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Seed priming by *Bacillus megaterium* L. to enhance the resistance of *Phaseolus vulgaris* L. seedlings to heavy metal stress

Enas F. Naji[1], Hiba F. Abdulfatah[1], Ahmed Sh.A. Lafi[2]

[1]*Department of Biology, College of Science, University of Anbar. Ramadi, 31001, Iraq*
[2]*Center of Desert Studies, University of Anbar, Ramadi, 31001, Iraq*

The buildup of heavy metals in the environment is one of the risks to the growth and development of plants globally, as well as to people if they consume plants contaminated with these heavy metals. Numerous countries have implemented diverse strategies to reduce the probability of these metals building up. Among these strategies, using biofertilizers to encourage seed is the most important. Thus, the purpose of this study was to determine how well *Bacillus megaterium* might increase the resistance of two types of *Phaseolus vulgaris* L. seeds (ASGROW and Sunray) to varying levels of nickel and cadmium (0, 100, and 200 mg.L^{-1}). Since cadmium is hazardous to living cells and an unneeded element, these two elements were selected. Within specific bounds, nickel is one of the essential trace elements for plant cells. It turns into a hazardous element if its concentration goes above those thresholds. After priming and without priming seeds exposed to heavy metals, measurements of the germination parameters (SRL, SSL, SFW, SDW, and proline content of the seedlings) were made. The following germination indicators—RL, SL, and SFW—were decreased by heavy metals. A proportionate rise in amount was noted as the levels of heavy metals increased. In contrast, heavy elements increase the dry weight of seedlings. These minerals also assisted in the build-up of proline in seedlings that had higher concentrations of heavy metals. By contrasting promoted and non-promoted seedlings, *Bacillus megaterium* significantly lessened the harmful effects of the heavy metals employed in the study.

Keywords: *Phaseolus vulgaris* L, cadmium, nickel, seed priming, *Bacillus megaterium*

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CORRESPONDENCE TO
Ahmed Sh.A. Lafi,
Center of Desert Studies,
University of Anbar, Ramadi, 31001, Iraq
ORCID: 0000-0002-8713-3257
Email. cds.ahmed.lafi@uoanbar.edu.iq
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INTRODUCTION

Phaseolus vulgaris L., commonly known as the common bean, is a widely distributed, common, and prominent member of the Fabaceae plant family. This seed legume is valued for its abundance in dietary fiber, protein, starch, and a variety of minerals, including folic acid, vitamin B6, and thiamine (Sánchez *et al.,* 2004). The selection of supplemental fertilizers that give plants the growth nutrients they require is a prerequisite for increasing agricultural output (Yilmaz *et al.,* 2023). Additional factors that have been proposed to contribute to the prevalence of heavy metal contamination include the exponential increase in population and enhanced material production, the rising demand for agricultural commodities, and the use of compost fertilizers and sewage sludge because of their inherent richness in certain components (Özyiğit *et al.,* 2021). These substances are extremely dangerous to all living things, including people, which is why their negative effects must be given the utmost consideration. Because they enter the food chain, heavy metals pose a threat to humans, plants, and other species as dangerous environmental contaminants. When it comes to heavy metal-contaminated soils, using plants that can survive, adapt, and absorb these metals is considered one method of remediating the soil (Budovich, 2021). Heavy metals are not removed from the body once they are ingested; instead, they build up in tissues including fat, skeletal muscles, osseous structures, and articular joints, which can lead to a variety of illnesses and problems in the body (Hu *et al.,* 2020; Okhotnikova *et al.,* 2021; Sesin *et al.,* 2021). The concentration of metallic elements within plants is contingent upon the particularities of their ecological surroundings, botanical classification, desiccation circumstances, preservation, conveyance, and manipulation. The inclusion of heavy metals within the substrate used for plant cultivation possesses the potential to yield noteworthy modifications in both the abundance and caliber of these organic compounds through the alteration of the physiological course of secondary metabolites (Nguyen *et al.,* 2021). Heavy metal ions are resistant to degradation and endure within the soil. When present in harmful amounts, they impair essential plant activities and metabolic processes, which reduces or prevents plant growth (Seneviratne et al., 2017; Saber et al., 2022; Ahmed et al., 2023; Nessem et al., 2023; Taha et al., 2024). Because there are so many obstacles to overcome when trying to remove pollutants with conventional methods, there has been a growing interest in using microorganisms that are resistant to heavy metals to decontaminate heavy metal-contaminated soil (Yang *et al.,* 2015). Plants have developed a wide range of

defensive mechanisms to protect their physiological systems from the harmful effects of heavy metal stress. These tactics include the following: lowering rhizosphere pH, binding metallothionein, eliciting various antioxidant enzymes, and ensnaring metals in vacuoles to reduce heavy metal accumulation (Clemens *et al.,* 2002; Greger 2004; Raza *et al.,* 2020).

