

The combined activity of *Bacillus cereus* and calcium oxide nanoparticles in chromium degradation

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ABSTRACT

Background: Chromium contamination in the soil and environment is a significant environmental concern due to its toxicity and is needed to be addressed. This study investigates the potential of *Bacillus cereus* and calcium oxide (CaO) nanoparticles for chromium remediation and plant growth promotion. **Methodology:** The bacterium's resistance to chromium and its plant growth-promoting Rhizobacterial (PGPR) characteristics was assessed by inoculating seeds into bacterial broth prior to germination. Calcium Oxide Nanoparticles were synthesized by using eggshell waste as calcium source by sol gel method. Seed germination was tested in two conditions: normal soil and chromium-contaminated soil, with and without *Bacillus cereus* and nanoparticles. **Results:** Results showed that while chromium inhibited seed germination in contaminated soil, the presence of *Bacillus cereus*, CaO nanoparticles, or combination enabled seed germination despite chromium toxicity. **Conclusion:** The study concluded that *Bacillus cereus* and CaO nanoparticles can degrade chromium and promote plant growth, offering a promising approach for soil remediation.

Keywords: *Bacillus cereus*, CaO nanoparticles, Chromium degradation, PGPR, Sunflower

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INTRODUCTION

Metals are an integral part of life and biological processes, however, some of them pose toxic effects on living organisms, especially human beings. Any such metal with prevalent toxic effects is termed as heavy metal, regardless of its atomic number and mass. These metals are naturally part of Earth's crust; however, the constant involvement of human activities has disturbed their normal balance resulting in toxic outcomes (Fulke et al., 2024). A significant increase in the heavy metal concentration in the environment has led to several toxic effects on human and plant health. There are several routes through which living organisms get exposed to these metals including dermal exposure, inhalation, ingestion, and mechanical damage (Fu and Xi, 2020). Some of these heavy metals are mandatory for the metabolism in

humans in small amounts, however, their concentrations higher than needed can lead to

problems in the functioning of metabolism (Fulke et al., 2024). These metals include copper, cobalt, iron, molybdenum, magnesium, selenium, and iron. Other heavy metals such as cadmium, mercury, thallium, lead, chromium, and silver are very toxic to the human body even in minute concentrations and are not required by the human body at any level. They can cause serious acute and chronic abnormalities in human metabolism (Engwa et al., 2019). Different heavy metals have distinct effects on the human body depending upon their composition (Haidar et al., 2023). For instance, exposure to chromium results in carcinogenic effects, exposure to lead results in intellectual abnormalities in children and exposure to thallium leads to alopecia. Toxicity of heavy metals

can also be organ specific, for example, some of them cause neurotoxicity while some cause nephrotoxicity. This is the reason that the hazardous effects of heavy metals outweigh their benefits to the living world (Mitra et al., 2022).

In a similar manner to humans, heavy metals have been harming the aquatic world for a very long time. Heavy metal-loaded food, when ingested by aquatic animals such as fish, inhibits the growth and development of these animals leading to the death of certain species. Disturbance in the natural food chains due to heavy metals has become a global concern. These metalloids cause the direct death of smaller organisms while causing the indirect death of bigger organisms by reducing food availability. For example, zinc causes hypoxia in smaller fishes leading to their death (Zaynab et al., 2022).

Heavy metal contamination in soil leads to the accumulation of these metalloids in plants, resulting in higher concentrations within the vegetation. Soil contamination mainly results in decreased microbial activity which is indicated by the decreased decomposition of litter or fertilizers in the soil. In addition to the useful microbes, sometimes heavy metals can destroy harmful microbes such as fungi and pesticides, which can help plant growth (Atuchin et al., 2023). It has been observed in many experiments that heavy metal toxicity and a decrease in microbial activity in the soil depends upon certain physical parameters such as pH, temperature, and oxygen availability (Haidar et al., 2023). Among other heavy metals chromium has significant adverse impacts on the soil. It is mainly caused by natural mechanisms such as volcanoes, however, anthropogenic activities are also involved in the contamination. Chromium is non-biodegradable in nature and it can either directly enter the soils or can contaminate the water bodies that are later used for irrigation in plant production. Chromium decreases plant production by reducing the production of photosynthesis pigments which leads to decreased autotrophic activity (Batool et al., 2024). Chromium is generally not required for plant growth or production at any level so it is dangerous for the plant at all concentrations. In addition to decreasing chlorophyll content, it also disturbs the enzyme activity in many plants. Some studies reported decreased protein production leading to decreased root & shoot biomass in certain plants (Singh et al., 2020). In addition to these toxicities, hexavalent chromium

