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Research Article

Endophytic *Burkholderia* sp. strain PsJN Improves Plant Growth and Phytoremediation of Soil Irrigated with Textile Effluent

The aim of this study was to determine whether the inoculation of plant growth-promoting bacteria to plants, vegetated in soil irrigated with textile effluent, influences plant biomass production, and soil remediation. Three different plant species (*Acacia ampliceps*, *Eucalyptus camaldulensis*, and *Leucaena leucocephala*) with and without bacterial inoculation were grown in soil irrigated with secondary treated textile wastewater for one year. An endophytic bacterium, *Burkholderia* sp. strain PsJN, possessing plant growth-promoting 1-aminocyclopropane-1-carboxylate deaminase activity was inoculated to plants. There was more plant biomass production (up to 12%) and contaminants removal (up to 29%) from soil with bacterial inoculation as compared to soil having non-inoculated plants. Enhanced plant growth and soil remediation activity are associated with the survival and colonization of the inoculated bacterium in the rhizosphere and endosphere of plants. The highest plant biomass production and contaminants removal from soil were observed in the treatment, in which *A. ampliceps* was inoculated with *Burkholderia* sp. strain PsJN. These results suggest that plant-bacteria partnerships can be applied to improve plant growth and soil remediation during the application of industrial effluent for plant biomass production in the arid regions.

Keywords: Bioremediation; Mineral nutrients; Organic matter; Plant–bacteria partnerships; Wastewater treatment

Received: January 4, 2013; *revised:* June 4, 2013; *accepted:* July 9, 2013

DOI: 10.1002/clen.201300006

1 Introduction

The use of treated wastewater for vegetation of trees and shrubs is a current practice all over the world [1–3]. One of the main benefits of this approach is that plants can absorb organic and inorganic pollutants present in wastewater and thus reduce the pollution load that treated wastewater could contribute to the surface water supply [4, 5]. Tree species such as *Acacia ampliceps*, *Eucalyptus camaldulensis*, *Dalbergia sissoo*, *Populus trichocarpa*, *Acacia nilotica*, and *Leucaena leucocephala* are well known for their speedy growth and potential to adapt to adverse soil and environmental conditions; as well as their ability to produce large amount of above ground biomass [6, 7]. Irrigation to these tree species by treated wastewater for biomass production may contribute to solve the fuel wood scarcity of suburban inhabitants [8]. However, several studies showed that wastewater inhibits the growth and biomass production of different crops/tree species and also affects physicochemical properties of the soil [6, 7].

Recently, it was proposed that the addition of plant growth-promoting bacteria (preferably endophytes) is a simple and effective strategy to enhance plant growth and remediation of contaminants from the soil [9, 10]. Endophytic bacteria can assist their host plant to overcome pollutant-induced stress responses and consequently improve plant growth and phytoremediation activity [11, 12]. Several endophytic bacteria can also survive and colonize in the soil surrounding the roots, where they can penetrate into their associated plant via the roots [13], however, it was also observed that some phyllosphere bacteria may be a source of endophytic bacteria [14, 15]. Plants provide nutrients and residency for endophytic bacteria. In return, endophytic bacteria can improve plant growth by different mechanisms including 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activity to modulate the ethylene levels in plants, reducing the growth and activity of pathogens through competition for nutrients and space, stimulation of plant resistance mechanisms, and synthesis of hydrolytic enzymes and biosurfactants [10]. Despite the beneficial effects of the endophytic bacteria on plants, application of such microorganisms has been rarely observed to improve biomass production during wastewater irrigation to plants.

One of these endophytic plant growth-promoting bacteria, *Burkholderia phytofirmans* strain PsJN [16], has been isolated from surface-sterilized onion roots. Strain PsJN shows high ACC deaminase activity and is therefore able to lower the ethylene level in a developing or stressed plant. Strain PsJN has been able to promote plant growth of non-natural hosts differentially in addition to

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Abbreviations: **ACC**, 1-aminocyclopropane-1-carboxylate; **STTW**, secondary treated textile wastewater

lowering biotic and abiotic stress. This bacterium can also thrive as an endophyte inside various plant hosts, including tomato, potato, and grapevine [17].

Wastewater irrigation to fast growing trees is an effective approach for improving biomass production and the remediation of pollutants from soil (irrigated with wastewater). The aim of the present study was to assess the potential of plant growth-promoting endophytic bacterium, *Burkholderia* sp. strain PsJN, to improve plant biomass production and contaminants removal from soil irrigated with STTW. Moreover, the potential accumulation of organic and inorganic pollutants in soil and plant was also investigated.