Biofertilizers, or biological additives, are among the most important technologies used (Ahmadi and Arain, 2021). These compounds are made up of microorganisms that can release plant growth regulators taken from natural sources and supply plants with vital nutrients. This use allows nutrients to be released continuously while reducing the need for chemical fertilizers. Additionally, it helps speed up metabolic reactions that transform nutrients into forms that plants can easily absorb, improving the plant's ability to absorb nutrients. As per Misra and Dadhich (2010). Plant growth promoting bacteria (PGPB) could have a substantial impact on soil properties and crop yield. They can also suppress plant diseases, reduce unfavorable environmental circumstances, and promote soil ecological variety (Santoyo *et al.,* 2021). A physiological technique known as "seed promoting" involves hydrating and dehydrating seeds to speed up the pre-germinative metabolic process, which in turn accelerates seed germination, seedling growth, and final crop output. even when faced with demanding circumstances (Ejaz *et al.,* 2019). Due to differences in metabolic activity, biochemical cell repair, enzyme activation, bolstering of the defense mechanism for antioxidants, and protein synthesis, primed seeds germinate more swiftly and uniformly than unprimed seeds (Zulfiqar, 2021). Seed priming can repair DNA breaks, activate germination inducers, decrease the imbibition time, and regulate moisture levels to produce uniform germination (Marthandan *et al.,* 2020). Numerous studies have demonstrated the potential of Bacillus sp. in the field of heavy metal (HM) bioremediation. Bacillus sp. can remove several kinds of heavy metals (HMs) and provides an environmentally sound, economical, and sustainable bioremediation method. According to Karmakar *et al.* (2024), the current manuscript concludes that Bacillus sp. might be a practical solution for dealing with heavy metal pollution in the future.

The goal of the current study was to determine whether Bacillus megaterium L. could improve bean seedling resistance to stress caused by the heavy metals used in the experiment.

MATERIALS AND METHODS

Isolation of *Bacillus megaterium*

A series of dilutions were prepared from soil sample taken from agricultural field of in Anbar Governorate. One milliliter of each dilution was then spread out on Pikovskaya medium, and the dishes were incubated at 30° C for five days following the appearance of bacterial colonies, as indicated by the appearance of a transparent ring indicating bacterial growth (El-Komy, 2005).

Bacterial colony purification

A streaking method was used on solid Pikovskaya medium in sterile conditions with the goal of obtaining individual, uncontaminated colonies of bacteria. The dishes were then incubated for five days at 30° C for microscopic inspection and biochemical testing, followed by a species-level diagnosis.

Diagnosis of bacterial isolates

Cultural tests: To make it easier to identify the bacterial isolates, the phenotypic characteristics of the growing colonies were examined. These properties include the colony shapes and colors as well as the nutritional agar surface's borders, transparency, and texture (Bremner, 1965).

Motility test: Tubes that contain a semi-solid medium, which is rich in nutrients, were inoculated with bacteria through the utilization of the stabbing technique. Following an incubation period of 48 hours at 30°C, the extension of growth in an outward direction from the area where the stabbing occurred serves as an indication of the isolate's capacity to migrate. (Collee *et al.,* 1996)

Microscopic tests: By taking a smear from the bacterial colonies that were growing on the culture media, bacterial specimens were examined under a microscope. Later, the smear was put on a glass slide and stained with a Gram stain, which was done to look at the shape, behavior, and reactivity of the bacterial cells to the dye.

Biochemical tests

Methyl red test: Bacteria were added to the Pikovskaya medium, and it was incubated for 48 hours at 30° ^{C}. It was mixed with five drops of methyl red reagent. A reduction in the medium's pH caused a red tint to appear, which signaled a successful outcome. Nonetheless, the continued presence of the yellow hue denotes a poor outcome (Collee, 1996).

Catalase test: A part of the bacterial culture on the culture medium was taken and put on a glass slide for this test. The bacterial culture on the slide was added to a 3% hydrogen peroxide solution. As soon as the isolate was able to produce the catalase enzyme, bubbles were discharged (Versalovic, 2011).

Oxidase tests: To support the colonies growing on the culture medium, cytochrome oxidase solution was added. The colonies' violet tint indicated that the test was positive (Gerhardt *et al.,* 1981).

Starch hydrolysis test: After spreading 0.1 milliliter of the bacterial suspension over the starch agar medium, the dishes were incubated for 24 hours at 30^{C}. After adding 1 milliliter of Logul's iodine solution to the colonies that were surrounded by a transparent zone, the medium turned blue everywhere but the transparent areas around the colonies. This indicates that the test was successful because the bacteria were able to produce the enzyme amylase, which breaks down starch (Versalovic, 2011).

