

Article

Field Studies on the Effect of Bioaugmentation with *Bacillus amyloliquefaciens* FZB42 on Plant Accumulation of Rare Earth Elements and Selected Trace Elements

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Abstract: This study is an investigation of the effect of soil bioaugmentation (inoculation) on a field scale with the commercially available product RhizoVital[®] 42, containing *Bacillus amyloliquefaciens* FZB4, on element bioavailability, plant biomass production, as well as accumulation of rare earth elements (REEs), germanium, and selected trace elements. *Zea mays* and *Helianthus annuus* were selected as test plants. Post-harvest, results showed inoculation increased biomass production of *Z. mays* and *H. annuus* by 24% and 26%, albeit insignificant at $p \leq 0.05$. Bioaugmentation enhanced *Z. mays* shoot content of P, Cd, and Ge by percentages between 73% and 80% (significant only for Ge) and decreased shoot content of REET, Pb, and Cu by 28%, 35%, and 59%, respectively. For *H. annuus* grown on bioaugmented soil, shoot content of Ca, Cu, Ge, REET, and Pb increased by over 40%, with a negligible decrease observed for Cd. Summarily, results suggest that bioaugmentation with *Bacillus amyloliquefaciens* FZB42 could enhance biomass production, increase soil element bioavailability enhance, and increase or reduce plant accumulation of target elements. Additionally, differences in P use efficiency could influence bioaugmentation effects on P accumulation.

Keywords: bioaugmentation; *Bacillus amyloliquefaciens*; RhizoVital[®] 42; bioavailability; phytoextraction; trace elements; germanium; rare earth elements



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1. Introduction

Many of the elements used in today's society are extracted from minerals [1]. Examples of such elements are germanium (Ge), rare earth elements (REEs), phosphorus (P), magnesium (Mg), calcium (Ca), cadmium (Cd), lead (Pb), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), chromium (Cr), nickel (Ni), etc. [2–4]. These elements could be classified as plant nutrients, heavy metals and essential raw materials, or critical raw materials (Ge and REEs) [1,5–7]. They are very useful in metabolic and physiological processes in plants as well as in chemicals and high-tech producing industries [8,9] but could become toxic at concentrations high concentrations in the environment [10–12]. Increased environmental concentrations of these elements sometimes come from their extraction from the soil via mining, as well as other activities such as excess fertilizer application. This often leads to severe environmental impact including contamination of soil and groundwater [1,6,13–16]. Such impact often leads to adverse effects on the biochemical and physiological processes in plants and causes a deterioration in soil physical and biological characteristics [17,18]. To remedy the adverse impact on the environment, especially soil, gentle remediation

options such as phytoremediation and bioaugmentation have been suggested as effective risk management approaches to reduce and limit the mobility and transfer of contaminants to organisms and other compartments of the environment [15,19] by stabilizing them or extracting them from soil using plants. In the same vein, a sustainable mining approach called phytomining—a strategy of the phytoremediation category of phytoextraction—which uses plants to extract raw materials from the soil, is suggested as a possible eco-friendly means of recovering raw materials from the soil in times of increasing demands for minerals. These eco-friendly phytotechniques are widely accepted and cost-effective, and they do not require the invasive/intensive processes associated with conventional chemical and physical remediation methods such as excavation and application of chemicals [20,21]. Besides the selection of plant species for phytoaccumulation purposes, which is important because plants have various capabilities for phytoremediation [22], another factor that is critical to the success of phytoremediation and phytomining is soil biological activity because of the roles microorganisms play in plant growth and availability of elements for accumulation by plants [23–25], which can be enhanced via bioaugmentation. The process of bioaugmentation introduces microorganisms capable of transforming and degrading contaminants to non-toxic or less toxic species [26,27]. Bioaugmentation of soil with plant growth-promoting rhizobacteria (PGPR) can increase plant growth and biomass production, in addition to increasing element availability in soil, which could improve the overall phytoextraction of elements from soil including nutrients [4,28,29]. Mechanisms involved in achieving these effects include, but are not limited to, phosphate and mineral solubilization, production of phytohormones and macromolecule-degrading enzymes, as well as volatile growth stimulants [30,31].

Several studies have reported the effects of bioaugmentation or inoculation of soil with PGPR on plant element accumulation. Pot and field studies by Kumar et al. [32] stated that PGPR identified as *Bacillus* sp., *Pseudomonas* sp. and *Rhizobium leguminosarum* increased nutrient accumulation, plant growth, and biomass either as single species or in consortia. *B. licheniformis* has been reported to have enhanced accumulation of Cu, Cd, Pb, and Cr [33], while *B. amyloliquefaciens* BSL16 has been reported to increase Cu accumulation and growth of rice seeds and tomato plants during Cu stress [34]. Schwabe et al. [35] reported increased shoot content of Ge and REEs upon inoculation with PGPR, and in another study, Rajkumar and Freitas [36] also observed that the inoculation of *Ricinus communis* with *P. jessenii* PjM15 or *Pseudomonas* sp. PsM6 enhanced biomass production and phytoextraction efficiency of zinc (Zn), nickel (Ni), and copper (Cu) by the production of indole-3-acetic acid (IAA) and solubilizing phosphate.

Additionally, based on similar chemical characteristics of elements, the bioavailability of some toxic elements and their effects on plants are linked to plant nutrient status and/or competition among elements at uptake channels or soil binding sites and the inoculation of soil/plants with PGPR does affect these elemental relationships. For example, Cd and Fe are chemically similar, and Cd pollution in the soil is many times associated with deficiency of Fe in soil [37,38]. This deficiency can be alleviated via soil inoculation with PGPRs, which could lead to the formation of bioavailable Fe-siderophore complexes, thus making Fe available for plant accumulation [39]. Furthermore, it has been reported that phosphorus mobilization can help mobilize toxic elements in soil [40], thus making them bioavailable for plant accumulation. Several studies have reported that PGPR solubilizes insoluble phosphates, thereby making P available for plant accumulation [41–43]. Thus, soil inoculation with PGPR to increase the abundance of PGPR above that normally found in soil, with the hope of mobilizing essential elements in soil such as Fe and P, which play critical roles in the availability of other elements, is considered a strategy for enhancing the bioavailability of some other elements in soil and their accumulation by plants. This is because processes involved in P and Fe mobilization could result in a change in rhizosphere chemistry and plant root structure in such a way that promotes the availability and accumulation of other elements [44–46]. Additionally, the mobilization of plant Fe and P is important because

many trace elements are bound to oxides of Fe [47] and phosphate compounds [48], and their accumulation by plants shows correlation with that of Fe [49].