2 Materials and methods

2.1 Secondary treated textile wastewater

The secondary (biological) treated textile wastewater (STTW) was collected in pre-cleaned plastic containers from different textile industries, located at Khurianwala, Faisalabad, Pakistan. Wastewater treatment processes are dissolved air flotation (primary treatment) and aerobic activated sludge (secondary treatment). Various physico-chemical characteristics (Tab. 1) of wastewater were determined using standard methods [18].

2.2 Bacterial strain

Burkholderia sp. strain PsJN, previously isolated from surface sterilized onion roots [16], was used in this study. This bacterium exhibited high ACC deaminase activity and was previously labelled with *gusA*10 gene [17] to observe its survival and colonization in the soil and plant interior. Bacterial culture was prepared by cultivating in a glass reactor (5 L capacity) containing Luria–Bertani (LB) broth containing spectinomycin (50 mg L^{-1}) for 24 h at 37°C. In order to recover the enriched bacterial cells, broth was centrifuged at 10 000 × *g* for 15 min and re-suspended in autoclaved normal saline (0.85% NaCl in distilled water). The optical density was adjusted with normal saline to 0.7 at 600 nm. The obtained bacterial cell suspension was the bacterial inoculum.

2.3 Experimental design

The experiment was performed at National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad (31°0.2′N, 73° 0.05′E), Pakistan. For the experiment an agricultural soil (pH 7.8, electrical conductivity 6.90 ds/m, total N 0.02%, 0.08% available P, 1.46% extractable K, 0.34% organic matter) was used. Air-dried and sieved soil was transferred in plastic pots (22 kg pot^{-1}). One-month old plants of *A. ampliceps*, *E. camaldulensis*, and *L. leucocephala* were transplanted into these pots (one plant per pot), acclimatized with canal water for one month and then irrigated with STTW for the next whole year. These plant species are introduced in Pakistan from Australia and have been used for the reclamation of salt affected lands in Pakistan and Australia. Different treatments were T0: STTW (without vegetation); T1: canal water; T2: STTW; T3: STTW and bacterial inoculation. Before STTW application, the soil of T3 treatment was inoculated with 200 mL bacterial culture of *Burkholderia* sp. strain PsJN::*gusA*10. The experiment was performed in a complete randomized design with three replicates of each treatment. Plants were irrigated with STTW and canal water whenever needed, typically three times per week. About 300 L STTW was applied into each pot of respective treatments.

2.4 Morphological observations of plants

After one year, pots were broken and carefully whole plant was removed from the soil. Root and stems lengths were measured with a measuring tap, and collar diameter was determined using a vernier caliper. Leaves removed from the stems were counted and roots were

Table 1. Characterization of secondary treated textile wastewater

Parameter	Unit	Value	NEQS
Color		Muddy gray	NG
pH		7.62 ± 1.4	6–10
Electrical conductivity	mS cm^{-1}	7.05 ± 1.7	NG
Chemical oxygen demand	mg L^{-1}	217 ± 34	150
Biochemical oxygen demand	mg L^{-1}	69 ± 16	80
Total organic carbon	mg L^{-1}	72 ± 13	NG
Total dissolved solids	mg L^{-1}	5020 ± 438	3500
Ammonia	mg L^{-1}	48.50 ± 8.6	40
Phenol	mg L^{-1}	0.74 ± 0.16	0.1
Chloride	mg L^{-1}	1874 ± 309	1000
Sulfate	mg L^{-1}	251 ± 68	600
Sulfide	mg L^{-1}	0.08 ± 0.01	1.0
Sodium	mg L^{-1}	1320 ± 145	NG
Potassium	mg L^{-1}	59.30 ± 8.6	NG
Calcium	mg L^{-1}	90 ± 9.8	NG
Magnesium	mg L^{-1}	45 ± 6.5	NG
Phosphate	mg L^{-1}	11.62 ± 0.2	NG
Iron	mg L^{-1}	6.6 ± 1.3	2.0
Nickle	mg L^{-1}	3.2 ± 0.7	1.0
Aluminum	mg L^{-1}	1.5 ± 0.3	NG
Chromium	mg L^{-1}	0.47 ± 0.6	0.1
Chlorine	mg L^{-1}	Nil	1.0

Each value is a mean of twelve different secondary treated textile wastewater samples (collected at one month interval) and values in parentheses indicate standard deviation among the samples; NG = not given in NEQS list, NEQS = National Environmental Quality Standards for irrigation, set by Government of Pakistan.

cut down from the collar diameter. Roots were washed with tap water and then with distilled water to eliminate adhering soil and other contaminants. Leaves, stems and roots were kept in an oven at 80°C for 72 h and their dried weights were determined [7].

