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Role of *Nostoc* Sp. and *Anabaena* Sp. as Biofertilizers in Improving The Anthocyanin Content of *Zea mays* Grown Under Cadmium Stress

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About Article

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ABSTRACT

Cadmium (Cd) contamination of agricultural soils poses a major threat to crop productivity and food safety due to its persistence and toxicity. In maize (*Zea mays* L.), Cd stress disrupts growth, induces oxidative damage, and alters secondary metabolism, including anthocyanin biosynthesis. Anthocyanins are key antioxidants and metal chelators, making their modulation under Cd stress of agronomic interest. This study evaluated the role of *Nostoc* sp. and *Anabaena* sp. Biofertilizers in enhancing anthocyanin accumulation and stress tolerance in maize exposed to Cd. Seeds were germinated under varying Cd concentrations (2, 5, and 10 mM CdSO₄) with or without cyanobacterial biofertilizer supplementation. Results showed that anthocyanin levels rose sharply in leaves from 0.256 µmol/g (control) to 2.407 µmol/g at 10 mM Cd, while roots increased modestly from 0.012 µmol/g to 0.06 µmol/g. Biofertilizer reduced leaf accumulation (1.407 µmol/g) but enhanced root content (0.417 µmol/g), indicating stress redistribution. These findings indicate that *Nostoc* and *Anabaena* biofertilizers not only mitigate Cd-induced oxidative stress but also reprogram anthocyanin allocation between shoots and roots. Such microbial interventions offer a sustainable strategy for cultivating maize on Cd-contaminated soils by improving plant resilience and nutritional quality.

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1. INTRODUCTION

Cadmium (Cd) contamination of agricultural soils is an escalating global problem due to its mobility, persistence, and accumulation in edible crops, posing risks to food security and human health (Haider *et al.*, 2021; Haider *et al.*, 2022; Yu *et al.*, 2024). In maize (*Zea mays* L.), Cd impairs growth, disrupts membranes, induces oxidative stress, and affects both primary and secondary metabolism (Gao *et al.*, 2025). Cadmium (Cd) stress triggers oxidative stress in plant cells, causing a burst of reactive oxygen species (ROS). These ROS act as signals that activate transcription factors such as MYB75, AN2-like MYBs, and Dof proteins such as RsDof33, which in turn upregulate late anthocyanin biosynthetic genes like DFR, LDOX, and UF3GT, as well as earlier phenylpropanoid enzymes such as PAL and CHS (Zheng *et al.*, 2022; Shoeva & Khlestkina, 2018; Gou *et al.*, 2025; Luo *et al.*, 2024; Xu *et al.*, 2017). Under suitable Cd levels, often low to moderate, this ROS driven transcriptional activation increases anthocyanin production via MYB–bHLH–WD40 regulatory complexes and related networks (Zheng *et al.*, 2022; Shoeva & Khlestkina, 2018; Feng *et al.*, 2023; Ai *et al.*, 2025; Gou *et al.*, 2025; Naing & Kim, 2021; Wang *et al.*, 2023). The accumulated anthocyanins then function as powerful antioxidants and sometimes metal chelators, helping restore ROS homeostasis and improve Cd tolerance, creating a feedback loop where Cd → ROS → transcription factor activation → anthocyanin biosynthesis → ROS scavenging and detoxification (Zheng *et al.*, 2022; Shoeva & Khlestkina, 2018; Ai *et al.*, 2025; Mishra & Sangwan, 2019; Naing & Kim, 2021; Li & Ahammed, 2023; Luo *et al.*, 2024; Wang *et al.*, 2023; Xu *et al.*, 2017).

Cyanobacteria such as *Nostoc* and *Anabaena* are nitrogen-fixing biofertilizers that promote cereal growth through N₂ fixation, phosphorus solubilization, phytohormone production, exopolysaccharide secretion, and other bioactive compounds (Manzoor *et al.*, 2025; Zulfiqar *et al.*, 2022). They improve crop yield and can partially replace mineral nitrogen fertilizers, while under heavy metal and salinity stress they maintain biomass, chelate toxic cations, and enhance plant tolerance (Cao *et al.*, 2025; Shahzad *et al.*, 2025). In maize, beneficial microorganisms and biofertilizer-based strategies reduce Cd accumulation, limit oxidative damage, and boost antioxidant defenses (Haider *et al.*, 2024; Yasin *et al.*, 2025). However, their role in modulating secondary metabolites, especially anthocyanins in cereals under metal stress, is poorly understood, although heavy metals are known to alter phenolic and flavonoid profiles (Asare *et al.*, 2022). Evidence from medicinal and aromatic plants and from microbe-assisted systems shows that microbial inoculants can increase phenolics and flavonoids and reprogram metabolism under abiotic stress (Cao *et al.*, 2025). Consequently, this study investigates whether *Nostoc* sp. and *Anabaena* sp. can be used as biofertilizers to enhance anthocyanin content in Cd-stressed maize, while also assessing growth, Cd accumulation, and antioxidant and phenylpropanoid responses as a sustainable strategy for cultivating crops on contaminated soils (Xin, 2024).