Urease test: The capacity of this isolate to produce the urease enzyme was demonstrated by the color change of the medium from yellow to pink on slant urea agar streaked with bacteria and cultured for 24 hours at 30° C (Collee *et al.,* 1996).

Voges-Proskauer test: Following a 48-hour incubation period at $30°^{C}$ and the inoculation of the medium with bacteria, 2.5 ml were transferred to a new vial along with 6 drops of α-naphthyl and 2 drops of KOH solution. The presence of red indicates a positive test result (Alexander, 2001).

Germination experiment

The study examined the impact of priming two types of common bean (*Phaseolus vulgalis*) seeds—ASGROW and Sunray—with Bacillus megaterium bacteria on their resistance to high concentrations (0, 100, and 200 $mg.L^{-1}$) of the heavy elements used in the investigation (cadmium and nickel). The experiment was conducted in the biology department's labs at University of Anbar, College of Science. After being sterilized with 10% sodium hypochlorite, the seeds—which were acquired from the Desert Studies Center, Department of Conservation Agriculture, University of Anbar—were soaked in bacterial nutrient broth for one night, dried, and then placed in sterile petri dishes with fifteen seeds each. The remaining dishes received the same volume of heavy metal solutions as a control treatment, and these dishes received 10 milliliters of DW per dish. After that, the watering schedule was determined by observing the dryness of the seeds and the demands of the plants. After that, it was incubated for 10 days at $27°^{C}$. After the germination period was over, measurements were made of the seedlings' fresh and dry weight, proline content, and shoot and root length.

Seedling proline content

Bates *et al.*'s (1973) estimation of proline content was used. Every seedling was homogenized in 10 mL of 3% sulphosalicylic acid "w/v" after weighing roughly 0.2 g. "1.25 g of ninhydrin dissolved with 30 ml of glacial acetic acid and 20 ml of 6 M of orthophosphoric acid heat the mixture for several minutes till the ninhydrin is fully dissolved" was the ninhydrin acid reagent added after centrifuging the extract for ten minutes at 10,000 rpm. Subsequently, the supernatant was mixed with 2 milliliters of ninhydrin solution. The contents were rapidly cooled in an ice bath after one hour in a $100°^{C}$ water bath. After each sample cooled and 4 mL of toluene was injected, the pink color was measured at 520 nm. A typical pure proline curve (0.1 mg mL-1) with different proline concentrations was created using toluene as a blank. The proline content of plant matter was estimated using a fresh weight and a standard curve as follows:

proline (µmoles /g)=[(µg proline/ mL × mL toluene) /115.5 µg / µmoles]/[(g sample)/5].

RESULTS

Seedling Root Length (SRL)

Heavy elements, cadmium, and nickel, have a pronounced negative impact, as shown by the data in Tables 1 and 2. As these heavy element concentrations rise, so does this influence. Both amounts of cadmium and nickel cause a considerable reduction in the root length of bean seedlings. Before the seeds were activated with bacillus bacteria, the percentages of drop from the control treatment (8.67 cm) were 88.4% and 92.27%, respectively, in response to the two concentrations of 100 and 200 $mg.L^{-1}$ cadmium. Following the activation phase, these percentages dropped to 67.5% and 87.8% in response to the concentrations when compared to the control treatment (12.33 cm). The grand mean values before and after the activation process (3.44 cm and 5.94 cm), respectively, demonstrate the efficacy of the seed activation procedure with bacteria. Under the concentration of 100 $mg.L^{-1}$, the root length of non-

Table 1. Seedling root length (cm) of non-activated and activated bean seedlings under different concentrations of cadmium

Seed condition	Non- activated				Activated			
	Cadmium Concentration (mg.L^{-1})			Average	Cadmium Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	7.33	1.00	0.50	2.94	9.00	4.00	1.67	4.89
Sunray	10.00	1.00	0.83	3.94	15.67	4.00	1.33	7.00
LSD $_{P\leq0.05}$	3.282			1.895	2.593			1.497
Average	8.67	1.00	0.67		12.33	4.00	1.50	
LSD $_{P\leq0.05}$	2.320				1.834			
Grand Mean	**3.44**				**5.94**			

Table 2. Seedling root length (cm) of non-activated and activated bean seedlings under different concentrations of nickel

Seed condition	Non- activated				Activated			
	Nickel Concentration (mg.L^{-1})			Average	Nickel Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	7.33	0.83	0.67	2.94	9.00	1.83	1.33	4.06
Sunray	10.00	1.00	0.67	3.89	15.67	1.33	0.83	5.94
LSD $_{P\leq0.05}$	3.355			1.937	1.767			1.020
Average	8.67	0.92	0.67		12.33	1.58	1.08	
LSD $_{P\leq0.05}$	2.372				1.249			
Grand Mean	**3.42**				**5.00**			

stimulated seedlings treated with nickel considerably decreased compared to the control treatment of 8.67 cm by 89.9% mg.L^{-1} and 92.27% at 200 mg.L^{-1} of concentration mg.L^{-1}. Following the application of bio fertilizer to the seeds, these percentages dropped once more, peaking at 87.1% and 91.2% in response to the concentrations. Upon comparing the total rates of root lengths before activation, which were determined to be 3.42 cm, and after activation, which were measured at 5, it is apparent that activation has a positive effect due to its ability to lessen the negative effects of the heavy metals used.