In most of these studies, the source of the inoculants has not been commercially available microbial formulation, nor have most of these studies been under field conditions. Additionally, very few of these studies have considered the effect of Fe acquisition strategies (strategies 1 and 2) and phosphorus accumulation efficiency of test plants in assessing the effect of bioaugmentation with PGPR under field conditions. Strategy 1 (mainly exhibited by non-graminaceous plants) involves the pumping of protons into soil and secretion of carboxylates, phenolics, and other compounds to acidify the rhizosphere and increase the solubilization of Fe, while strategy 2 (mainly exhibited by graminaceous plants) involves the secretion of phytosiderophores by plants into the soil for solubilization of Fe [50,51]. Additionally, *H. annuus* has been reported to have a higher P uptake efficiency than *Z. mays* [52]. Thus, how bioaugmentation with commercially available PGPR affects Fe and P bioavailability and accumulation by these plants of different Fe acquisition strategies and different levels of P uptake efficiency is important for phytoextraction, especially when plants are grown under field conditions.

Therefore, the general aim of this study was to elucidate the effects of soil inoculation with commercially available microbial formulation RhizoVital®42, containing spores of *B. amyloliquefaciens* FZB42, on bioavailability, accumulation of sum of rare earth elements represented as REET, Ge, selected macronutrients, and trace elements (P, Ca, Fe, Cd, Pb, and Cu) under field conditions, using *Helianthus annuus* (forb, strategy 1 plant) and *Zea mays* (grass, strategy 2 plant) as test plants, with a special interest in the effects of differences in P use efficiency and Fe acquisition strategies on effects of inoculation on plant P and Fe accumulation behaviour. We hypothesized that inoculation with commercially available microbial formulation RhizoVital®42 containing spores of *B. amyloliquefaciens* FZB42 would increase target element bioavailability in soil, as well as their concentration and accumulation in plants.

2. Materials and Methods

2.1. Field Site Characterization

Soil samples were collected randomly throughout the fields at depths of between 15 and 20 cm for purposes of soil characterization. Physicochemical properties of soil, total element concentrations, and concentrations of readily available soil element fractions are reported in Table 1. Soil conductivity, determined according to methods stated in Wiche et al. [53], was 351 $\mu\text{S}/\text{cm}$, and pH ranged from 4.9 to 6.2, at different plots across the field, but with an average of 5.6, which is marginally in the optimal range for soil microbial functions and nutrient availability but not for bioavailability of many PTEs and REEs [54,55]. Total concentrations of Cd and Pb (Table 1) were more than the threshold values allowed for European soils as reported by Tóth et al. [56], due to previous mining activities in the region of Freiberg, Germany. However, the soil was still productive and fertile. Concentrations of water-extractable fractions of investigated are shown in Table 1. Concentrations of PTEs were in the order of $\text{Cu} > \text{Pb} > \text{Cd}$. The total sum of REEs was much higher than that of Ge, a pattern similar to observations of Okoroafor et al. [57] for readily available concentrations of these elements in the soil. For selected nutrients, concentrations were in the order of $\text{Ca} > \text{Fe} > \text{P}$, respectively (Table 1).

Table 1. Soil Physicochemical parameters and concentration of elements in soil.

| Soil physicochemical parameters | | |
|--|---------------------|-----------------------------|
| Soil physicochemical parameters | Value | |
| pH value in aqueous solution | 5.6 | |
| Conductivity | 351 μ S/cm | |
| Organic matter content | 7.3% | |
| Nitrate concentration | 160.0 mg/kg | |
| Ammonium concentration | 1.4 mg/kg | |
| Phosphate concentration | 42.9 mg/kg | |
| Total concentration (μ g/g) and water-soluble concentration (mean \pm SE, $n = 6$) | | |
| | Total concentration | Water-soluble concentration |
| P | 477 \pm 38 | 1.26 \pm 0.41 |
| Ca | 2689 \pm 115 | 48 \pm 5.6 |
| Fe | 23,570 \pm 1937 | 7.40 \pm 0.78 |
| Cu | 31 \pm 1.8 | 0.19 \pm 0.012 |
| Cd | 1.9 \pm 0.08 | 0.0052 \pm 0.0003 |
| Pb | 228 \pm 18 | 0.12 \pm 0.007 |
| REET | 109 \pm 11 | 0.03 \pm 0.002 |
| Ge | 0.63 \pm 0.03 | 0.001 \pm 0.0001 |

2.2. Plant Growth Experiment and Soil Inoculation

Zea mays and *Helianthus annuus* were grown on the agricultural fields of Fachschulzentrum Zug, Freiberg, on small sized plots of 2.25 m \times 2.25 m size, with each plot separated from the next by 1.5 m gap. Plots were assigned to plant species and treatment according to a randomized design. Within each small-sized plot, plants were sown in three rows with a 75 cm gap between each row, with a distance of between 10 and 20 cm between each individual plant stand. Plants grown on non-inoculated soil served as the reference for those grown on soils inoculated with *B. amyloliquefaciens* FZB42. Plot inoculation rate of approximately 0.04% (4 mL inoculum in 10 L water) per application was used, with the source of inoculum being the commercially available product RhizoVital[®] 42 (supplied by ABiTEP GmbH Berlin, Germany), which contains 2.5×10^{10} CFU (colony forming units) of *B. amyloliquefaciens* FZB42 per milliliter. The growing period lasted for 17 weeks, starting from 27 May 2020, and soils were inoculated twice within this period, with the first inoculation taking place on 15 July 2020 and the last inoculation two weeks after the first.

