Research Article

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Consortium of plant growth-promoting bacteria improves spinach (*Spinacea oleracea* L.) growth under heavy metal stress conditions

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Abstract

BACKGROUND: Heavy metal contamination results in oxidative stress to plants and leads to decreased plant growth and development. Affected plants cannot be efficiently used in phytoremediation studies and their potential may be significantly reduced. In many cases, bioaugmentation with plant growth-promoting bacteria is used as a strategy to alleviate this stress condition.

RESULTS: Isolates were found to possess 1-aminocyclopropane-1-carboxylate (ACC) deaminase, to produce indole-3-acetic acid (IAA) siderophores and to solubilize phosphates, while identification revealed that the isolates belonged to the genera *Pseudomonas* and *Bacillus*. Out of the 17 isolates, ten were found to possess ACC deaminase activity, producing 1–3.2 μmol mg^{-1} h^{-1} of α-ketobutyrate and siderophores of catechol and hydroxamate type. A higher quantity of IAA was observed in the case of isolate SGPI 41 (65 μg mL^{-1}). In addition, the inoculation of consortia of isolates led to decreased accumulation of Cd, Pb and Zn in the whole plant but at the same time increased the plant biomass by up to 100% compared with the un-inoculated control.

CONCLUSIONS: The use of beneficial bacteria possessing plant growth-promoting traits is a very useful approach to alleviating heavy metal stress to plants and can be successfully applied in phytoremediation strategies.

Keywords: PGPB; IAA; ACC-deaminase; *Pseudomonas*

INTRODUCTION

Nowadays, agriculture faces serious problems related to expectations of obtaining higher crop production, while environmental conditions are becoming less favorable and more stressed with each passing day owing to contamination and climate change. In this situation, the agricultural sector is looking for new environmentally friendly approaches in order to solve the problems. One option is to improve the naturally occurring interactions in the plant rhizosphere between the roots and the soil microbiota. Soil microbial communities are the main factor in the soil, and their activities can be defined as very important for sustainable crop production.[1] At the same time, some authors have pointed out the increase in stress produced by salinity, drought, high temperature, use of chemicals and air and soil contamination.[2,3]

Soil industrial contamination with heavy metals on agricultural lands is a common problem in many countries. Some of them are essential for plants because they play an important role in plant metabolism as micronutrients (Cu and Zn). However, in high concentrations, heavy metals have a negative, even toxic effect on plants and their use to remedy such contaminated soils could be problematic.[4] In our case, the studied site had been contaminated over the years with Pb, Cd and Zn from a non-ferrous metal smelter. The stress produced by those metals in the soil has diverse effects on plants related to decrease in growth, even death. It is known that soil microorganisms are able to promote plant growth in symbiotic relationships with the roots, alleviating stress conditions in different ways, thus improving the phytoremediation potential of plants.[5] Plant growth-promoting bacteria (PGPB) are free-living, symbiotic or endophytic and possess various abilities that characterize them as beneficial for crops or plants as a whole.[5,6] They promote plant well-being through various mechanisms such as production of phytohormones, molecular nitrogen fixation from the atmosphere, phosphorus solubilization, production of siderophores or enzymes and expressing activity against phytopathogens.[1,7,8] Depending on the strains' abilities, the introduction of PGPB tolerant to heavy metals in the plant rhizosphere could result in increased growth, higher biomass, phytopathogen resistance, etc.[1,9] In that sense, the production of indole-3-acetic acid (IAA) is widespread among soil bacteria and plays an important role in interactions with plant roots.[5,10] There is a lot of evidence of its positive influence on plant growth under heavy metal stress conditions.[11] In some studies, researchers report that IAA stimulates the length of lateral roots and changes in root architecture in *Arabidopsis* plants,[12] while in others the increased root and shoot biomass is attributed to soil application of PGPB.[13] More recently, Etesami and Alikhani[14] found a stimulation effect of these effective bacteria on rice