2.5 Enumeration of inoculated bacterium *Burkholderia* sp. strain PsJN::*gusA*10

For rhizosphere soil, roots were agitated and soil still attached to roots was sampled. Rhizosphere bacteria were obtained by mixing 3 g rhizosphere soil with 9 mL of normal saline and agitated (100 rpm) for 1 h at 30°C. Surface sterilized roots and shoots (2 g of each) were ground in mortal and pestle containing 6 mL normal saline. When soil and plant particles were settled, serial dilutions up to 10^{-3} were plated on M9 medium [19] containing succinate, acetate and citrate, each at a concentration of 2 g L^{-1}, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (100 μg mL^{-1}), isopropyl-β-D-galactopyranoside (100 μg mL^{-1}), and spectinomycin (100 μg mL^{-1}). Cycloheximide (100 mg L^{-1}) was added to prevent fungal growth. The plates were incubated at 30°C for 72 h and then put at 4°C for 2 days. Blue colonies were counted on each plate. Furthermore, the identity of the isolates (blue colonies) with the inoculant strain (*Burkholderia* sp. strain PsJN::*gusA*10) was confirmed by restriction fragment length polymorphism analysis of the 16S-23S rRNA intergenic spacer region [20].

2.6 Chemical analysis of plants and soil

The chlorophyll contents of plants under different treatments were determined by extracting fresh leaves in 80% acetone and measuring the color intensity of the extract at 445, 645 and 663 nm wavelength using UV/Vis spectroscopy. The total chlorophyll contents were calculated by using the formulae of Arnon [21]. Separately, 10 g dried leaves, stems and roots were ground to pass through a 0.2 mm sieve and digested (1 g of each) with mixture of sulfuric acid (H_2SO_4), nitric acid (HNO_3) and perchloric acid ($HClO_4$) [22]. The filtrate was used for the determination of metals accumulation in plants. Soil samples collected at the end of experiment were analyzed for various physicochemical properties. For the estimation of metals, air-dried soil samples were digested with nitric acid, hydrochloric acid and hydrofluoric acid as described by Rump [23]. Atomic absorption spectrometry was used for the estimation of Ca, Mg, Cr, Fe, Ni, and Al whereas Na and K were analyzed by a flame photometer. Soil organic matter was estimated by the partial oxidation method [24]. All of the samples were analyzed in triplicates. Blanks were prepared in the same way as the sample solution in all cases.

2.7 Statistical analysis

Data analyses for plant growth parameters, chlorophyll contents and mineral composition of soil and plants were performed by using SPSS software package (SPSS, USA). One-way analysis of variance was used to compare treatments. After testing homogeneity of variance, Duncan's test was applied for analysis of variance.

3 Results and discussion

3.1 STTW characteristics

The physicochemical characteristics of STTW are shown in Tab. 1. The chemical oxygen demand, total dissolve solids, ammonia, phenol, chloride, iron, nickel, and chromium contents of treated textile wastewater were higher than the permissible limits set by Government of Pakistan [7] for irrigation purposes.

3.2 Growth, biomass production, and chlorophyll contents

The combined use of plants and plant growth-promoting bacteria is a promising approach to enhance the remediation of polluted soils in conjunction with sustainable production of biomass. Plant growth-promoting bacteria can improve plant's adaptation to pollutants such as dyes, oil, phenol, and heavy metals by the virtue of their ACC deaminase activity which reduces stress responses in developing plant leading to improved plant growth and development in contaminated soil [20, 25, 26].

In this study, collar diameter, root length, stem length, number of leaves, dry biomass, and chlorophyll contents of plants irrigated with STTW (T2 and T3) were significantly less than of those irrigated with canal water (T1) (Tab. 2). It might be attributed to the toxicity of heavy metals, dye and other chemicals present in STTW. Previous findings also revealed that application of industrial effluent significantly decrease plant biomass production [5, 7, 27]. Due to wastewater

Table 2. Effect of secondary treated textile wastewater and bacterium inoculation on plant development