2.3. Incubation Experiment

In addition, an incubation experiment was conducted to assess the effect of inoculation on the bioavailability of elements without influence from plants. For this, 30 mL of deionized water was added to each of two sets of eight replicates of 1 g of homogenized soil in 50 mL uncorked Teflon tubes. To one set, 200 μ L of commercially available product RhizoVital[®] 42 containing *B. amyloliquefaciens* FZB42 was added, and to the other set, nothing was added. The setup was allowed to stay for 6 days, after which the tubes were shaken and centrifuged at 5000 rpm, and the supernatant was collected for measurement of trace elements using ICP-MS.

2.4. Sample Preparation and Analysis

2.4.1. Soil Samples

Assessment of total element concentrations was performed via modified aqua-regia digestion. To this end, 100 mg of fine soil dried at 105 $^{\circ}$ C was mixed with 200 μ L water and four acids (900 μ L hydrochloric acid, 300 μ L nitric acid, 300 μ L hydrofluoric acid, and 150 μ L perchloric acid) and subjected to microwave digestion, with the ratio of acid mixture guided by Uddin et al. [58]. Determination of bioavailable element fractions of uncultivated soil samples, as well as root soils (collected from the roots of each harvested

individual plant in a plot and then aggregated and harmonized as one sample), was carried out via single-step extraction using deionized water and 0.1 M calcium chloride (CaCl_2) as extractant. Water-soluble element fractions of the total concentration of elements in soil were obtained by shaking approximately 1 g of soil, dried at 105 °C in 5 mL of distilled water for 24 h, while calcium-chloride-extractable fractions were obtained by shaking 2 g of soils dried at 105 °C in 20 mL of 0.1 M CaCl_2 for 3 h, according to methods described by Petruzzelli et al. [59], after which resulting mixtures from both extraction methods were centrifuged at 5000 rpm, and the supernatant was collected for element concentration determination via ICP-MS.

2.4.2. Plant Samples

The plants were harvested using a 75 cm² quadrant, and the dried above-ground biomass of plants was obtained from the quadrant extrapolated to 1 m². Plants were dried at 60 °C in an oven (model SIM 500, Memmert, Schwabach, Germany) for 48 h to obtain constant weight. Subsequently, the dry mass of the samples was determined and ground to a fine powder using an ultra-centrifugal mill (model ZM1000, Retsch, Haan, Germany). Then, 100 mg of the dried plant sample were weighed out for digestion in the microwave (MLS-ETHOS plus, MLS GmbH, Dorsten, Germany), according to Krachler et al. [57]. Before digestion, the samples were mixed with 200 µL ultra-pure water and 1.9 mL nitric acid and left overnight to react, before adding 600 µL 4.9% hydrofluoric acid. After digestion, samples were transferred into 15 mL centrifuge tubes, and the volume was made up to 10 mL. For measurement of trace elements, Ge, and REEs using ICP-MS (model X Series 2, Thermo Fisher Scientific, Dreieich, Germany), 1 mL each from the diluted samples was further transferred to 15 mL Teflon tubes, before adding 100 µL of internal standard solutions containing 1 mg/L of rhodium and rhenium according to Krachler et al. [60], and subsequently, they were made up to 10 mL.

2.5. Determination of Concentration of Elements in Soil and Plant Samples

The resulting solutions from microwave digestion of plant and soil samples, water, and CaCl_2 soluble extraction and incubation experiments were diluted, and element concentrations were determined using ICP-MS (model X series 2, Thermo Fisher Scientific, Dreieich, Germany). ICP-MS was equipped with a concentric glass nebulizer and cyclonic spray chamber. The torch operated at 1400 W. During the analysis, the cerium oxide (CeO) rate was less than 2%, and the $\text{B}^{++}/\text{B}^{+}$ ratio was less than 1%. Internal standards were 10 µg/L Rh and Re in concentrated nitric acid [53]. Possible interferences, especially on europium (Eu) by barium oxide (BaO), were monitored and corrected according to Pourret et al. [61]. Accuracy of the analytical process for plant and soil samples was checked by using certified soil and plant reference materials (GBW 07406, GBW 07407, NCS ZC73032, NCS ZC73030) [62,63]. The results deviated by less than 10% of the certified values.

2.6. Statistical Analysis

The statistical differences between treatments for each plant species with respect to element concentration, accumulation, and biomass production were evaluated using Welch's analysis of variance (ANOVA) test at the significance level of $p \leq 0.05$, using IBM SPSS Statistics 26 software.

3. Results

3.1. Effect of Inoculation on Element Bioavailability in Soil

Incubation experiment to check for effects of inoculation without the influence of plants on element bioavailability showed that inoculation increased concentration/bioavailability of Fe, Cu, Cd, Pb, and REET in soil solution by 15%, 67%, 57%, 38%, and 17%, while concentration on Ca decreased by 18%. However, the effects were only statistically significant for Cu (Figure 1).

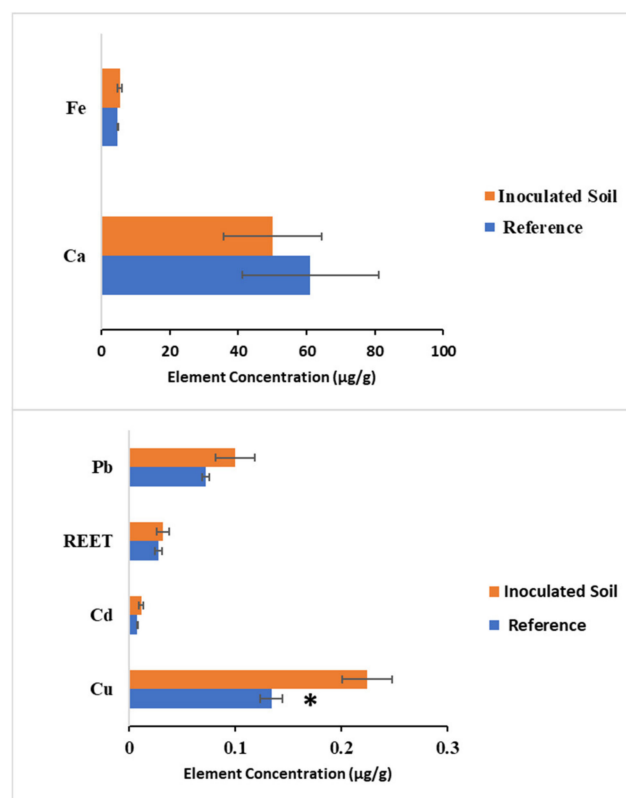


Figure 1. Incubation experiment: effect of soil inoculation on element concentration in aqueous extract per dry weight of soil. Significant difference ($p \leq 0.05$) between means indicated by asterisks * (mean \pm SE, $n = 3$).