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growth and development. On the other hand, PGPB possessing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase are able to utilize ACC as sole carbon source of N, converting it to α-ketobutyrate and ammonia, thus decreasing the concentration of ethylene in the plant and overcoming the root growth reduction in heavy metal stress conditions. This theoretical model[15] is confirmed by a number of studies on crops such as lentil,[16] tomato,[17] maize and mung bean,[18] barley and oats,[19] etc. In another study, after inoculation of Pb-tolerant bacteria expressing ACC deaminase, a significant increase in *Brassica napus* root and shoot biomass was found.[20] This evidence was observed in several studies of heavy metal-contaminated soils after inoculation of PGPB, confirming the possitive influence of ACC deaminase enzyme in tolerant bacteria.[21,22] Morever, some authors have suggested that exopolysaccarides produced by bacteria have an important role in bacterial tolerance to heavy metals;[22] for example, *Pseudomonas putida* binds 100% of Cd in solution owing to these compounds.[23] Some PGPB in Fe-deficient environments produce siderophores that can improve many-fold the Fe complexes absorbed by plants. There are many testimonies of the very positive influence of bacterial siderophores on plant growth and development. The biomass of okra (*Abelmoschus esculentus* L.) was increased, including root and shoot length, after inoculation of siderophore-producing isolates, but at the same time they showed a negative effect against the phytopathogens *Rhizoctonia solani* and *Fusarium oxysporum*.[24] Wheat plants were affected positively by inoculation of a consortium of rhizobacteria, increasing germination, root and shoot length and plant biomass and showing a negative effect against *Fusarium solani*.[25] At the same time, some PGPB are able to solubilize P in the soil, converting it to plant-available form. These bacteria have been suggested to play an important role in plant nutrition but also in heavy metal immobilization.[26] Available P in the soil resulting from PGPB solubilization activity has affinity to available heavy metals, thus forming insoluble soil complexes and reducing the plant uptake of metals. There are not many findings demonstrating reduction of Pb, Cd and Zn accumulation due to PGPB application, but in any case, bacterial plant growth promotion is due to the complex effect of multiple mechanisms, whose understanding is the key to their successful use and application in agricultural practices.

Spinach is a crop of great agricultural importance that is an indicator of good soil health. It could be an efficient indicator of soil contamination in toxicity studies or phytoremediation experiments to prove the alleviation effect of microorganisms. Various authors have used spinach as an important test plant for accumulation of heavy metals.[27] One of the intentions of this study was to grow spinach as an indicator plant for the state of the soil, heavy metal accumulation and their toxic effect.

The aim of this study was to isolate rhizosphere bacteria able to promote the growth of spinach plants in heavy metal-contaminated soils.

EXPERIMENTAL

Isolation of metal-tolerant rhizobacteria

The heavy metal-contaminated site is situated in the South-Central region of Bulgaria, town of Kuklen, Plovdiv district. The contamination was historically air-spread by a non-ferrous metal plant over the arable calcareous soil in the region. It was heterogeneous with metals represented by Pb, Zn and Cd.

Whole plants of the following species during flowering were randomly collected from the heavy metal-contaminated site, together with rhizosphere-adhering soil: *Lamium amplexicaule* L., *Capsella bursa-pastoris* L., *Geranium molle* L., *Valerianella* sp. and *Cirsium* sp. Rhizosphere soil from each plant was taken and added to sterile distilled water. A 5 g portion of each sample was mixed with 45 mL of sterile saline water (0.85%, w/v) in a flask and shaken for 30 min at 150 rpm. Ten-fold serial dilutions were prepared from each sample, and aliquots from each dilution and sample were inoculated on nutrient agar medium in duplicate. The medium was supplemented with 10 ppm Pb (as $Pb(NO_3)_2$), 50 ppm Zn (as $ZnSO_4$), 2 ppm Cd (as $CdCl_2$) and 100 mg L^{-1} cyclohexamide, using concentrated sterile solutions. Plates were incubated at 28 °C for 24–48 h.