can also lead to mutagenesis and oxidative imbalance in the plants resulting in malfunctioned growth (Batool et al., 2024). Hampered production and growth of plants is a great concern due to which there are several solutions advised to fight the heavy metal contamination in the soils. The chromium remediation can be of many types i.e., biological, physical or chemical. However, all of these techniques have their disadvantages and advantages so studies are still being conducted to find the most suitable technique which has the highest output with the lowest input (Prasad et al., 2021). Remediation is the process of removal of certain chemical or biological contaminant that is hazardous to a particular specie (Blenis et al., 2023). There are many experiments in which chemical effluents were used to remove chromium from the soil by its reduction. Chemical methods are used to remove the hexavalent chromium from the soil by decreasing the pH. A decrease in PH affects the mobility of hexavalent chromium to a greater extent resulting in its removal from a particular area of soil (Paul et al., 2024). Several reducing agents for chromium are being used in the chemical leaching methods such as EDTA and FeSO_4 at several temperatures. In addition, several acids such as oxalic acid or acetic acid can also be used as reducing agents. An increase in the concentration of the reducing agents increases the removal of hexavalent chromium to an extent, however, at a certain point the concentration variation does not affect the removal of heavy metal from the soil (Wang et al., 2021). Moreover, nanotherapy is another trending technique being used for remediation of heavy metals. It is based on the nano-sized particles which are synthesized using different techniques such as the top-down technique and bottom-up techniques. Nanoparticles exhibit a larger surface area-to-volume ratio as compared to their counterparts because of their very small size. Nanoparticles have been used in drug delivery, remediation, therapeutic operations, and mechanics for a long time (Haleem et al., 2023). Nanoparticles can also be used in industrial applications as well as in treating the waste materials generated from industries. For instance, nanoparticles are being used for wastewater treatment which means they can remediate industrial effluent including heavy metals. Calcium oxide nanoparticles derived from eggshell waste are specifically used for wastewater treatment in many studies. They also exhibit conductivity and electrical properties (Habte et al.,

2019). Calcium oxide nanoparticles prepared from eggshell waste are also used in this study for chromium degradation in the soil.

Biological methods for chromium degradation are also important and in trend. Many microbes i.e., bacteria can degrade chromium and other heavy metals as they can consume these metalloids as their carbon source (Wang et al., 2023). Bacteria are prokaryotic organisms with an average diameter ranging between 0.5-1 micrometres, however, some of them can even be 50 micrometres. The very small size of the bacterial cell enables it to grow rapidly. Bacteria can be of different shapes depending on their genetic properties, however, in general, they are of the following three shapes i.e., rods, spherical, and spiral. Being prokaryotes, bacteria do not have any double membrane-bounded structures in their cell, however, they have got a cell wall to protect the shape of the cell. Bacteria are divided into two main groups based on their cellular composition that is gram-negative bacteria or gram-positive bacteria (Ansari et al., 2011). This type of remediation involving bacteria is called bioremediation, as living organisms are being used as the way of remediation. In many studies these microbes especially, bacteria are being used as chromium degradation tools for several purposes. For instance, recently these bacteria have been used in chromium degradation as plant growth promoters. This way biological ways to degrade chromium are a great benefit to the environment. Primarily these bacteria degrade heavy metals by using them as an energy source for their metabolism. Reportedly, the bacterial genera that can be used in heavy metal degradation include *Bacillus spp.*, *pseudomonas spp.*, *Azotobacter spp.*, *Arthrobacter spp.*, *alkaligenes spp.*, *Rhodococcus spp.*, and *Methanogens* (Pham et al., 2022). In addition to the singular effects of microbial strains on chromium degradation, synergistic effects of these bacterial strains are also utilized for the remediation. It is observed that a group of bacteria act more efficiently for the remediation of chromium or any other heavy metal than a single bacteria. Bacteria show different biosorption levels based on several constituents such as peptidoglycan, functional groups, and several proteins that might be present in them (Gómez-Godínez et al., 2023). This research study specifically focused on chromium degradation Using bacteria and nanoparticles. Several studies have already used *Bacillus* species for promoting the