Additionally, calcium chloride extractable mobile fractions of rare earth elements and trace elements in root soils collected at harvest showed bioavailability/concentration of P, Cu, Cd, Pb, Ge, and REET increased in root soils of *Z. mays* grown on inoculated plots by 52%, 12%, 31%, 36%, 25%, and 9%, respectively (significant only for Cd), with a negligible effect on Fe concentration. Conversely, the concentration of P, Fe, Cu, Cd, Pb, Ge, and REET decreased in soils of inoculated *H. annuus* by 13%, 45%, 30%, 15%, 53%, 59%, and 40%, respectively (upper section of Table 2).

Using water as the extractant, bioavailability/concentration of Fe, Cd, Pb, Ge, and REET increased in root soils of *Z. mays* grown on inoculated plots increased by 15%, 99%, 36%, 29%, and 17%, respectively, while changes for Ca, P, and Cu were more or less negligible. Additionally, concentrations of Cu, Cd, Pb, and REET in inoculated *H. annuus* plots decreased by 11%, 41%, 31%, and 15%, respectively, while concentration changes for Fe and Ge were less than 9% and, therefore, considered negligible. Concentrations of P and Ca increased by 53% and 28%, respectively. However, none of the effects were statistically significant (lower section of Table 2).

Table 2. Concentration ($\mu\text{g/g}$) of elements in root soils (upper section of the table =calcium chloride extraction; lower section of the table =water extraction). Mean \pm SE, $n = 3\text{--}4$, NIL = reference, R = inoculated Soil. Statistics ^a means asymptotically distributed F statistic for Welch's ANOVA.

| Species | Treatment | P | Fe | Cu | Cd | Pb | Ge | REET | |
|--------------------------|------------------------|--------------|--------------|-------------|--------------|-----------------|-----------------|--------------------|----------------|
| <i>Zea mays</i> | NIL | 16.90 ± 6.26 | 1.68 ± 0.21 | 0.49 ± 0.02 | 1.51 ± 0.10 | 1.02 ± 0.52 | 0.0023 ± 0.0006 | 0.60 ± 1.87 | |
| | R | 25.62 ± 6.95 | 1.78 ± 0.33 | 0.55 ± 0.07 | 1.97 ± 0.06 | 1.39 ± 0.68 | 0.0029 ± 0.0004 | 0.65 ± 0.27 | |
| | Statistic ^a | 0.87 | 0.07 | 0.63 | 16.63 | 0.18 | 0.71 | 0.03 | |
| | <i>p</i> value | 0.40 | 0.81 | 0.50 | 0.02 | 0.69 | 0.46 | 0.88 | |
| <i>Helianthus annuus</i> | NIL | 24.21 ± 4.90 | 2.74 ± 0.82 | 0.58 ± 0.11 | 1.85 ± 0.10 | 2.29 ± 1.15 | 0.0080 ± 0.0060 | 0.92 ± 0.37 | |
| | R | 21.10 ± 4.47 | 1.51 ± 0.10 | 0.41 ± 0.04 | 1.57 ± 0.07 | 1.08 ± 0.62 | 0.0033 ± 0.0011 | 0.55 ± 0.23 | |
| | Statistic ^a | 0.22 | 2.222 | 2.205 | 4.951 | 0.844 | 0.585 | 0.722 | |
| | <i>p</i> value | 0.661 | 0.271 | 0.254 | 0.094 | 0.423 | 0.52 | 0.449 | |
| Species | Treatment | P | Ca | Fe | Cu | Cd | Pb | Ge | REET |
| <i>Zea mays</i> | NIL | 0.96 ± 0.43 | 40.15 ± 7.24 | 5.87 ± 0.56 | 0.17 ± 0.011 | 0.004 ± 0.00014 | 0.104 ± 0.019 | 0.00050 ± 0.000098 | 0.029 ± 0.0022 |
| | R | 0.94 ± 0.27 | 41.75 ± 7.90 | 6.73 ± 1.50 | 0.18 ± 0.017 | 0.007 ± 0.0037 | 0.141 ± 0.068 | 0.00064 ± 0.000083 | 0.034 ± 0.0071 |
| | Statistic ^a | 0.001 | 0.022 | 0.291 | 0.204 | 0.948 | 0.284 | 0.427 | 1.292 |
| | <i>p</i> value | 0.978 | 0.889 | 0.633 | 0.678 | 0.433 | 0.641 | 0.571 | 0.321 |
| <i>Helianthus annuus</i> | NIL | 0.88 ± 0.18 | 46.27 ± 3.87 | 6.29 ± 0.40 | 0.18 ± 0.00 | 0.0063 ± 0.0024 | 0.13 ± 0.03 | 0.00056 ± 0.000065 | 0.035 ± 0.004 |
| | R | 1.34 ± 0.61 | 59.17 ± 6.65 | 5.85 ± 0.75 | 0.16 ± 0.02 | 0.0037 ± 0.0003 | 0.09 ± 0.02 | 0.00051 ± 0.000033 | 0.029 ± 0.001 |
| | Statistic ^a | 0.537 | 2.811 | 0.275 | 0.627 | 1.192 | 1.618 | 0.422 | 1.795 |
| | <i>p</i> value | 0.509 | 0.16 | 0.625 | 0.482 | 0.385 | 0.283 | 0.562 | 0.297 |