Characterization of isolates

Morphological and biochemical characterization

The morphological and biochemical characters of examined isolates were studied using *Bergey's Manual of Determinative Bacteriology*.[28] Gram staining was done according to standard procedures for cultures in the exponential phase of growth. Morphological characteristics were studied by phase contrast microscopy. Finally, the tolerance of isolates to the heavy metals Pb, Zn and Cd was studied by growing cultures of each isolate in exponential phase on nutrient agar medium supplemented with different metal concentrations, studied separately. The concentration ranges used were as follows: 100–1000 ppm for Pb, 100–300 for Zn and 0–250 ppm for Cd. Growth on nutrient agar without metals was used as control.[29]

Determination of siderophore production

The production of siderophores was determined by the modification made by Pérez-Miranda *et al.*[30] of the method of Schwyn and Neilands.[31] The method consists in the use of an overlay technique whereby a modified chrome azurol S (CAS) medium is cast upon a culture agar plate (O-CAS). The CAS medium for 1 L of overlay was as follows: CAS 60.5 mg, hexadecyltrimethylammonium bromide 72.9 mg, piperazine-1,4-bis(2-ethanesulfonic acid) 30.24 g and 1 mmol L^{-1} $FeCl_3 \cdot 6H_2O$ in 10 mL of 10 mmol L^{-1} HCl, with agarose (0.9%, w/v) as gelling agent. Siderophore detection was achieved when 10 mL (standard, 80 mm diameter Petri dishes) overlays of this medium were applied over nutrient agar plates containing the studied isolates.

ACC deaminase activity assay

ACC deaminase activity was determined by measuring the concentration of α-ketobutyrate resulting from degradation of ACC.[15] Initially the isolates were grown separately in nutrient agar medium till medium exponential phase. After centrifugation, the cells were re-grown in minimal medium containing ACC as sole N source. The α-ketobutyrate produced by the degradation was determined by measuring the absorbance of samples at 540 nm and comparison with a standard curve of α-ketobutyrate (0.1–1.0 μmol L^{-1}). The ACC deaminase activity was expressed as μmol α-ketobutyrate mg^{-1} protein h^{-1}.

Quantification of IAA

The isolates were propagated in liquid Bacto Pseudomonas F.[32] Equal aliquots were transferred into 20 mL of the same medium supplemented with the following concentrations of L-tryptophan (taken from a filter-sterilized 4 mg mL^{-1} stock solution prepared in warm water; Sigma): 0, 300, 500 and 900 μg mL^{-1}. After incubation

for 24 h, the density of each culture was measured spectrophotometrically at 550 nm, then the bacterial cells were removed from the culture medium by centrifugation (3400 × g, 4 °C, 10 min). A 0.2 mL sample of the supernatant was mixed vigorously with 0.8 mL of Salkowski's reagent (150 mL of concentrated H_2SO_4, 250 mL of distilled water, 7.5 mL of 0.5 mol L^{-1} $FeCl_3 \cdot 6H_2O$)[33] and left for reaction at room temperature for 20 min before measuring the absorbance at 535 nm. The concentration of IAA in each culture medium was determined by generating a standard curve for samples containing IAA.

Other PGP traits

The phosphate solubilization abilities of the isolates were investigated as described by Georgiev *et al.*[34]

Biolog® analysis

Identification of the isolates was performed through Biolog® Gen III (Biolog Inc., Hayward, CA, USA). The system is based on the sole carbon source utilization patterns of the isolates, inoculating and incubating the GN/GP plates for 7 days at 25 ° and reading the color from the first 24 h using an automated reader model (Tmax Molecular Device, Biolog, Inc.). The methodology was performed according to the instructions of the manufacturer.

Soil and compost

The soil used in this study was collected from a heavy metal-contaminated area near the town of Kuklen and non-ferrous metal-producing smelter (Plovdiv region, Bulgaria) from a depth of 0–30 cm, after removing the top layer. It is the same site from which the plants for bacterial isolation were taken. The soil was cleaned from organic rests and stones. The compost was produced from waste microbial biomass and wood waste (bark and sawdust). Both soil and compost were analyzed to determine their basic characteristics (Table 1).

