a significant portion of the adverse effects of industrial pollution, highlighting its importance as a sustainable strategy for improving maize productivity in degraded environments.

Table 6. Effect of Site and Biofertilization Treatments on Growth Traits and Grain Yield of Maize

Treatment	Plant Height (cm)	Shoot Dry Weight (g $plant^{-1}$)	Grain Yield (g $plant^{-1}$)
S1B0	142 f	88 f	96 f
S1B1	158 d	106 d	118 d
S1B2	171 b	126 b	142 b
S1B3	183 a	148 a	168 a
S2B0	118 h	64 h	58 h
S2B1	134 g	79 g	76 g
S2B2	149 e	95 e	98 e
S2B3	164 c	118 c	128 c

Note: *Means followed by the same letter within each column are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).*

Conclusion

1. Co-inoculation of Azotobacter and mycorrhizal fungi (B3) exhibited a true synergistic effect in both contaminated and uncontaminated areas, with this synergy particularly enhanced under metal stress conditions in contaminated areas.
2. All biofertilizer treatments increased dust capture efficiency on plant foliar surfaces by increasing leaf area, while simultaneously reducing lead and cadmium concentrations in plant tissues by up to 56–59% in co-inoculated areas. This reduction was attributed to two complementary mechanisms: direct fungal fixation by mycorrhizal fungi and bacterial chelation and biodilution by Azotobacter.
3. Biofertilizer treatments improved chlorophyll content and relative water content, reducing the physiological need for excessive ascorbic acid synthesis by decreasing internal oxidative stress. This was reflected in a significant increase in the APTI index, peaking with the interaction treatment.

4. The relative response to co-fertilization in yield characteristics was significantly higher in contaminated areas (121% increase) than in uncontaminated areas (75% increase), suggesting that this fertilization may be a preferred mitigation measure, especially in contaminated industrial environments.

Recommendations

1. When cultivating yellow maize in agricultural areas surrounding industrial zones in Karbala Governorate, we recommend adopting co-fertilization with Azotobacter and mycorrhizal fungi (by seed coating) as a routine practice.
2. The effects of co-fertilization and crop responses may differ between controlled pot conditions and openfield conditions; therefore, these results (obtained from pot experiments) need to be verified in subsequent field trials.
3. Future research should isolate and characterize native strains of Azotobacter and mycorrhizal fungi adapted to Iraqi soil conditions and contamination levels, and compare them with standard and commercially available strains.

4. The relative response to co-fertilization in yield characteristics was significantly higher in contaminated areas (121% increase) than in uncontaminated areas (75% increase), suggesting that this fertilization may be a preferred mitigation measure, especially in contaminated industrial environments.

Recommendations

1. When cultivating yellow maize in agricultural areas surrounding industrial zones in Karbala Governorate, we recommend adopting co-fertilization with Azotobacter and mycorrhizal fungi (by seed coating) as a routine practice.
2. The effects of co-fertilization and crop responses may differ between controlled pot conditions and openfield conditions; therefore, these results (obtained from pot experiments) need to be verified in subsequent field trials.
3. Future research should isolate and characterize native strains of Azotobacter and mycorrhizal fungi adapted to Iraqi soil conditions and contamination levels, and compare them with standard and commercially available strains.