Seedling Shoot Length (SSL)

When compared to the control treatment, Tables 3 and 4 show that both nickel and cadmium have a suppressive effect on the elongation of bean seedling shoots. This effect intensifies as the heavy element concentration rises. The differences between the two elements' concentrations were also highly significant. At 200 mg.L^{-1} cadmium, the maximum amount of inhibition was seen, reaching a value of 81.5% before activation. This percentage dropped to 76.7% once the seeds were subsequently triggered by bacteria. When the concentration was fixed at 100 mg.L^{-1} cadmium, the inhibitory percentage before activation was measured at 67.6%. After activation, this percentage decreased to 63.3%.
Activation with bacteria has lessened the inhibitory effect of cadmium by comparing the magnitudes of the total seedling length rates, both before (5.44 cm) and after (6.31 cm) the activation procedure. Regarding the nickel treatments, the length of the seedling shoot was significantly reduced by 73.8% before activation when the concentration was adjusted at 100 mg.L^{-1}. This percentage then dropped to 71.8% because of the activation procedure. Conversely, an inhibitory impact of 75.3% was observed when dose was raised to 200 mg.L^{-1}. That percentage, however, dropped to 70.4% after the activation procedure. Looking at the average values for the overall averages of the effects that nickel had on the seedling lengths, which are recorded in the tables at 5.44 cm before activation, it is significant that this value increased to 6.22 cm after activation. This demonstrates the biofertilizer's ability to lessen the negative effects of this potent heavy metal.

Seedling fresh weight (g. plant $^{-1}$)

The results of the statistical analysis displayed in Tables 5 and 6 support earlier findings about the detrimental effects of heavy metals on the fresh weight values of bean seedlings, which significantly decreased when compared to the control treatment. When cadmium was applied, the weights of seedlings that were not stimulated with Bacillus bacteria were significantly lower than those of the control treatment, weighing 1.198 g. plant^{-1}. This fresh weight decline was expressed as percentages

Table 3. Seedling shoot length (cm) of non-activated and activated bean seedlings under different concentrations of cadmium

Seed condition	Non- activated				Activated			
	Cadmium Concentration (mg.L^{-1})			Average	Cadmium Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	9.67	3.67	1.67	5.00	12.00	3.67	2.83	6.17
Sunray	12.00	3.33	2.33	5.89	11.67	5.00	2.67	6.44
LSD $_{P\leq0.05}$	2.011			1.161	1.637			0.945
Average	10.83	3.50	2.00		11.83	4.33	2.75	
LSD $_{P\leq0.05}$	1.422				1.158			
Grand Mean	**5.44**				**6.31**			

Table 4. Seedling shoot length (cm) of non-activated and activated bean seedlings under different concentrations of nickel

Seed condition	Non- activated				Activated			
	Nickel Concentration (mg.L^{-1})			Average	Nickel Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	9.67	2.00	1.67	4.44	12.00	2.33	2.33	5.56
Sunray	12.00	3.67	3.67	6.44	11.67	4.33	4.67	6.89
LSD $_{P\leq0.05}$	1.828			1.055	1.569			0.906
Average	10.83	2.83	2.67		11.83	3.33	3.50	
LSD $_{P\leq0.05}$	1.292				1.109			
Grand Mean	**5.44**				**6.22**			

Table 5. Seedling fresh weight (g. plant $^{-1}$) of non-activated and activated bean seedlings under different concentrations of cadmium.