3.2. Effects of Inoculation on Biomass, Concentration, and Accumulation of Investigated Elements by Plants

Zea mays grown on plots inoculated with *B. amyloliquefaciens* FZB42 produced 20% higher shoot biomass than plants grown on uninoculated plots. Similarly, *H. annuus* grown on inoculated plots produced 26% higher biomass than those grown on uninoculated plots (Figure 2). However, these effects of inoculation observed were not statistically significant. Inoculating plots with *B. amyloliquefaciens* FZB42 resulted in varying effects on the accumulation of elements by *Z. mays*. *Zea mays* grown on inoculated plots showed higher shoot content of P, Cd, and Ge by 73%, 80%, and 75%, respectively (significant for Ge,) while shoot content of Cu, Pb, and REET reduced by 59%, 35%, and 28%, respectively, while effect on Ca and Fe shoot content was negligible (Figure 3). Observations were similar for concentrations of elements in *Z. mays* grown on inoculated plots—plants grown on inoculated soils showed reduced concentrations of Ca, Fe, Cu, Pb, and REET by 20%, 19%, 71%, 40%, and 24%, respectively, and increased concentrations for Cd and Ge by 42% and 59%, respectively (Table 3). In addition, concentrations of P, Ca, Cu, and Cd increased by 134%, 48%, 22%, and 62% in roots of *Z. mays* grown on inoculated soils, while concentrations of Fe, Pb, Ge, and REET decreased by between 7% and 21% (Table 3). For *H. annuus* grown on inoculated plots, results showed that inoculation had negligible effects on P, Cd, and Fe accumulations but increased accumulations of Ca, Cu, Pb, Ge, and REET by 45%, 141%, 78%, 40%, and 66%, respectively; however, none was statistically significant (Figure 4). Concentrations of elements in *H. annuus* cultivated in inoculated plots showed that inoculation resulted in the decrease in P and Cd concentrations by 13% and 29%, respectively, while it increased concentrations of Cu, Ge, and REET by 18%, 20%, and 42%, respectively, with effects on Ca and Pb being more or less negligible (Table 3). Comparing element concentrations in *H. annuus* and *Z. mays* grown on uninoculated soils (Table 3), concentrations of elements were at least 40% higher in *H. annuus* (significantly higher for Ca and Cu) except for Ge, which was higher in *Z. mays*. Observations were similar for plants grown on inoculated soils, with concentrations of Ca, Fe, Cu, Pb, and REET being significantly higher in *H. annuus* than in *Z. mays*.

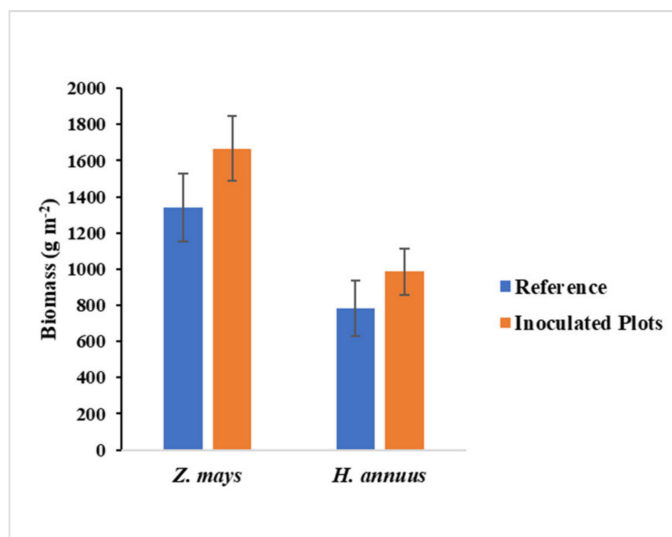


Figure 2. Effect of soil inoculation on biomass production by *Zea mays* and *Helianthus annuus*. Differences between means are not significant at $p \leq 0.05$ (mean \pm SE, $n = 3$ –4).

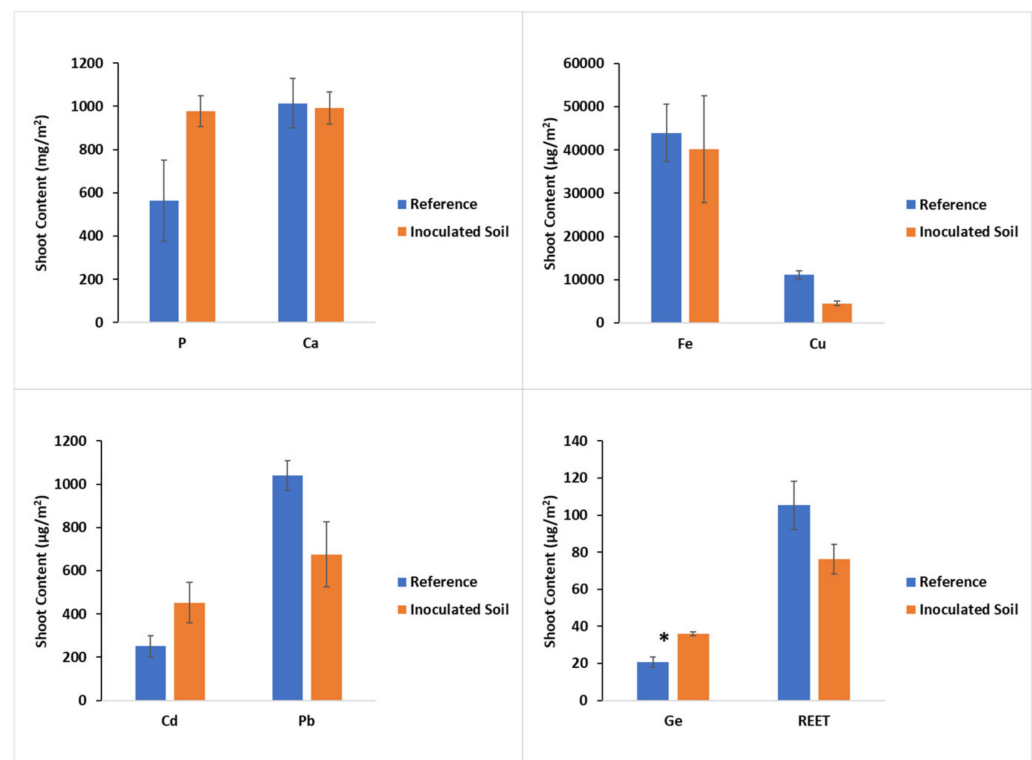


Figure 3. Effect of soil inoculation on element accumulation in above-ground biomass of *Zea mays*. Significant difference ($p \leq 0.05$) between means indicated by asterisks * (mean \pm SE, $n = 3$).