growth of plants. It is usually found in soil, food, or environment and is facultative anaerobic in nature. It has a gram-positive cell wall and produces spores. This bacterium is pathogenic towards human beings and produces many foodborne illnesses because of its spore production. Its pathogenicity leads to serious gastrointestinal infections in humans leading to vomiting, nausea, and diarrheal illnesses (McDowell et al., 2022). However, this bacterium has chromium degradation ability as it can use chromium as its energy source for its metabolism. This is why it improves plant growth and soil quality wherever it is present. *Bacillus cereus* has been used in several studies for the degradation of chromium such as in soil and tanneries. *Bacillus cereus* converts chromium (VI) into chromium (III) by utilizing it as a food source. Chromium (III) is much less dangerous as compared to its chromium (VI) form which is why its conversion into chromium (III) is known as the degradation of chromium (Wang et al., 2023).

This study mainly aimed at assessing potential of *Bacillus cereus* and CaO nanoparticles for chromium remediation as well as plant growth promotion both individually and in combination.

MATERIALS AND METHODS

Isolation and confirmation of bacterial colonies

Bacillus cereus colonies were taken from the Lahore Garrison University research lab. Colonies were grown using Luria Broth at standard conditions and were incubated at 37° Celsius for 24 hours (Tallent et al., 2012). White-colored colonies were then stained by gram staining for further confirmation. Confirming chromium resistance is a crucial step before using the bacterial strain for chromium degradation so *Bacillus cereus* colonies were tested for chromium resistance by growing on the chromium-containing media. This medium was prepared by adding one percent chromium solution in Nutrient Agar.

Plant growth promoting rhizobacteria (PGPR) characterization

Plant Growth Promoting Rhizobacteria characterization is a testing procedure in which bacterial strains are checked for certain properties such as Nitrogen Fixation and Indole-3 Acetic Acid Production (Santoyo et al., 2021).

Nitrogen fixation

Bacillus cereus was checked for nitrogen fixation using Jensen's media. This media is a nitrogen-free

basal media and is used for checking the nitrogen fixation ability of bacteria. For this purpose, *B. cereus* was grown on Jensen's media and plates were incubated for five days at 37°C (Kumar et al., 2014).

IAA production test

Secondly, *Bacillus cereus* was tested for the indole acetic acid production. Most IAA-producing bacteria use tryptophan as a precursor for the production pathway (Laird et al., 2020). This is why the isolate was tested by growing in Jensen's broth containing 100 µg/ml of Tryptophan. Tubes were incubated at 37°C for 2 days in a shaking incubator to check the survival of the auxin-producing cells. After two days of incubation, the broth was centrifuged to check the auxin production. Cells were centrifuged at 15,000 rpm for about 60 seconds. Pellets were removed and the supernatant was taken for further testing of IAA presence. The supernatant was mixed with about two ml of Salkowski reagent. This mixture was incubated at room temperature for 30 minutes in the darkness (Mohite, 2013).

Preparation of nanoparticles

Waste egg shells were thoroughly washed with tap water until cleaned and then washed twice with distilled water to remove any impurities that might be present. Once cleaned the egg shells were heated in the oven for two hours at 160°C to remove moisture from them. Once egg shells were dried they were ground properly in sterilized mortar and pestle until converted to powdered form. These powdered egg shells were stored in a polyethene bag and placed in an airtight container until used for the preparation of calcium oxide nanoparticles (Ahmed et al., 2022).

CaO nanoparticle synthesis by Sol-gel method

Although there are several methods for the preparation of nanoparticles, the Sol-gel method is a very easy, cheap, least time-consuming, and cost-effective procedure as compared to others. In this study, calcium oxide nanoparticles were synthesized using the prepared eggshell powder, sodium hydroxide and hydrochloric acid. For this purpose, a flask completely covered with brown paper was taken and 250ML of 1M HCL was added to it. 12.5 grams of eggshell powder was measured using a weighing balance, mixed in 1M HCL solution and kept at room temperature until further processed (Bokov et al., 2021). Solid calcium carbonate was added to the solution to form the metal salt which means calcium