Seed condition	Non- activated				Activated			
	Cadmium Concentration (mg.L^{-1})			Average	Cadmium Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	1.235	0.718	0.660	0.871	1.270	0.958	0.640	0.956
Sunray	1.161	0.768	0.709	0.879	1.419	0.819	0.844	1.027
LSD $_{P\leq0.05}$	0.2426			0.1401	0.2723			0.1572
Average	1.198	0.743	0.685		1.344	0.888	0.742	
LSD $_{P\leq0.05}$	0.1715				0.1925			
Grand Mean	0.875				0.991			

Table 6. Seedling fresh weight (g. plant $^{-1}$) of non-activated and activated bean seedlings under different concentrations of nickel

Seed condition	Non- activated				Activated			
	Nickel Concentration (mg.L^{-1})			Average	Nickel Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	1.235	0.671	0.679	0.861	1.270	0.712	0.858	0.947
Sunray	1.161	0.860	0.939	0.986	1.419	1.094	0.946	1.153
LSD $_{P\leq0.05}$	0.3970			0.2292	0.3340			0.1929
Average	1.198	0.765	0.809		1.344	0.903	0.902	
LSD $_{P\leq0.05}$	0.2807				0.2362			
Grand Mean	**0.924**				**1.050**			

of 37.9% and 42.8%, respectively, for concentrations of 100 and 200. In contrast, the weights of the activated seedlings decreased by 33.0% and 44.7% in comparison to the 1.344 g. plant^{-1} control treatment. These concentrations described were applied. By contrasting the general rates of wet seedling weights before activation (0.875 g. plant^{-1}) and after activation (0.991 g. plant^{-1}) the activation method' efficiency is demonstrated. Under concentrations of 100 and 200 mg.L^{-1} nickel, respectively, the fresh weight of non-activated bean seedlings decreased by 36.1% and 32.4% in relation to the 1.198 g. plant^{-1} control treatment. Activated seedlings showed a comparable reduction percentage for both

concentrations, measuring 32% compared to 1.344 g. plant^{-1} for the control treatment. These percentages showed very little change from what was seen before activation. The activation process somewhat increased these weights when comparing the overall mean fresh weights before activation (0.924 g. plant^{-1}) and after activation (1.050 g. plant^{-1}).

Seedling Dry Weight (SDW) (g. plant $^{-1}$)

Tables 7 and 8 clearly show the impact of heavy metals and biofertilizer activation on the dry weight of seedlings. The dry weights increased significantly when heavy metals were present, with percentages of increase in the dry weights before activation process reaching 28.8% and 53.8% under 100 and 200 mg.L^{-1} cadmium and 39.4% and 82.2% under 100 and 200 mg.L^{-1} nickel, respectively. These differences were significant for both concentrations and for each element from the control treatment, which accounted for 0.152g plant^{-1}. However, none of these values differed significantly from the control treatment of 0.161 g. plant^{-1}. As a result, the percentage growth rates following the seed activation process were 24.2% and 8.6% in response to 100 and 200 mg.L^{-1} cadmium and 36.6% and 26.7% in response to 100 and 200 mg.L^{-1} nickel. It is evident by looking at the percentage values and overall average values in the two tables that the amount of increase in dry weights has been lessened by the seed activation process.

Seedlings proline content (µmole.g plant^{-1})

Tables 9 and 10 clearly demonstrate the impact of the heavy elements used in this study, with cadmium treatments prior to activation resulting in significantly higher amounts of proline formed in the bean seedlings (1.7335 µmole.g plant^{-1} under 100 mg.L^{-1} cadmium and 6.1472 µmole.g plant^{-1} under 200 cadmium) compared to 1.6282 µmole.g plant^{-1} under the control treatment. Proline aggregation in seedlings decreased because of the seed activation process. In particular, there were no appreciable variations in the amount of proline generated at 200 mg.L^{-1} cadmium (1.581 µmole.g plant^{-1}) in comparison to the control treatment, which had 1.404 µmole.g plant^{-1}. With regard to the nickel treatments, it was shown that proline buildup occurred at concentrations of 100 and 200 mg.L^{-1} in non-activated seedlings treated with nickel, and 4.5363 µmole.g plant^{-1} in non-activated seedlings. These values were considerably different from the 1.6252 µmole.g plant^{-1} proline level of the control seedlings. The proline content of the bacterially activated seedlings showed a discernible decline, with the values coming close to but still differing greatly from the control treatment. It is evident how important activation was in preventing the buildup of proline in the seedlings by looking at the values of the general rates for the cadmium and nickel treatments and comparing them with one another.

DISCUSSION

Heavy metals are the main abiotic factors that cause stress in plants (Atis and Akar, 2018). It has been demonstrated that heavy metals affect a wide variety of plant processes, with a clear negative impact on the germination process of seeds. They have the capacity to cause damage to a plant's root system (Singh, Thakur, 2014). Either by creating free radicals, which cause oxidative stress (Shah *et al.,* 2010), or by replacing nutrients and necessary metals (Henry, 2000). A plant's life cycle includes a seed phase, which is protected from many pressures. However, it becomes vulnerable to stress soon after it consumes water and passes through the succeeding vegetative developmental stages. To maintain their protective state until external conditions become conducive for following developmental processes, seeds are therefore thought to actively monitor external elements including light, temperature, and nutrients (Karssen 1982; Pritchard *et al.* 1993; Bungard *et al.* 1997). The original theory was based on the finding that seeds could still germinate when exposed to high quantities. However, at far lower concentrations of these metallic elements, the growth of the seedlings after the seed coat rupture was severely hindered (Li *et al.,* 2005). According to ABDULFATAH and NAJI (2023), salt stress decreased the amount of chlorophyll in oat leaves, which has a detrimental effect on plant growth and cell division.