Table 3. Concentration ($\mu\text{g/g}$) of elements in shoots and roots. Mean \pm SE, $n = 3$ –4, NIL = reference, R = inoculated Soil. Statistics ^a means asymptotically distributed F statistic for Welch’s ANOVA. A = *Zea mays*. B = *Helianthus annuus*. SC = shoot concentration. RC = root concentration. vs. = compared with.

| Species | Treatment | P | Ca | Fe | Cu | Cd | Pb | Ge | REET |
|------------------|-----------------------------------|----------------|-------------------|----------------|-----------------|-----------------|-----------------|-------------------|------------------|
| A (SC) | NIL | 2336 \pm 286 | 4952 \pm 1061 | 172 \pm 39 | 64 \pm 13.7 | 1.04 \pm 0.23 | 4.39 \pm 1.04 | 0.07 \pm 0.003 | 0.49 \pm 0.094 |
| | R | 2534 \pm 361 | 3985 \pm 79 | 138 \pm 31 | 18 \pm 2.2 | 1.48 \pm 0.29 | 2.64 \pm 0.60 | 0.11 \pm 0.010 | 0.37 \pm 0.059 |
| | Statistic ^a p value | 0.14 0.73 | 0.82 0.46 | 0.39 0.56 | 10.53 0.08 | 1.17 0.35 | 1.96 0.24 | 6.08 0.13 | 1.03 0.37 |
| A (RC) | NIL | 130 \pm 14.0 | 1974 \pm 164 | 2386 \pm 424 | 6.69 \pm 1.29 | 1.41 \pm 0.08 | 25.4 \pm 4.02 | 0.13 \pm 0.022 | 13.05 \pm 1.87 |
| | R | 304 \pm 106 | 2916 \pm 330 | 1891 \pm 316 | 8.18 \pm 0.23 | 2.29 \pm 0.42 | 22.3 \pm 2.82 | 0.12 \pm 0.014 | 10.58 \pm 1.55 |
| | Statistic ^a p value | 2.67 0.24 | 6.55 0.09 | 0.88 0.41 | 1.29 0.37 | 4.31 0.17 | 0.42 0.56 | 0.11 0.76 | 1.03 0.37 |
| B (SC) | NIL | 3265 \pm 532 | 32,822 \pm 1199 | 437 \pm 77 | 114 \pm 6.5 | 12.0 \pm 4.4 | 11.2 \pm 2.4 | 0.019 \pm 0.002 | 1.4 \pm 0.3 |
| | R | 2829 \pm 84 | 35,253 \pm 1568 | 417 \pm 85 | 135 \pm 33 | 8.6 \pm 3.6 | 11.8 \pm 2.8 | 0.022 \pm 0.005 | 2.0 \pm 0.4 |
| | Statistic ^a p value | 0.66 0.50 | 1.52 0.27 | 0.03 0.87 | 0.37 0.58 | 0.37 0.58 | 0.02 0.89 | 0.41 0.55 | 1.81 0.24 |
| A vs. B (NIL SC) | Statistic ^a | 2.366 | 302.893 | 9.506 | 11.284 | 6.304 | 6.820 | 7.367 | 10.695 |
| | p value | 0.220 | <0.001 | 0.055 | 0.047 | 0.128 | 0.088 | 0.113 | 0.061 |
| A vs. B (R SC) | Statistic ^a | 0.516 | 396.341 | 9.206 | 12.357 | 3.782 | 10.175 | 3.090 | 20.161 |
| | p value | 0.542 | <0.001 | 0.039 | 0.038 | 0.146 | 0.042 | 0.220 | 0.018 |