chloride. This solution was further used for the synthesis of nanoparticles by adding NaOH solution. 250 ml distilled water was added in another autoclaved and sterilized flask. 10 grams of sodium hydroxide pellets were added into this distilled water. Sodium hydroxide pellets were measured using an accurate weighing balance. The solution was mixed until it was turbid and heat production was observed. Once the NaOH solution was prepared, it was mixed with the HCl and eggshell powder solution slowly and drop by drop. Solutions were then mixed by stirring continuously using a glass stirrer. Once the mixing was done, the solution-containing flask was covered with the porous membrane and placed at room temperature for the next 24 hours to allow the condensation to happen. This whole procedure was performed at room temperature (Habte et al., 2019). After 24 hours of condensation, water was removed from the prepared solution using Whatman's filter paper filtration. This obtained precipitate was washed with distilled water twice. To remove moisture completely, the precipitates were placed in a hot air oven for the next 24 hours at 60°C.

Calcination

Calcination of the dried powder was performed in a muffle furnace because it provides very high temperatures. The powder was kept in the furnace for one hour at 900°C and then the furnace was turned off to let the powder cool down (Bano & Pillai, 2020).

Characterization of synthesized nanoparticles

Fourier transform infrared spectroscopy FTIR was used for characterization of the calcium oxide nanoparticles. FTIR technique uses the infrared absorption spectrum to identify the chemical bond in the molecules (Eid, 2022). The absorption spectrum of synthesized calcium oxide nanoparticles was recorded.

Pot preparation

The germination soil was taken from the ground of Lahore Garrison University. The soil was autoclaved to remove any harmful pathogens that might be present in it (Hu et al., 2020). 30 plastic pots were taken and labelled accordingly. 170 grams of soil on average was added in each pot using a weighing balance. A total of 8 groups of the pots were prepared out of which two were control and six were experimental (Table 1).

To check the chromium degradation, soil used for the experimental groups 1, 3, and 5, and control group 2 was stressed with a 1% chromium solution. Solution was prepared by adding 1 gram of potassium dichromate in 100 grams of distilled water. 50 micrograms of chromium per gram of the soil (50µg/1g) was added to the pots using a sterile micropipette. Additionally, 3 grams of calcium oxide nanoparticles were added into the pots of groups 5, 6, 7, and 8. Nanoparticles were found to remediate soil only when added 2.5 grams or more per pot in a separate control experiment. The nanoparticles were then added to the respective experimental groups.

Sowing seeds in the pots

Sunflower (*Helianthus annuus*) seeds were purchased from a local store in Lahore. Seeds were washed with distilled water and then dipped into 1% mercuric chloride solution for 30 seconds for disinfection. Mercuric chloride is considered a good disinfectant that can efficiently remove surface bacteria (Darkazanli and Kiseleva, 2021). Half of the seeds were inoculated with *Bacillus cereus* by dipping into the bacterial broth. Pots were differentiated based on whether they will have inoculated seeds or not. Groups 3, 4, 7, and 8 were labelled and sowed with inoculated seeds. Pots in groups 1, 2, 5, and 6 were sowed with the seeds washed with mercuric chloride.

Germination and watering

Sterilized water was used for watering the seeds in order to assure the sole activity of *B. cereus*. At first day, enough water was added to moist and drench all the soil in the cups. After day one, 2ml of autoclaved water was added to all the pots daily until clear germination was observed on day 7. Percent germination was recorded and the grown seedling was harvested after seven days. The length of the shoot and root of all the seedlings and groups was measured in centimetres. All the experimental and control experiments were performed by making 2 sets of each. Average results were noted with representative plants used as observation materials.

RESULTS

Clear white-colored colonies grown on plates appeared purple/blue under the light microscope indicating their gram-positive nature. The growth of the colonies on the chromium plate further confirmed the isolation of *Bacillus cereus*.

Nitrogen is an essential factor in the growth of crops and plants because they cannot utilize environmental nitrogen directly. Some of the bacterial species can fix the nitrogen present in the soil according to the plants' needs. Bromothymol blue was added in the Jensen's Media to trace nitrogen fixation. Nitrogen Fixation test for *Bacillus cereus* resulted in blue color production proving that the bacterial isolate has plant growth-promoting potential. Furthermore, Salkowski reagent used for the IAA production by bacteria grown in Jensen's media contains 0.5M Ferric Chloride (FeCl₃) and 35% Perchloric acid. It produces pink color in the presence of Indole-3 Acetic Acid. Once 30 minutes incubation at room temperature was done after centrifugation and the addition of the Salkowski reagent, a pinkish-red shade was produced. This confirmed that *Bacillus cereus* is capable of producing IAA or auxin and hence exhibits PGPR characteristics.