The length of the bean seedlings' roots and shoots clearly decreased in the current study, and this decrease is consistent with an increase in the concentration of the heavy metals utilized in the investigation. These findings are consistent with those of Başdinç and Çirka (2021) and Sharifi-Rad (2017), who used nickel and cadmium on Hyssopus offieinalis to achieve the same outcomes. Phaseolus vulgaris's decreased growth performance may be explained by a decrease in meristematic cells as well as an inhibitory effect on the activities of hydrolytic enzymes present in the cotyledon and endosperms. It is the harmful effects of heavy metals that cause this inhibition. The mobilization of stored nutrients is

Table 7. Seedling dry weight (g. plant $^{-1}$) of non-activated and activated bean seedlings under different concentrations of cadmium

Seed condition	Non- activated				Activated			
	Cadmium Concentration (mg.L^{-1})			Average	Cadmium Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	0.1567	0.1613	0.2603	0.1928	0.174	0.240	0.169	0.194
Sunray	0.1480	0.2310	0.2083	0.1958	0.147	0.161	0.180	0.163
LSD $_{P\leq0.05}$	0.04647			0.02683	0.0891			0.0515
Average	0.1523	0.1962	0.2343		0.161	0.200	0.175	
LSD $_{P\leq0.05}$	0.03286				0.0630			
Grand Mean	**0.1943**				**0.178**			

Table 8. Seedling dry weight (g. plant $^{-1}$) of non-activated and activated bean seedlings under different concentrations of nickel

Seed condition	Non- activated				Activated			
	Nickel Concentration (mg.L^{-1})			Average	Nickel Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	0.157	0.223	0.324	0.235	0.174	0.187	0.235	0.198
Sunray	0.148	0.201	0.230	0.193	0.147	0.253	0.174	0.191
LSD $_{P\leq0.05}$	0.0832			0.0480	0.1160			0.0670
Average	0.152	0.212	0.277		0.161	0.220	0.204	
LSD $_{P\leq0.05}$	0.0588				0.0820			
Grand Mean	**0.214**				**0.195**			

Table 9. Proline content (µmole.g plant^{-1}) of non-activated and activated bean seedlings under different concentrations of cadmium

Seed condition	Non- activated				Activated			
	Cadmium Concentration (mg.L^{-1})			Average	Cadmium Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	0.8927	0.9350	3.2407	1.6894	1.594	3.519	2.173	2.429
Sunray	2.3637	2.5320	9.0537	4.6498	1.213	1.925	0.989	1.376
LSD $_{P\leq0.05}$	0.04060			0.02344	0.3455			0.1995
Average	1.6282	1.7335	6.1472		1.404	2.722	1.581	
LSD $_{P\leq0.05}$	0.02871				0.2443			
Grand Mean	**3.1696**				**1.902**			

Table 10. Proline content (µmole.g plant^{-1}) of non-activated and activated bean seedlings under different concentrations of nickel

Seed condition	Non- activated				Activated			
	Nickel Concentration (mg.L^{-1})			Average	Nickel Concentration (mg.L^{-1})			Average
Variety	0	100	200		0	100	200	
ASGROW	0.8927	5.2660	7.1960	4.4516	1.594	1.937	1.547	1.693
Sunray	2.3637	3.8067	1.4567	2.5423	1.213	2.622	1.996	1.944
LSD $_{P\leq0.05}$	0.05356			0.03093	0.4179			0.2413
Average	1.6282	4.5363	4.3263		1.404	2.279	1.772	
LSD $_{P\leq0.05}$	0.03788				0.2955			
Grand Mean	**3.4969**				**1.818**			

made possible by hydrolytic enzymes, which enable the nutrients to reach the radicle and plumule and therefore encourage the growth of seedlings (Shafiq *et al.,* 2008). The rapid nutrient material degradation in seeds that occur when heavy metals are present could also be the reason for the Phaseolus vulgaris growth characteristics decline (Heidari and Sarani, 2011; Nasr, 2013). The observed reduction in shoot length could perhaps be explained by an overabundance of heavy metal salts inside the cell wall, which could lead to modifications in metabolic pathways and limitations on the flexibility of the cell wall (Naseer, 2001). However, an increase in the high-density element concentrations in the root