Growth experiment

The treatments in the present growth experiment were prepared by mixing contaminated soil with compost (3:1, v/v). In our previous studies, we found that soil rhizobacteria were able to promote plant growth and alleviate heavy metal stress to plants, which was expressed mainly as increased plant biomass.[29,35] Before starting the experiments, the soil/compost mixture was left to stabilize for 30 days. Each treatment consisted of three rectangular pots with a volume of 2000 cm^3 each. They were sown with 20 spinach (*Spinacia oleracea* L. var. Matador) seeds treated with corresponding PGPB solutions for 2 h. The seeds were previously surface sterilized using 1.5% (v/v) sodium hypochlorite and thoroughly washed with sterile distilled water.

During vegetation, corresponding solutions of PGPR were added once. The pots were situated in an open air greenhouse in the middle of March 2017 and the experiment continued for 60 days. During vegetation, the plants were watered with distilled water to maintain the water-holding capacity at around 60%.

Six selected bacterial isolates that expressed desired PGP traits were used separately or in consortium as shown in Table 2.

Preparation of inoculum

Each isolate was grown separately in nutrient broth medium till late log growth phase. The inoculum needed for each culture was centrifuged (3400 × g, 4 °C, 10 min). The obtained pellets

Table 1. Characteristics of soil and compost used in study

Item	EC(dS m^{-1})	pH	Soil	Total N (g kg^{-1})	Total P (g kg^{-1})	Bioavailable N (mg kg^{-1})	Bioavailable P (mg kg^{-1})	TOC (g kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Cd (mg kg^{-1})
Soil	0.2 ± 0.03	6.6 ± 0.2	Loamy	1.52 ± 0.1	0.42 ± 0.03	17.3 ± 0.7	36.6 ± 1.5	10.1 ± 0.5	579 ± 19.3	468 ± 27.1	18.8 ± 0.9
Compost	0.2 ± 0.03	6.9 ± 0.2	fluvisoil	21.52 ± 0.7	8.3 ± 0.2	645 ± 14	2610 ± 58	342.7 ± 11	0.84 ± 0.1	18.6 ± 0.1	0

Table 2. Experimental design

Treatment	Control	1	2	3	4	5	6	7	8	9
Isolates	–	Consortium of 32, 32b, 41, 41b	Consortium of 32, 32b, 41, 44	Consortium of 32, 32b, 44, 44b	32	32b	41	41b	44	44b

were washed first in 20 mmol L^{-1} $MgCl_2$ to remove ions and then in distilled water. Bacterial suspensions in distilled water were adjusted by spectrophotometry at 550 nm, knowing the growth curve of viable cells. The final concentration was approximately 5×10^7 colony-forming units (CFU) mL^{-1}. Consortia were prepared by mixing the corresponding isolates (Table 2).

Determination of heavy metals in soil, compost and plant tissues

Total soil and compost heavy metal concentrations were assessed using the method described by Karstensen *et al.*[36] The heavy metal concentration in spinach plants (roots, stems, leaves) was determined through the method of wet mineralization.[37] All tissues were carefully washed with distilled and deionized water, dried at 85 °C for 24 h, powdered and weighed for analysis. Digestion was done in an autoclave using 100 mL Schott bottles, 0.25 g of plant sample, 10 mL of deionized water, 3 mL of HNO_3 (65%, v/v) and 2 mL of H_2O_2 (35%, v/v). After cooling, solutions were made up to 50 mL. Measurements were made by atomic absorption spectroscopy.

Statistical analyses

The data obtained in the present study were subjected to analysis of variance (ANOVA), while the means were compared by one-way ANOVA. Significance at the 5% level was tested by Duncan's multiple range test using SPSS Statistics V.18. Some of the results are presented after calculation of the mean and standard error using Excel V.14.