Morphological characteristics of isolate

Morphological characteristics of the isolate were observed on the plate. Colonies were white in color and margins were smooth. Colonies were granular in form with a raised elevation. The texture was smooth to mucoid while the size was small to moderate.

Characterization of synthesized CaO nanoparticles

- Peaks obtained for the calcium oxide nanoparticles were 1397.53 cm⁻¹, 871.41 cm⁻¹, 711.72 cm⁻¹, 588.15 cm⁻¹, 574.70 cm⁻¹, 559.13 cm⁻¹, 536.16 cm⁻¹, and 531.68 cm⁻¹.
- Peaks obtained for calcium carbonate solution were as follows; 1397.53 cm⁻¹, 871.41 cm⁻¹, 711.72 cm⁻¹. The first main peak which is 1397.53 showed COO⁻ groups in a class of carboxylic acid salts. Moreover, the rest of the sharp bands showed the Co bonds present in the CaCO₃.
- Eggshell powder was used as a control in this spectroscopy and hence it proved that there were different functional groups on the calcium carbonate and calcium oxide nanoparticles when compared to the eggshell powder.

Germination

Seeds were observed for germination for seven consecutive days in order to record the pattern. There was no clear germination in any of the pots for first 3 days. On day 4, mild germination was observed in pots that contained no chromium as well as pots that contained either *B. cereus*/nanoparticles or both, regardless of chromium stress. Root and shoot length

increased significantly by day 7. There was no germination observed in Control 2, pot containing chromium with no mediator present (Table 2).

Harvesting

All of the seedlings were harvested on day 8th after 7 days of watering. Harvesting was done gently such that roots were not damaged. Shoot and root lengths were measured in cm using a measuring tape. The average weight of the seedling was measured using a weighing balance.

DISCUSSION

The persistence of chromium in soil is a serious environmental threat which requires innovative remediation. To address this, there have been a lot of studies aiming at remediating chromium in several possible ways so that they can be degraded to a less harmful form. This study was conducted to assess the chromium degradation ability of the bacterial strain *Bacillus cereus* as well as the nanoparticles derived from eggshells. Although both bacteria and nanoparticles have been used for remediation widely but this study used the synergistic effect of biological and chemical method to remediate the chromium, particularly. Although *Bacillus cereus* is known to have ability to degrade heavy metals such as chromium, there is no study using calcium oxide nanoparticles as the chromium detoxifier. However, the growth trends in the results depicted that calcium oxide nanoparticles can be used as potential chromium degraders as there were visible growth patterns when sunflower seeds were inoculated with CaO nanoparticles despite the chromium stress in the soil. As shown in Table 3, sunflower seeds sowed in the chromium loaded soil (control; group 6) without microbe and nanoparticles showed no growth at all, even after seven consecutive days of watering. This suggested that chromium in the soil inhibited seed germination as there was visible growth in normal agricultural soil (control; group 1). There are several possible explanations for how chromium ions can interact with the seed or a plant to inhibit its germination and growth. Toxic chromium ions can interfere with seed germination, nutrient uptake by the roots, root length, shoot length, and photosynthesis by posing an alteration in the synthesis of photosynthetic pigments, leaf growth, stem growth, and plant biomass. The abundance of chromium in the