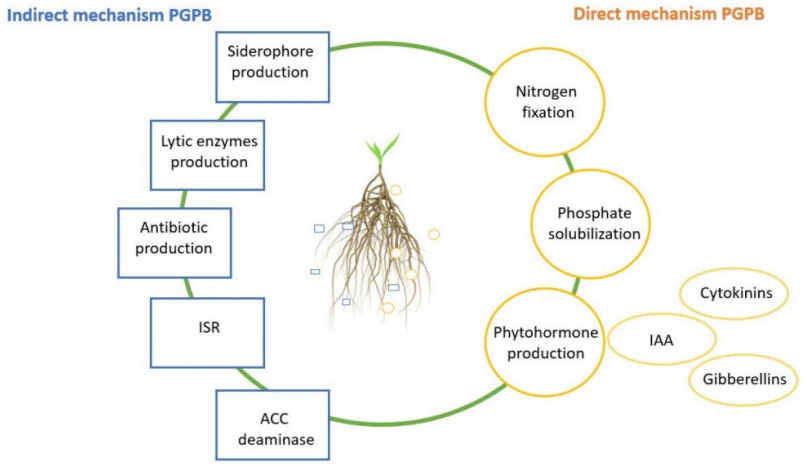

Figure 1. Direct and indirect mechanisms of PGPB **(**Kulkova etall., 2023).

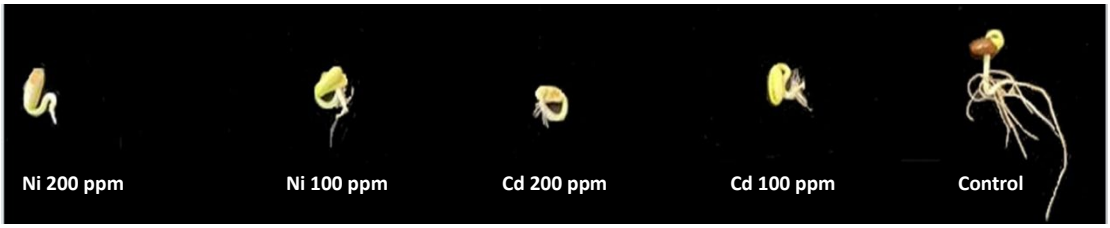

Figure 2. Non activated seedlins of *Phaseolus vulgaris* (ASGROW) under different heavy metals concentrations

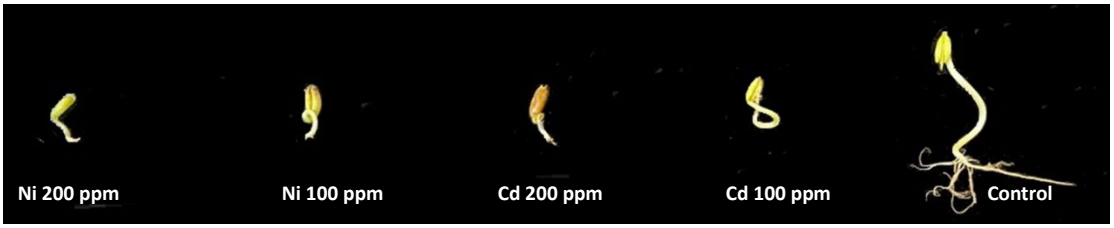

Figure 3. Activated seedlins of *Phaseolus vulgaris* (ASGROW) under different heavy metals concentrations.

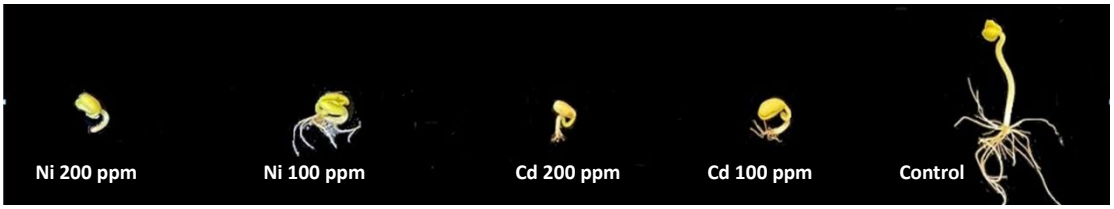

Figure 4. Non activated seedlins of *Phaseolus vulgaris* (Sunray) under different heavy metals concentrations.