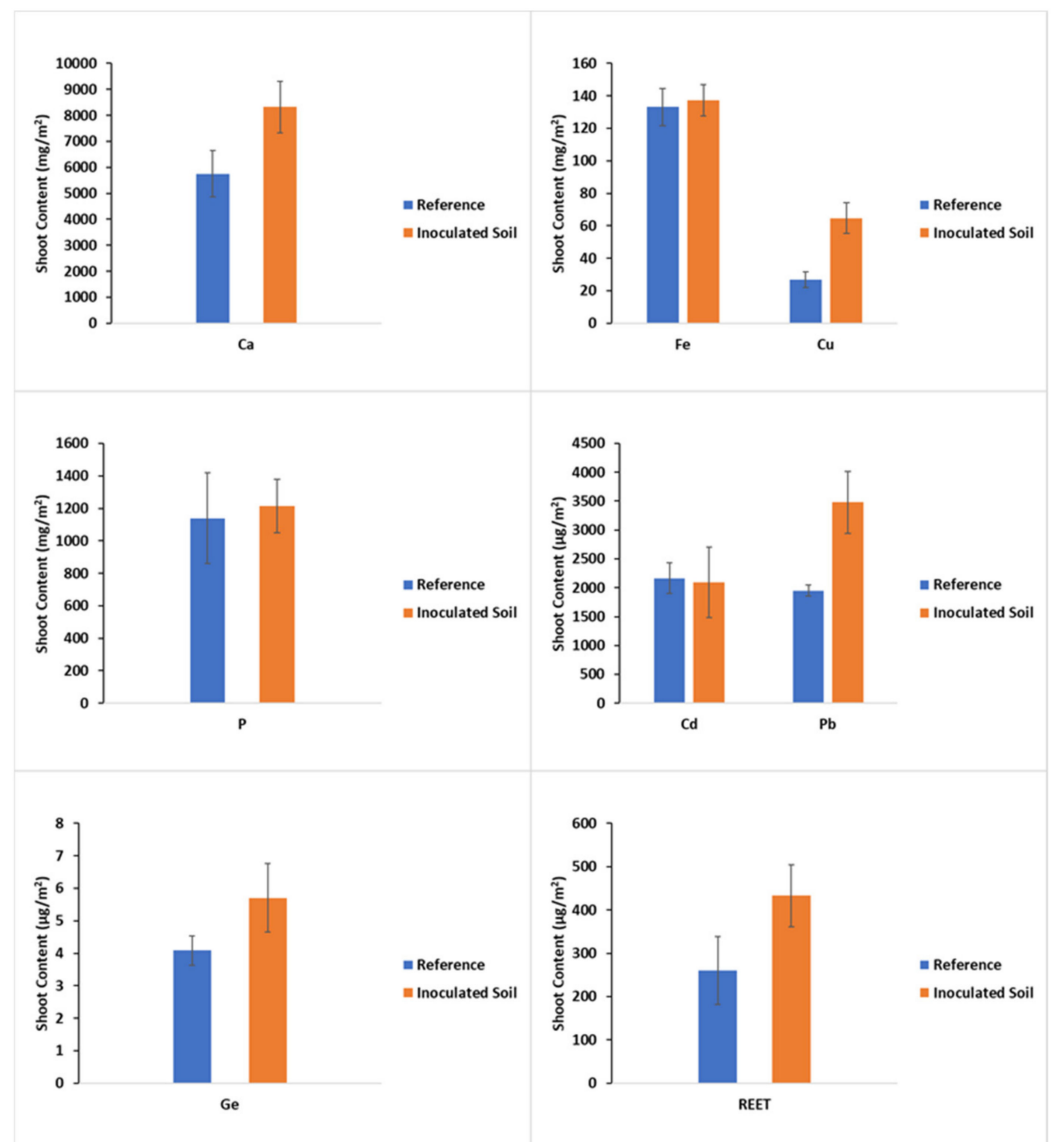


Figure 4. Effect of soil inoculation on element accumulation in above-ground biomass of *Helianthus annuus* (mean \pm SE, $n = 3\text{--}4$).

4. Discussion

4.1. Effect of Soil Inoculation on Biomass Production

Mineral nutrient uptake is critical for plant biomass production and based on classification of plant mineral nutrient sufficiency contained in Fässler et al. [64], *H. annuus* and *Z. mays* grown under uninoculated and inoculated soil conditions were sufficient in the mineral nutrients P, Ca, and Fe, with *H. annuus* having a significantly higher concentration of Ca and Fe than *Z. mays*, as indicated in Table 3, likely because of its Fe acquisition strategy (Strategy 2) and its reported higher phosphate use sufficiency. Given the sufficiency of nutrients in both plants under both soil conditions (unamended and inoculated soil), the non-significant effect of soil inoculation with *B. amyloliquefaciens* FZB42 on the accumulation of these mineral nutrients by both test plants is not surprising. This suggests that the non-significant but considerably higher plant biomass production of both plants grown on inoculated soil is mainly not caused by increased nutrient accumulation. Rather, the increased biomass production was most likely caused by plant growth-promoting properties related to synthesizing of phytohormones such as gibberellins, cytokinins, and auxins, and enzymes such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase [65,66]. This result is in tandem with the results of Gowtham et al. [67] and Alami et al. [68], which

revealed increased plant growth upon inoculation with PGPR, as well as those of Okoroafor et al. [57], which showed that soil inoculation with *B. amyloliquefaciens* FZB42 promoted the growth of *Z. mays* (strategy 2 plant) and *F. esculentum* (strategy 1 plant) under laboratory conditions. In addition, *Z. mays* producing higher biomass per square meter, compared with *H. annuus*, agrees with the results of laboratory studies reported by Okoroafor et al. [49].

4.2. Effect of Soil Inoculation on Bioavailability of Elements in Root Soils

Incubation experiment without plant influence showed that the PGPR *B. amyloliquefaciens* FZB42 is capable of increasing the bioavailability of most elements considered in this study, including Fe, even though results were significant for only Cu. This increased concentration of Cu and other elements could be a result of solubilization of Cu and other elements by low-molecular-weight organic compounds and protons released by the metabolic activities of *B. amyloliquefaciens* FZB42 [69]. The higher amount of Cu most likely results from the solubilization of Cu bound to organics, as Cu is considered to have a high affinity for soil organic matter [70]. This (increased concentrations of elements) is consistent with the reports of Fang et al. [71], in which bacteria with plant growth-promoting traits increased the concentrations of water-extractable Cu, Zn, Pb, Cd, and Fe, etc., thus indicating the potential of *B. amyloliquefaciens* FZB42 for solubilizing some soil elements.

Bioavailability of elements root soils of *Z. mays* and *H. annuus* grown on inoculated soils—revealed by distilled water and CaCl_2 extractable concentrations of elements—were different for some elements and also different from results of incubation experiment for some elements. In roots soils of *Z. mays*, soil inoculation increased the concentration levels of many of the elements considered in this study, including Fe. This is likely because substances released by the PGPR *B. amyloliquefaciens* FZB42 and PGPR influenced root exudates secretion by *Z. mays*, affecting soil chemistry in a way that promoted the solubility of Cd and other elements, thus increasing their bioavailability [72]. Conversely, in root soils of *H. annuus* grown on inoculated plots, the reduced concentration of most elements investigated in this study is suggestive of a reduced bioavailability for these elements. However, we believe that the reduced concentrations were not necessarily a result of immobilization of some of these elements in the soil but a result of depletion of the easily bioavailable fractions of these elements upon their increased root absorption/accumulation by *H. annuus* (which is indicative of increased bioavailability), without their immediate replacement in soil solution by transfer from other less available soil element fractions. Therefore, it is possible that substances released by *B. amyloliquefaciens* FZB42 and *H. annuus* root exudates caused an increased bioavailability of some of these elements, leading to the observed increased accumulation of some of these elements by *H. annuus*.