RESULTS

Seventeen bacterial isolates tolerant to heavy metals were chosen on the basis of their tolerance and morphology (Table 3). The morphological tests revealed the existence of both Gram-positive and Gram-negative representatives among the isolates. At the same time, only one isolate was characterized as coccus, while some of the others were spore-forming. Taking into account that our goal is to isolate PGPB capable of improving plant growth in heavy metal-contaminated soils, the tolerance to metals present in the medium was a very important part of the selection procedure, and the results are shown in Table 3. In addition, the characterization tests revealed the existence of all studied PGP properties, but only six isolates were able to express all traits (SGPI 32, 32b, 41, 41b, 44 and 44b).

An important ability of PGPB is the production of phytohormones. All isolates in our experiment were able to produce IAA in the presence of tryptophan, but SGPI 32, 32b, 41 and 41b showed higher productivity (Fig. 1). In that sense, SGPI 41b showed the best productivity, especially at the highest concentration of tryptophan (900 μg mL^{-1}). It was more than 200% higher than that of the highest producer from the rest of the isolates (SGPI 32). The majority of the strains showed a highest IAA rate of only 10 μg mL^{-1} or less, even at maximum tryptophan concentration.

Finally, after the tests, six isolates were selected for identification using Biolog® features and further PGP experiments. The similarity of the identification was sufficiently high in each case to identify the isolate.

The characteristics of the isolates tested with Biolog® are in common with bacteria belonging to the genera *Pseudomonas* (five isolates) and *Bacillus* (one isolate). The morphological characterization presented in Table 3 matched the identification results. The isolates were identified as follows: 32 and 32b as *Pseudomonas putida*, 41 and 44b as *Pseudomonas fluorescens*, 41b as *Bacillus thuringiensis* and 44 as *Pseudomonas synxantha*.

Following the identification of isolates, they were applied in plant growth experiments with contaminated soil. The highest concentration of Zn was observed in leaves and generally in the control treatment (Table 4).On the other hand, a substantial reduction of accumulated Zn was found in treatments (T) 1 and 2, where the difference was statistically significant compared with the control and other treatments. It seems that the consortia of PGP isolates from T-1 and T-2 led to lower accumulation of Zn in the whole spinach plant (reduction of 24–33% in T-1 and 23–27% in T-2).

The results for accumulation of Pb in spinach showed higher values in control plants, including leaves, stems and roots. The highest influence of PGPB on accumulation of Pb was observed in T-1 and T-2, where the decreases in accumulation were 45 and 46% in leaves, 41 and 56.6% in stems and 69.5 and 67% in roots respectively. As the most toxic of the studied metals, Cd influenced the growth and development of plants at a higher rate than the other metals studied. In our case, the highest accumulation was observed in plants grown in the control treatment, with the exception of leaves in T-9. The accumulation in leaves, stems and roots of T-1 was reduced by 69, 59 and 59% respectively compared with the control. It seems that bacterial consortia from T-1 and T-2 decreased the metal accumulation in test plants much more than the bacterial populations from other treatments.

The accumulation of heavy metals had a negative influence on spinach plant growth and development. Figure 2 shows the shoot fresh weight of plants at the end of the experiment. There is a clear, statistically proven difference between the control and the other treatments. The plants in T-1–T-5 showed higher biomass than the control: in T- 2 and T-3 the biomass of shoots was 100% more, while in T-1, T-4 and T-5 it was increased by about 75%. In contrast, in treatments with application of only one isolate, from T-6 to T-9, a reduction in growth, highest in T-6 and T-7, was observed.

DISCUSSION

It is known that plant roots release diverse compounds that attract microbial populations in the nearby area called the rhizosphere. At the same time, these consortia 'compensate' the plant by applying various tools or mechanisms which benefit its growth and health.[1] The mechanisms are basically related to improving the availability of nutrients to the plant, protecting against phytopathogens or ameliorating stress conditions.[5,21] In addition, some heavy

Table 3. Morphological characteristics, plant growth-promoting traits and heavy metal tolerance of isolates