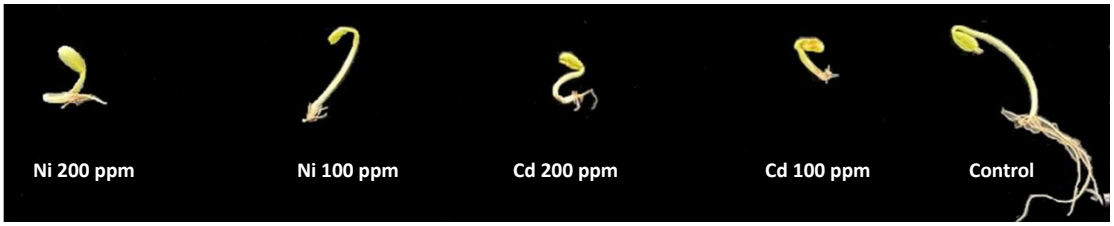

Figure 5. Activated seedlins of *Phaseolus vulgaris* (Sunray) under different heavy metals concentrations.

environment could lead to a reduction in the plant's ability to absorb water and other vital nutrients, an obstruction of enzymatic activities, a decrease in cellular metabolism, a stop to growth, an acceleration of senescence, or even the plant's death (Souguir *et al.,* 2008; Peralta *et al.,* 2000; Cheng and Huang, 2006).

According to the current study's findings, bean seedlings' fresh weight significantly decreased when cadmium and nickel concentrations were employed. According to Ertekin *et al.* (2020), using cadmium and nickel at increasing concentrations (0, 100, 200, 400, and 800 mg L^{-1}) severely affects the growth indicators of some plant species, such as sorghum seedlings in terms of relative root length (RRL), relative shoot length (RSL), root fresh weight (RFW), and shoot fresh weight (SFW). In essence, heavy metals cause the production of free radicals and reactive oxygen species (ROS), which lead to uncontrolled oxidation and radical chain reactions. Consequently, this leads to the degradation of cellular macromolecules, including proteins, lipids, and nucleic acids (Phaniendra *et al.,* 2015). The results of the current study showed that the stress of utilized metals caused a definite excess in the proline content of the seedlings. These results concur with those of Sasi *et al*. in 2019. According to Li *et al.* (2010) and Chai *et al.* (2014), enhanced compatible solute synthesis is essential for cellular osmotic regulation, which preserves water potential equilibrium and stabilizes biological macromolecule structure under oxidative stress.

It can be concluded from the study that adding bacteria to seeds improved their influence and decreased the number of heavy metals on all the study's indicators. The capacity of bacterial genera, like Bacillus, to improve plant growth and productivity is well known. Through the development of several methods, bacterial isolates recovered from rhizosphere soils have successfully adapted to withstand numerous heavy metal stressors (Jha, 2016). Numerous advantageous characteristics of plant growth-promoting bacteria (PGPB) either directly or indirectly boost plants. A few of these include the production of "auxins, e.g., indole-3-acetic acid (IAA)", "cytokinins", and "gibberellins", as well as "aminocyclopropane1-carboxylic acid deaminase (ACC)", atmospheric nitrogen fixation, phosphorus solubilization, lytic enzymes (chitinase, cellulase, protease, glucanase), siderophore production, induced systemic resistance (ISR), and antibiotic lipopeptides" (Figure 1) (Saxena et al., 2020; Dobrzy´nski *et al.,* 2022). The following strategies fall under the category of "Heavy Metal Bioremediation Strategies Found in *Bacillus*": biosorption, bioremediation by extracellular polymeric substances (EPS), bioaccumulation, bioprecipitation, and biological removal of heavy metals using plant growth-promoting bacteria (Wróbel, 2023).

Because of their ability to produce biosurfactants, siderophores, and enzymes that can sequester, solubilize, and convert heavy metals (HMs), Bacillus species are among the most effective microorganisms used in bioremediation. (Karmakar, 2024). *B. cereus* strains can promote plant development in the face of abiotic stresses such salt, drought, and heavy metal contamination, according to Kulkova *et al.* (2023). Moreover, *B. cereus* strains produce antibiotic lipopeptides and extracellular enzymes, or they induce systemic resistance, which permits an indirect encouragement of plant growth. The addition of *Pseudomonas aeruginosa*, *Bacillus subtilis*, and Beauveria bassiana to the soil resulted in reduced cadmium acumulation in rice (Oryza sativa L.) and increased plant growth and biomass (Siripornadulsil and Siripornadulsil, 2013; Suksabye *et al.,* 2016). According to research by Wang *et al.* (2019a), biofertilizers efficiently bound soil Cd and lessened its harmful effects on tobacco leaf. Wang et al. (2019b) suggested that the pH of the soil was raised by the alkaline properties of the bio fertilizer treatments, which may have contributed to the lower concentration of bioavailable Cd. Additionally, the induced changes in soil parameters, such as pH, DTPA-Cd, total P, and organic carbon, influenced the population of soil bacteria.

CONCLUSIONS

The results of this study indicate that using biofertilizers to prime bean seeds enhanced the seedlings' tolerance to cadmium and nickel stress. This was demonstrated by comparing several parameters before and after activation. For best outcomes, it is advised that bio fertilizers be sprayed directly onto the soil or through foliar spraying in future research.

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