agricultural soil can also result in the DNA strand break and chromosome aberration of particular plant species (Paul et al., 2024). However, It was observed that when seeds were inoculated with *Bacillus cereus* before sowing into the chromium-loaded soil, not only they germinated but also grew the shoot and root. Compared to control 2 in which soil contained only chromium without any *Bacillus cereus*, the only possible explanation for the germination of the seeds is that this bacterial strain can remediate or degrade the chromium into a less toxic form allowing the seed to germinate. The suggested mechanism from the pathway studies, with which microorganisms remediate heavy metals involves the following steps. First of all hexavalent chromium ions get attached to the ligand of the cell membrane of microorganisms. This results in the formation of a metal-ligand complex which resides on the surface of the cell membrane. On the detection of a complex molecule formed at the surface of the cell, transporter proteins present at the cell membrane get activated. This activation results in the transportation of this metal-ligand complex into the cytosol. Inside cytosol, metal-binding amino acids such as methionine interact with the complex resulting in the precipitation and methylation of the ions. This results in a change in the composition of the metal ions resulting in the degradation. Most of the time chromium hexavalent ions are changed into chromium trivalent ions. This is a less toxic form and poses less threat to human and other organisms' health. However, these metals can also inhibit the growth of the microorganism when present at relatively higher concentrations (Ayele and Godeto, 2021). In a similar manner, group 6 (Table 3) where chromium-loaded soil was treated with CaO Nanoparticles, visible germination and growth was observed. This indicated that CaO nanoparticles can also be used to detoxify this chromium similarly. However, the mechanism which is used by nanoparticles is different compared to the microbes. Nanoparticles are very small molecules having diameters ranging from 1 to 100 nanometers and have a very high volume-to-surface area ratio. This allows them to perform activities which their mega counterparts are unable to do. They chemically react with heavy metals resulting in the conversion of the toxic ions into non-toxic forms (Calderon and Fullana, 2015).

As in table 3, the first four groups showed significant seed germination as well as plant shoot and root length. These groups contained no chromium in the soil in which the seeds were germinated. However, the growth trend indicates that the soil cups which contained *B. cereus*, nanoparticles or the combination of both showed more growth as compared to the control i.e., only soil. Group 1 with normal soil and seeds showed significant growth because there was no chromium stress present in the soil to begin with. Groups 2, 3 and 4 also had stress free soil but with the presence of potential remediators. Group 2 had *Bacillus cereus*, group 3 had calcium oxide nanoparticles, while group 4 had a combined effect of *Bacillus cereus* and nanoparticles. Groups 2 and 3 showed almost similar growth which suggested that bacterial strain and nanoparticles posed similar effects on the plant growth. Group 4 has significantly higher shoot and root length as compared to the first three groups which means that combined and synergistic effects of bacteria and nanoparticles potentially increased plant growth. The Trend for the number of seeds germinated in the soil cups also increased from group 1 to group 4. However, there were zero seeds germinated in group 5 (Control 2) indicating that chromium stress did not allow seeds to germinate at all. In groups 6, 7, and 8, seeds germinated despite the presence of chromium in the soil suggesting that bacterial strain and nanoparticles were effective in the remediation of chromium ultimately resulting in the germination of seeds.

The growth trend of the shoot length in all the seedlings; Group 1 had significant shoot lengths because there was no stress and similarly group 2, 3 and 4 had significantly good shoot lengths. It can be seen that group 2 had a little more shoot length as compared to group 1 which is because of the bacterial strain and its PGPR characteristics while group 3 had more positive effects on the shoot length as compared to the bacterial strain. This indicated both bacterial strain and nanoparticles were proven to improve the growth however they affect the plant differently. Root length trends were similar to the shoot length. Groups 1, 2, 3, and 4 showed increasing root length respectively. These groups did not contain chromium in the soil but increased root length in microbe and nanoparticle-containing soil further demonstrated plant growth-promoting activity.

Plant Growth Promoting Rhizobacteria (PGPR) testing of the *Bacillus cereus* showed that it is capable of nitrogen fixation as well as Indole Acetic Acid IAA production. Production of indole acetic acid by bacteria poses physio-biochemical activity on plants by modification of root and shoot. Plant interaction with these plant growth-promoting rhizobacterial species results in the mitigation of drought resistance. This is why the Plant-microbe interaction when the microbe is PGPR is beneficial for plant growth. As *Bacillus cereus* was tested positive for indole-3 acetic acid production and nitrogen fixation, the root and shoot length increase is potentially because of these PGPR characteristics. Most of the time rhizobacteria tend to colonize the roots because these bacteria are capable to produce many metabolites and enzymes which help plants fight against drought conditions (Ahmad et al., 2022). Another important factor in plant growth is nitrogen fixation. Compromised soil health by the toxic materials from the environment leads to decreased nitrogen availability for plant growth. Another reason is that nitrogen present in the environment is needed to be fixed before it can be used by the plants for their needs. This indicated that one of the reasons for increased shoot and root length in the pots with *Bacillus cereus* was its ability to fix nitrogen (Soumare et al., 2020).