	Isolate (SGPI)																
Character	11	12	13	14	21	21b	22	23	31	31b	32	32b	41	41b	43	44	44b
Morphological characteristics																	
Gram reaction	−	−	+	+	−	+	+	+	−	+	−	−	−	+	−	−	−
Cell shape	Rod	Rod	Coccus	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Colony color	White	White	White	White	White	Gray	Gray		Gray			Gray					Gray
Spores	−	−	−	−	−	−	−	+	+	−	−	−	−	−	+	−	−
Plant growth-promoting traits																	
Phosphate solubilization	+	−	−	−	+	−	−	−	+	−	+	+	+	+	+	+	+
IAA production	−	−	−	+	+	+	+	−	−	−	+	+	+	+	−	+	+
Siderophore production	−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+
ACC deaminase activity[a]	−	−	2 ± 0.17	−	−	1 ± 0.08	−	1.3 ± 0.1	−	−	2.1 ± 0.2	1.9 ± 0.2	2.4 ± 0.3	1.8 ± 0.2	3.2 ± 0.2	1.4 ± 0.2	2.7 ± 0.3
Maximum tolerance of heavy metals (ppm)																	
Pb	700	700	600	700	700	700	600	600	600	700	600	700	500	700	700	700	800
Zn	150	150	100	150	150	150	200	100	150	200	100	150	200	150	150	200	200
Cd	150	70	20	20	70	70	100	20	100	100	150	200	20	20	20	70	70

[a] In units of μmol α-ketobutyrate mg^{-1} protein h^{-1}; values represent mean ± standard deviation of three replicates.

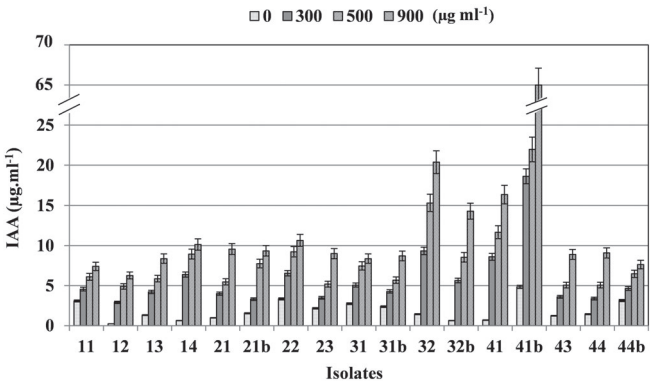

Figure 1. IAA production from studied strains under different tryptophan concentrations. Bars shown the mean and the standard error ($n = 3$).

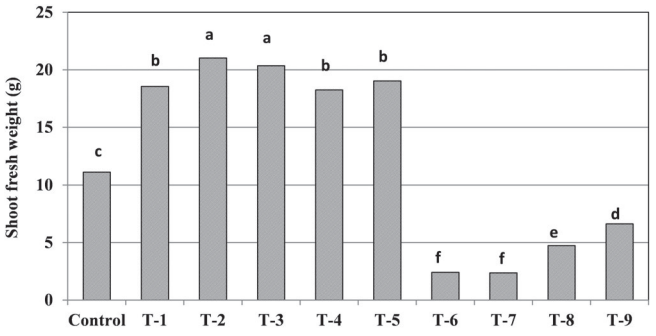

Figure 2. Fresh weight of shoots of spinach plants at end of experiment. Mean values with the same letter do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

metals are needed as micronutrients in enzymes, but in higher concentrations they may be harmful to plants.[1] The result may constitute a problem for the phytoremediation strategy that could have success in overcoming toxicity effects on plants. The toxicity of heavy metals negatively influences plant nutrition, including Fe[38] and cannot be easily distinguished from the symptoms of a lack of that element. Production of siderophores from the isolates is a prerequisite for improved nutrition and state of the spinach plant. As a consequence, the studied isolates responded positively to the phosphate solubilization assays, which is a very good sign concerning nutrition, because P is an important element involved in almost all metabolic processes, including energy conversion, respiration, photosynthesis, etc.[26] In our study, these beneficial properties led to a significant increment in the biomass of spinach (Fig. 2) in treatments with consortia of bacterial isolates, but also in single bacterial populations of *P. putida* SGPI 32 and *P. putida* SGPI 32b. These results were also attributed to the expression of ACC deaminase and IAA formation (Table 3, Fig. 1) facilitating plant growth and development. PGPB which are able to utilize ACC and to produce siderophores are very beneficial to plants in environmental stress conditions, enhancing indices of growth in various ways, especially those of roots and shoots[37] owing to improved plant nutrition.[5] ACC deaminase activity was quantified as the formation of α-ketobutyrate from 1 to 3.2 µmol mg^{-1} h^{-1}. In literature sources, results are very diverse, ranging from 0.06 to 2.66 µmol mg^{-1} h^{-1} in one report and from 6.62 to 147.59 µmol mg^{-1} h^{-1} in another.[39,40]