Treatment	Collar diameter (cm)	Root length (cm)	Shoot length (cm)	No. of leaf	Dry biomass (g)	Chlorophyll (mg g^{-1})
A. ampliceps						
T1	1.69 ± 0.34^{a}	38.5 ± 3.6^{a}	137.3 ± 7.5^{a}	462 ± 26.4^{c}	376.5 ± 14.6^{a}	24.2 ± 2.2^{a}
T2	1.14 ± 0.30^{de}	21.6 ± 2.8^{e}	102.5 ± 8.5^{de}	403 ± 19.8^{d}	316.9 ± 10.8^{c}	17.8 ± 1.9^{bc}
T3	1.47 ± 0.28^{bc}	29.8 ± 3.2^{c}	116.8 ± 6.6^{c}	437 ± 32.3^{cd}	351.6 ± 14.5^{b}	19.2 ± 2.0^{b}
E. camaldulensis						
T1	1.65 ± 0.24^{ab}	36.7 ± 2.3^{ab}	128.2 ± 5.4^{b}	258 ± 16.8^{e}	263.8 ± 10.2^{d}	25.1 ± 2.6^{a}
T2	1.08 ± 0.32^{e}	19.4 ± 1.8^{e}	95.1 ± 6.8^{e}	206 ± 22.6^{f}	206.4 ± 12.9^{f}	20.0 ± 1.9^{b}
T3	1.32 ± 0.25^{cd}	26.8 ± 2.6^{d}	108.4 ± 6.2^{d}	236 ± 20.2^{ef}	231.6 ± 11.4^{e}	22.6 ± 2.3^{a}
L. leucocephala						
T1	1.40 ± 0.31^{c}	35.3 ± 1.5^{b}	126.1 ± 7.1^{b}	592 ± 34.9^{a}	158.3 ± 8.6^{g}	15.7 ± 1.8^{c}
T2	0.98 ± 0.30^{e}	19.8 ± 1.7^{e}	98.5 ± 6.9^{e}	507 ± 27.7^{b}	133.8 ± 7.3^{h}	9.8 ± 1.4^{d}
T3	1.0 ± 0.27^{e}	20.1 ± 1.9^{e}	98.6 ± 8.8^{e}	507 ± 32.3^{b}	135.2 ± 9.5^{h}	10.2 ± 1.6^{d}

T1, canal water; T2, treated textile wastewater; T3, treated textile wastewater and *Burkholderia* sp. strain PsJN::*gusA*10.
Means in the same column followed by the same letter are not significantly different at a 5% level of significance, $n = 3$; results are given ± the standard error of three replicates.

irrigation, increased level of metals in soil influences transportation and balance distribution of essential elements among different plant parts via competitive uptake [28, 29]. The decrease in chlorophyll levels in plant leaves might be due to the high concentrations of heavy metals in plant tissues, which are reported to interfere with the protochlorophyllide reductase complex, and the production of levulinic acid [27].

Plants inoculated with plant growth-promoting bacterium, *Burkholderia* sp. strain PsJN::*gusA*10 (T3), exhibited significantly higher root length (1.5–38%), stem length (0–14%), number of leaves (0–14%), collar diameter (2–29%), dry biomass (1–12%) and chlorophyll contents (4–13%) as compared to those of the plants irrigated with STTW only (T2). This might be due to the plant growth promoting ACC deaminase activity of *Burkholderia* sp. strain PsJN. ACC deaminase, commonly found in plant growth-promoting bacteria, cleaves the plant ethylene ACC, thereby lowering the ethylene level in a developing or stressed plant and consequently improve plant health, growth and biomass production and phytoremediation efficiency [9, 10]. These findings are in agreement with previous studies showing that the plant growth-promoting bacteria having ACC deaminase activity can enhance plant growth and phytoremediation efficiency [20, 25]. The abundance of *Burkholderia* sp. strain PsJN in different compartments (rhizosphere, shoot and root) of plants showed positive correlation with plant biomass production ($r = 0.89$) and metal removal ($r = 0.74$) from soil. Bacterial population and activity may serve as makers of function: in the case of population of hydrocarbon-degrading bacterial population, strong positive correlations have previously been observed between phytoremediation efficiency and bacterial population [20].

3.3 Bacterial survival and colonization

Plant growth and removal of contaminants from soil were associated with the bacterial survival and colonization in the rhizosphere and endosphere of plants vegetated in the contaminated soil [9–12]. Inoculated strain (*Burkholderia* sp. strain PsJN::*gusA*10) was successfully recovered from the rhizosphere and endosphere of all three tested plants (Fig. 1). Restriction fragment length polymorphism analysis revealed that all the isolates (blue colony forming units on selective medium) were the inoculated strain *Burkholderia* sp. strain PsJN::*gusA*10. However, higher number of *Burkholderia* sp. strain PsJN::*gusA*10 was observed in the rhizosphere as compared to endosphere. Enumeration of inoculated bacterium in the rhizosphere and endosphere of three plants showed that bacterium colonization varied among three different plants, however, maximum (5.1×10^5) *Burkholderia* sp. strain PsJN::*gusA*10 population was observed in the rhizosphere of *A. ampliceps*. Many earlier studies also revealed that different plant species host distinct bacterial populations in their rhizosphere and endosphere, which is most likely due to the release of different types of organic compounds in soil by different plants [20, 30].