Similarly, in addition to remediating the chromium present in the soil, nanoparticles are also capable of enhancing plant growth by several mechanisms. It is proven in several studies that nanoparticles can be a good platform to transfer agrochemicals in plants. This results in an increase in plant growth and biomass as well as increasing resistance to stress conditions. In current studies, nanoparticles are also being used as a gene delivery vehicle for enhanced and controlled plant growth for achieving certain characteristics (Mali et al., 2020). Calcium nano oxides provide a calcium source to the plant which helps plants in resisting heavy metal uptake. In addition to this, they also adjust the acidity of the plant soil by changing the pH, which results in enhanced nutrient uptake thus leading to better plant growth. However, it is also observed that long-term exposure to the nanoparticles, especially metal oxide nanoparticles, can lead to reduced nutritional quality in the plants (Rui et al., 2018). This technique is, however, yet to be studied at larger scale with large sample size to assess its potential at industrial scale.

CONCLUSION

It was observed from this research study that both *Bacillus cereus* and calcium oxide nanoparticles can degrade chromium to its non-toxic form indicated by plant growth in chromium stressed soil. Additionally, combination of *Bacillus cereus* and CaO nanoparticles was found to be more effective in chromium degradation as compared to *Bacillus cereus* and nanoparticles individually. Moreover, roughly a 15% increase was observed in shoot & root length as well as biomass in the seedlings which germinated in mediator(s) containing soil as compared to the seedlings grown in normal soil. Conclusively, this research study suggested that the synergistic effect of *Bacillus cereus* and eggshell derived calcium oxide nanoparticles is a potential cost-effective technique for both chromium remediation and enhanced plant growth by modifying growth mechanisms and stress resistance patterns.

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Statement of conflict of interest

None to declare.

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Table 1: Pot Preparation

	Group	Chromium	<i>Bacillus Cereus</i>	CaO Nanoparticles
1.	Control 1	No	No	No
2.	Control 2	Yes	No	No
3.	Experimental 1	Yes	Yes	No
4.	Experimental 2	No	Yes	No
5.	Experimental 3	Yes	No	Yes
6.	Experimental 4	No	No	Yes
7.	Experimental 5	Yes	Yes	Yes
8.	Experimental 6	No	Yes	Yes

Table 2: Germination Trend of Sunflower

Day	Control 1	Control 2	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6
Day 1	No G	No G	No G	No G	No G	No G	No G	No G
Day 2	No G	No G	No G	No G	No G	No G	No G	No G
Day 3	Clear G	No G	No G	Clear G	No G	Clear G	No G	Clear G
Day 4	Clear G	No G	Little G	Clear G	Little G	Clear G	Little G	Clear G
Day 5	Clear Shoot	No G	Clear G	Clear Shoot	Clear G	Clear Shoot	Clear Ger.	Clear Shoot
Day 6	Shoot growth	No G	Clear Shoot	Shoot growth	Clear Shoot	Shoot growth	Clear Shoot	Shoot growth
Day 7	Shoot growth	No G	Shoot growth	Shoot growth	Shoot growth	Shoot growth	Shoot growth	Shoot growth

Table 3: Growth Trends in different pots of Sunflower

STRESS	Plant Group	Remediators in Soil	Percentage of seeds germination	Average root Length (cm)	Average Shoot Length (cm)	Seedling Average Weight (g)
WITHOUT CHROMIUM	Group 1	Control	100%	0.85±0.15	4±0	0.19±0.01
	Group 2	<i>B. cereus</i>	100%	0.5 ±0.0	4.75±0.25	0.285±0.015
	Group 3	Cao NP	100%	0.9±0.1	3.9±0.1	0.455±0.025
	Group 4	Combination	100%	0.7±0.1	5.75±0.25	0.185±0.015
WITH CHROMIUM	Group 5	Control	0%			
	Group 6	<i>B. cereus</i>	100%	0.15±1	1.45±0.25	0.36±0.02
	Group 7	Cao NP	50%	0.2±0.0	2±0	0.4±0.0
	Group 8	Combination	100%	0.9±0.1	2.95±0.15	0.21±0.09