4.3. Effect of Soil Inoculation on Plant Concentration and Accumulation of Investigated Elements

Increased concentrations of P and Cd in *Z. mays* shoots agree with the findings of Braud et al. [73] that soil inoculation increased the accumulation of PTEs, and this may be connected to increased concentration of P and Cd in roots of *Z. mays*. This (increased shoot and root concentration) is also reflected in the increased accumulation of P and Cd in *Z. mays*, and it is not surprising, as the increase in concentration and accumulation is likely connected to increased bioavailability of elements—an effect of inoculation on the bioavailability of elements, as earlier discussed. Decreased concentrations of Fe, Pb, and REET upon inoculation in shoots are connected to decreased concentrations of these elements in roots, and this is further reflected in the decreased accumulations of these elements by *Z. mays* upon inoculation with RhizoVital® 42. This suggests that it is likely that not all species or forms of Fe, Pb, and REET designated as bioavailable were available for plant accumulation. Additionally, increased concentrations of Ca and Cu in plant roots upon inoculation were not reflected in the concentration and accumulation of these elements by *Z. mays* for which a decrease was observed. This is suggestive of immobilization of Cu and Ca in the roots of *Z. mays*, possibly caused by ions of these elements forming complexes

with various chelators such as organic acids and being immobilized in cell walls and/or vacuoles [74] and thus not transferred to plant shoots.

For *H. annuus*, increased concentrations of Ca, Cu, Pb, Ge, and REET in plant shoots grown on inoculated soil, which was reflected in the increased accumulations of the same elements by *H. annuus*, does not agree with results on the bioavailability of elements such as Ca, Cu, Pb, and Ge, as revealed by both water and calcium chloride extraction. It could be that the increased accumulation is a result of the increased mobilization of these elements by substances released by bacteria and plant roots, irrespective of reduced concentrations of these elements in root soils, for which possible reasons were explained in Section 4.2 of this paper. Additionally, the decrease in P, Cd, and Fe concentrations in *H. annuus* grown on inoculated soils is also reflected in the content of these elements in plant shoots, as the effects on accumulation were mostly negligible (decrease/increase under 6%).

Similar effects on P and Cd shoot concentration and content in both plants upon inoculation with *B. amyloliquefaciens* FZB42 are suggestive of a relationship between P and Cd, which may be connected to mobilization and immobilization of Cd–phosphate complexes [75] by substances released in soil by plants and/or *B. amyloliquefaciens* FZB42. In the same vein, similar patterns observed for the effects of inoculation on Ca and REET, as well as Pb and Cu concentrations and accumulations by both plants, are likely related to the similar relationship between Ca and REEs in plants due to chemical similarities [76,77] and similarity in the source of origin for Pb and Cu in the geochemical system [78,79], perhaps anthropogenic contamination resulting from Cu–Pb alloy processing [80]. This agrees with a strong correlation between Cu and Pb reported in soil and plant shoot content reported by Okoroafor et al. [49] and Koniecznyński et al. [81]. In contrast, results for the effect of inoculation on Ge and Cu accumulation by *Z. mays* disagree with results of a similar study under laboratory conditions by Okoroafor et al. [57]. However, results for the effect of inoculation on Pb accumulation by *Z. mays* in this study agree with results reported for Pb by Okoroafor et al. [57] under laboratory conditions.

Furthermore, both test plants having different Fe acquisition strategies and levels of phosphorus use efficiency was reflected in the higher concentrations of all elements (except Ge) in *H. annuus*, compared with those in *Z. mays* (concentration differences significant for Ca, Fe, Cu, Pb, and REET) when grown in both uninoculated and inoculated soil conditions (Table 3). However, the effects of inoculation on plant concentrations of Fe and Ge were similar in both plants, while opposing effects of inoculation on plant concentrations were observed for the rest of the elements investigated. This suggests that it is less likely that plants having different Fe acquisition strategies influence the effect of inoculation on both Fe and Ge concentration and accumulation in plants. Contrastingly, opposing effects observed for plant P concentration and accumulation in both test species are suggestive of a possible influence of plant phosphorus use efficiency on the effect of inoculation on plant P concentration and accumulation. Differences in the effect of inoculation on P, Ca, Cu, Cd, Pb, and REET concentrations and accumulations might be connected to differences in root structure, biomass, quantity, the composition of root exudates, etc. of both plants, influenced by *B. amyloliquefaciens* FZB42, and how these factors impact the rhizosphere chemistry and activities, as well as speciation/availability of elements in soils and to plants [50,51,68,72,82,83].

In conclusion, important points to note based on the results of this study are as follows: (1) Although most effects of soil inoculation with *B. amyloliquefaciens* FZB42 in this study were not statistically significant, soil inoculation with *B. amyloliquefaciens* FZB42 showed potential for increasing biomass production of *H. annuus* and *Z. mays*, as well as their accumulation of Ge and some other trace elements, partially confirming our hypothesis (hypothesis was not true for all elements). (2) The effect of inoculation on the bioavailability of elements in soils did not necessarily translate to the same effect of inoculation for plant accumulation of elements. (3) Differences in the effects of inoculation on the concentrations of elements in roots and shoots of plants, as shown for *Z. mays*, is an indication that inoculation could possibly lead to enrichment of some elements in plant roots but not in

the shoots due to immobilization in roots. (4) Element concentration and accumulation patterns suggest that *H. annuus*—a strategy 1 plant with reportedly higher phosphorus use efficiency—is a better choice for phytoaccumulation of most elements considered in this study, compared with *Z. mays*, a strategy 2 plant with reportedly lower phosphorus use efficiency. (5) Effects of soil inoculation with PGPR on Fe and P concentrations and accumulations in plants suggest that difference in plant phosphorus use efficiency could influence inoculation effects on plant P concentration and accumulation, while the difference in plant Fe acquisition strategy is less likely to influence inoculation effects on plant Fe accumulation and concentration.

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