The soil we investigated had been contaminated over the years with Pb, Cd and Zn that were the objects of the study. On the other hand, we chose the spinach species as a test plant, not for the purpose of remediating the soil, but to use it as a sensitive indicator of the contamination. This can be considered a new way of exploring quickly the applicability of different approaches in phytoremediation. Confirming the strategy, researchers found that the population of *P. putida* KNP9 could successfully decrease uptake of Cd and Pb and stimulate plant growth of mung bean (*Phaseolus vulgaris*) owing to PGP traits, while others reported the effect on Ni in chickpea attributed to *Pseudomonas* sp.[41,42] In our case, the incorporation of consortia of PGPB resulted in improved growth expressed as shoot biomass (Fig. 2), but also in decreased heavy metal accumulation in all plant parts (Table 4). Plant biomass was increased by 80–100% in the case of bacterial consortia, taking into account that the production of IAA is higher compared with other isolates (Fig. 1), but also that the isolates expressed an ACC deaminase capability.

In our case, the addition of compost led to a decrease in metal absorption by spinach thanks to the carbon content, but also improved the state of the plant owing to the available nutrients that were many times higher than the soil content. Nevertheless, the role of PGPB in the amelioration of heavy metal toxicity and stress to plants is related to all traits.[43]

In conclusion, the selected bacterial isolates were able to promote the growth of spinach as a test plant under stress conditions produced by the heavy metals Pb, Cd and Zn. In addition, a decrease in the accumulation of these elements was

Table 4. Accumulation of Cd, Pb and Zn in different parts of spinach plants. Data represent the mean of three replicates analyzed by Duncan's multiple range test ($P = 0.05$)

	Cd (mg kg^{-1})			Pb (mg kg^{-1})			Zn (mg kg^{-1})		
Treatment	Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots
Control	30.4a	28.1a	24.2a	60.7a	24.2ab	20.6a	296.9a	133.8a	125.1a
T-1	9.4c	12.3d	12.3d	33.3d	14.3cd	8.2e	224.5f	88.7e	90.2f
T-2	14.6bc	12.5cd	12.8cd	32.8d	10.5d	8.9de	227.9f	91.9e	86.1f
T-3	10.4c	12.7cd	12.7cd	31.8d	14.1cd	9.5de	232.1ef	106.2d	95.1def
T-4	12.5bc	14.3bcd	14.3bcd	56.5a	16.6bcd	11.3cde	254.4cd	106.4d	110.6cd
T-5	27.3a	16.2bcd	16.2bcd	48.3b	20.1abc	13.0cde	230.5ef	112.8cd	92.3ef
T-6	14.4bc	20.6bc	20.6bc	44.5bc	21.7abc	12.7cde	264.4c	126.4ab	108.3cde
T-7	16.7bc	10.5d	10.4d	49.1b	20.4abc	20.3a	282.5b	120.8bc	115.1bc
T-8	21.4ab	16.7bcd	16.7bcd	48.5b	14.6cd	16.3bc	242.4de	104.3d	129.5a
T-9	40.3d	20.3abc	13.6cd	44.4b	14.2bcd	14.2bcd	242.6de	120.5bc	98.4cdef

observed, most highly pronounced in the treatments with consortia of bacteria. All these findings are attributed to the PGP traits of the isolates, but also to the incorporation of compost. However, further research is needed to reveal the intimate mechanisms through which the isolates protect the plants in each case study.

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