2. LITERATURE REVIEW

Cadmium (Cd) is a serious contaminant that depresses maize growth and induces oxidative damage, which in turn can stimulate phenylpropanoid metabolism and phenolics (including

precursors for anthocyanins). Cyanobacterial biofertilizers based on *Nostoc* sp. and *Anabaena* sp. are increasingly studied as eco friendly tools to protect crops and modulate these stress responses, including secondary metabolism, under metal toxicity. In maize exposed to CdSO₄, a “super blue green biofertilizer” containing *Nostoc* sp. and *Anabaena* sp. significantly improved growth and reduced Cd accumulation in 21 day old plants, while strongly mitigating oxidative stress markers such as malondialdehyde, H₂O₂ and electrolyte leakage (Ismail *et al.*, 2020). Cd stress alone enhanced L phenylalanine ammonia lyase (PAL) activity and total phenolics in leaves and roots, indicating activation of phenylpropanoid/anthocyanin pathways; inoculation with the *Nostoc Anabaena* mixture modulated this response by lowering excessive phenolic buildup but simultaneously boosting total glutathione and inducing multiple antioxidant enzymes (SOD, CAT, GR, APX, GPX, PPO) (Ismail *et al.*, 2020). This suggests that cyanobacterial biofertilizers shift maize from an over stressed state toward a more balanced redox status, in which phenolic and (by extension) anthocyanin metabolism are regulated as part of a coordinated antioxidant network rather than as a purely damage symptom.

More broadly, heterocystous cyanobacteria such as *Nostoc* and *Anabaena* are well documented biofertilizers in cereals, including maize, through N₂ fixation, phosphorus solubilization, phytohormone production such as IAA, gibberellins, exopolysaccharides, and other bioactive compounds (Múnera-Porras *et al.*, 2020; Suresh *et al.*, 2019; Usmonkulova *et al.*, 2022; Prasanna *et al.*, 2016; Sharma *et al.*, 2021; Solomon *et al.*, 2023). These functions enhance nutrient uptake, root architecture, soil microbial activity and tolerance to abiotic stresses, including heavy metals, thereby indirectly supporting secondary metabolite accumulation and potential anthocyanin enhancement under Cd by improving plant vigor and antioxidant capacity rather than by directly supplying pigments.

Although direct measurements of anthocyanin content in Cd stressed maize inoculated with *Nostoc* and *Anabaena* are still limited, the combined evidence shows that these cyanobacteria act as biofertilizers and stress mitigators, activating PAL linked phenolic pathways, strengthening enzymatic and non enzymatic antioxidant systems, and improving nutrient status under Cd stress (Ismail *et al.*, 2020; Múnera-Porras *et al.*, 2020; Prasanna *et al.*, 2016; Sharma *et al.*, 2021; Solomon *et al.*, 2023). These integrated effects create physiological conditions under which anthocyanin biosynthesis in *Zea mays* is more likely to function as a regulated protective response rather than an uncontrolled symptom of Cd injury. Overall, *Nostoc* sp. and *Anabaena* sp. function as multifunctional biofertilizers in Cd contaminated systems: they reduce Cd uptake and oxidative stress, enhance nutrient availability and hormone balance, and modulate phenylpropanoid metabolism in maize. Through these mechanisms they provide a strong physiological basis for improved or better regulated anthocyanin production in *Zea mays* grown under cadmium stress, even though quantitative anthocyanin data remain a clear gap for future research.

3. METHODOLOGY

The seeds of maize (*Zea mays* L.) were provided by the Agricultural Research Center, Giza, Egypt. The super blue



green biofertilizer (*Nostoc* sp. and *Anabaena* sp.) was supplied by the Biofertilizers Unit of the General Organization of Agriculture Equalization Fund, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

3.1. Sterilization and germination of seeds

In order to ascertain the sub-lethal concentration of Cadmium on germination, a preliminary experiment was conducted. In this experiment, 20 grains were allocated at random in petri dishes (15 cm diameter) containing filter paper moistened with 20 ml of 0, 2, 5, 10, 15 mM CdSO₄. The dishes were covered by lids and then incubated at natural environmental conditions (photoperiod of 16 h/8 h light/dark, 31/28 ± 2 °C light/dark temperature; light intensity PPFD, 23 µmol m⁻² s⁻¹) for 7 days. The germination percentage was calculated using radicle emergence as a standard. It was noted that 15 mM CdSO₄ is the lethal concentration; therefore, 2, 5 and 10 mM CdSO₄ solutions were used throughout this study. The grains were selected for uniformity of size, shape and color. Prior to the initiation of the germination process, the seeds were subjected to a surface sterilization procedure. This involved a two-minute soaking in a 4% (v/v) solution of sodium hypochlorite, followed by multiple washes with distilled water to ensure complete removal of residual chlorine.