References

1. Figlioli, F., Sorrentino, M. C., Memoli, V., Arena, C., Maisto, G., Giordano, S., & Spagnuolo, V. (2019). Overall plant responses to Cd and Pb metal stress in maize: Growth pattern, ultrastructure, and photosynthetic activity. Environmental Science and Pollution Research, 26(2), 1781-1790. https://doi.org/10.1007/s11356-018-3743-y
2. Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I. (2021). Nitrogen fixing Azotobacter species as potential soil biological enhancers for crop nutrition and yield stability. *Frontiers in microbiology*, *12*, 628379.
3. Sagar, A., Sayyed, R. Z., Ramteke, P. W., Ramakrishna, W., Poczai, P., Al Obaid, S., & Ansari, M. J. (2022). Synergistic effect of Azotobacter nigricans and nitrogen phosphorus potassium fertilizer on agronomic and yield traits of maize (Zea mays L.). Frontiers in Plant Science, 13, 952212. https://doi.org/10.3389/fpls.2022.952212
4. Severo, H. C. A. R., Arauco, A. M. S., Nunes, R. W. F., Monteiro, G. N., Duarte, M. H. F., Silva, A. P. M., Ferreira, A. C., Luz, M. R., Miranda, R. S., Araújo, A. S. F., & Costa, E. M. (2025). Co-inoculation of arbuscular mycorrhizal fungi and Bacillus subtilis enhances morphological traits, growth, and nutrient uptake in maize under limited phosphorus availability. Scientific Reports, 15, 25448. https://doi.org/10.1038/s41598-025-10038-6
5. Singh, S. K., Rao, D. N., Agrawal, M., Pandey, J., & Naryan, D. (1991). Air pollution tolerance index of plants. Journal of Environmental Management, 32(1), 45-55.
6. Alhesnawi, A. S. M., Alsalman, I. M., & Najem, N. A. (2018). Evaluation of air pollution tolerance index of some plants species in Kerbala city, Iraq. Journal of Pharmaceutical Sciences and Research, 10(6), 1386-1390.
7. Aquilanti, L., Favilli, F., & Clementi, F. (2004). Comparison of different strategies for isolation and preliminary identification of Azotobacter from soil samples. Soil Biology and Biochemistry, 36(9), 1475-1483. https://doi.org/10.1016/j.soilbio.2004.04.024
8. Rocha, I., Ma, Y., Souza-Alonso, P., Vosátka, M., Freitas, H., & Oliveira, R. S. (2018). Seed coating with inocula of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria for nutritional enhancement of maize under different fertilization regimes. Archives of Agronomy and Soil Science, 65(1), 31-43. https://doi.org/10.1080/03650340.2018.1479061
9. Tao, J., & Liu, X. (2024). Does arbuscular mycorrhizal fungi inoculation influence soil carbon sequestration? Biology and Fertility of Soils, 60(2), 213–225. https://doi.org/10.1007/s00374024-01793-1
10. Liu, Y., Qian, J., Lu, B., Hu, J., He, Y., Shen, J., & Tang, S. (2024). Arbuscular mycorrhizal symbiosis enhances the accumulation of plant-derived carbon in soil organic carbon by regulating the biosynthesis of plant biopolymers and soil metabolism. Plant Physiology and Biochemistry, 217, 109230. https://doi.org/10.1016/j.plaphy.2024.109230
11. Ram, S. S., Majumder, S., Chaudhuri, P., Chanda, S., Santra, S. C., & Chakraborty, A. (2015). A review on air pollution monitoring and management using plants with special reference to foliar dust adsorption and physiological stress responses. Critical Reviews in Environmental Science and Technology, 45(23), 2489-2522.
12. Redondo-Bermúdez, M. D. C., Gulenc, I. T., Cameron, R. W., & Inkson, B. J. (2021). 'Green barriers' for air pollutant capture: leaf micromorphology as a mechanism to explain plants capacity to capture particulate matter. Environmental Pollution, 288, 117809. https://doi.org/10.1016/j.envpol.2021.117809

13. Dhalaria, R., Kumar, D., Kumar, H., Nepovimova, E., Kuca, K., Islam, M. T., & Verma, R. (2020). Arbuscular mycorrhizal fungi as potential agents in ameliorating heavy metal stress in plants. Agronomy, 10(6), 815. https://doi.org/10.3390/agronomy10060815
14. Zhang, H., Li, X., Xu, Z., Wang, Y., Teng, Z., An, M., Zhang, Y., Zhu, W., Xu, N., & Sun, G. (2020). Toxic effects of heavy metals Pb and Cd on mulberry (Morus alba L.) seedling leaves: Photosynthetic function and reactive oxygen species (ROS) metabolism responses. Ecotoxicology and Environmental Safety, 195, 110469. https://doi.org/10.1016/j.ecoenv.2020.110469
15. Akram, N. A., Shafiq, F., & Ashraf, M. (2017). Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. Frontiers in plant science, 8, 613.
16. Abdou, A., Alkhateeb, O., Eldin, H., Ghazzawy, H., Albadrani, M., Al-Harbi, N., ... & Abdelaal, K. (2023). Application of plant growth-promoting bacteria as an eco-friendly strategy for mitigating the harmful effects of abiotic stress on plants. *Phyton*, *92*(12), 3305.
17. Kunal, Pranaw, K., Kumawat, K. C., & Meena, V. S. (2023). Plant growth-promoting rhizobacteria (PGPR) and plant hormones: an approach for plant abiotic stress management and sustainable agriculture. *Frontiers in Microbiology*, *14*, 1285756.
18. Wahab, A., Muhammad, M., Munir, A., Abdi, G., Zaman, W., Ayaz, A., ... & Reddy, S. P. P. (2023). Role of arbuscular mycorrhizal fungi in regulating growth, enhancing productivity, and potentially influencing ecosystems under abiotic and biotic stresses. *Plants*, *12*(17), 3102.