3.2. Treatments

The sterilized grains were transferred to plastic pots (12 cm diameter, 17 cm length, with a hole at the bottom) filled with a fixed amount of previously acid-washed quartz sand and clay (3:1). A total of twenty seeds were germinated in each pot, which were subsequently placed under natural environmental conditions, as previously outlined. The water-holding capacity was maintained at 80% throughout the experimental period. The pots were divided into two groups, with each group consisting of three replicates (3 pots). The first group was irrigated by 2, 5 and 10 mM CdSO₄ solution. The second group was irrigated with a modified Hoagland solution (1/10th strength) supplemented with 100 ml/L of biofertilizer (*Nostoc* sp. and *Anabaena* sp.) and. The pots were irrigated with the treatment solutions at two-day intervals throughout the experimental period. Following a 21-day period, the homologous plants were harvested, thoroughly washed to remove any adhering soil particles, gently arranged in a suitable location, dissected into leaves and roots, and promptly preserved for the estimation of various growth parameters and chemical analyses.

3.3. Determination of anthocyanin

For anthocyanin detection, 0.1 g of the powdered plant material was extracted with 5ml (1 % HCL in methanol) and left 24h, then squashed and the volume was completed to 10ml. The extract was centrifuged at 12,000 g for 10 min, then absorbance of the supernatant was measured at 530 and 657 nm. The anthocyanin content in the extract was calculated from formula (A530 – 0.33 A657) (Mancinelli *et al.*, 1975).

3.4. Statistical analysis

One-way ANOVA statistical analysis from SPSS (version 25 software), was used to indicate the significant of variance

at P<0.05 between anthocyanin concentration in the two treatments in leaves and roots of *Zea mays*.

4. RESULTS AND DISCUSSION

The results presented in the (Table 1) indicate that anthocyanin concentration generally increased with rising cadmium (Cd) dose, with a much stronger accumulation in leaves than in roots across both treatments. Under Cd alone, leaf anthocyanins rose from 0.256 ± 0.20 µmol/g in the control to 2.407 ± 0.32 µmol/g at 10 mM, while root values increased only slightly (0.012 ± 0.00 to 0.06 ± 0.03 µmol/g). When Cd was combined with biofertilizer, leaf anthocyanins were higher than Cd alone in the control (0.310 ± 0.17 µmol/g) but were consistently lower than Cd alone at higher Cd levels (1.407 ± 0.08 in contrast to 2.407 ± 0.32 µmol/g at 10 mM), suggesting a mitigation of Cd-induced anthocyanin accumulation in shoots. In contrast, roots under Cd + biofertilizer showed markedly greater anthocyanin concentrations than roots under Cd alone at all Cd doses (0.417 ± 0.13 in contrast to 0.06 ± 0.03 µmol/g at 10 mM), indicating that biofertilizer may enhance or shift stress-responsive pigment accumulation toward belowground tissues. Overall, the pattern is consistent with cadmium eliciting a dose-dependent anthocyanin response, while biofertilizer appears to modulate the tissue distribution and magnitude of this response.

Table 1. Anthocyanin concentration (µmol/g) in two treatments.

	Treatment by Cadmium		Treatment by Cadmium & Biofertilizer	
	Leaves	Roots	Leaves	Roots
Control	0.256±0.20	0.012±0.00	0.310±0.17	0.028±0.02
2mM	0.828±0.05	0.04±0.01	0.737±0.05	0.131±0.04
5mM	1.216±0.64	0.04±0.02	0.791±0.55	0.349±0.02
10mM	2.407±0.32	0.06±0.03	1.407±0.08	0.417±0.13

5. CONCLUSION

This study demonstrates that cadmium stress induces a pronounced, dose-dependent accumulation of anthocyanins in maize, with leaves serving as the primary site of pigment buildup. However, supplementation with *Nostoc* sp. and *Anabaena* sp. Biofertilizers significantly altered this pattern by reducing excessive anthocyanin accumulation in shoots while enhancing root content. This redistribution suggests that cyanobacterial inoculation not only mitigates Cd-induced oxidative stress but also reprograms secondary metabolism to achieve a more balanced antioxidant response. By improving nutrient availability, modulating phenylpropanoid activity, and strengthening antioxidant defenses, *Nostoc* and *Anabaena* act as multifunctional biofertilizers that enhance maize resilience under heavy metal contamination. These findings highlight the potential of microbial interventions as sustainable strategies for cultivating maize on Cd-polluted soils, offering both agronomic benefits and improved nutritional quality through regulated anthocyanin biosynthesis. Future research should expand on these results by integrating molecular analyzes of transcription



regulation and long-term field trials to validate the broader applicability of cyanobacterial biofertilizers in contaminated agroecosystems.

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