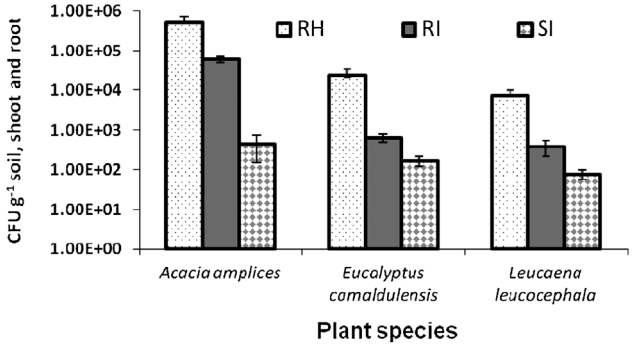

Figure 1. *Burkholderia* sp. strain PsJN::*gusA*10 population in the rhizosphere, and in the root and shoot of three different plant species.

3.4 Mineral nutrient concentration in plants

The availability and uptake of nutrients from the soil and their transportation to different plant parts influence the nutrients concentrations in the plant compartments. In this study, amount of mineral nutrients in roots, stems and leaves of three different plants was significantly increased with STTW application (Tabs. 3–5). Higher metal contents in the plants of T2 and T3 as compared to T1, showing a positive correlation between water and nutrient availability and plants nutrient concentrations. Similarly, high concentrations of nutrients were observed in different plants irrigated with textile and other industrial effluents [31–33]. Overall, leaves had the highest concentration of nutrients followed by the root and stem in all of the three treatments.

The maximum mineral nutrient concentration was observed in the plants inoculated with *Burkholderia* sp. strain PsJN::*gusA*10 (T3), and among three inoculated plants, *A. ampliceps* and *E. camaldulensis* absorbed and accumulated significantly more mineral nutrients as compared to *L. leucocephala*. The increased amount of nutrients in plants was probably due to enhanced nutrients solubilization activities of the inoculant strain in the soil. In *A. ampliceps*, *E. camaldulensis*, and *L. leucocephala* plants, the translocation and accumulation of studied metals was better in upper parts of the plant except Fe, Ni and Al showing highest levels in the roots of *A. ampliceps*. Higher levels of nutrients in leaves as compared to stems and roots were also observed in previous studies [5, 7, 34, 35]. The comparatively higher concentration of Fe, Ni and Al in roots than stems and leaves might be due the strong binding of these metals in the roots which resulted in poor translocation of these ions to the upper ground parts.

3.5 Soil characteristics

Irrigation with wastewater may increase mineral nutrients level in soil, and may also contribute to the accumulation of organic matter in soil [4, 7]. In the present study, STTW irrigation enhanced the level of micronutrients, macronutrients and organic matter in the vegetated and unvegetated soil (Tab. 6). An increase in the value of EC of the soil of T2 and T3 treatments might be due to the high amount of salts in the STTW. Moreover, the soil of T2 and T3 treatments had two- to threefold increase in concentration of soil organic matter, Na, K, Fe, Ni, Al, and Cr compared to that in T1 and the results are similar to those observed previously [36]. A significant decrease in soil organic matter in T3 was believed to be due to enhanced biodegradation of organic matter. Inoculated *Burkholderia* sp. strain PsJN::*gusA*10 enhanced plant growth and especially root length and biomass production which consequently enhanced bacterial population and biodegradation activity in the soil. The combined use of plants and microorganisms has been proved to be more efficient for the remediation of soil contaminated with different organic pollutants as compared to individual plants [37–39]. The nutrients released by the plant root system enhanced the

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Table 3. Accumulation of mineral nutrients in various parts of *A. ampliceps*

Treatment	Na (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (mg kg^{-1})	Ni (mg kg^{-1})	Al (mg kg^{-1})	Cr (mg kg^{-1})
Roots								
T1	2.14 ± 0.23^{e}	1.43 ± 0.32^{e}	1.20 ± 0.15^{f}	1.18 ± 0.13^{ef}	428 ± 26.8^{d}	15.8 ± 1.6^{e}	18.6 ± 2.1^{e}	10.2 ± 0.14^{e}
T2	5.22 ± 0.37^{c}	3.80 ± 0.25^{c}	3.17 ± 0.16^{d}	0.92 ± 0.10^{f}	740 ± 23.0^{b}	56.4 ± 4.2^{c}	46.4 ± 3.8^{b}	160.8 ± 5.2^{c}
T3	7.15 ± 0.24^{b}	4.49 ± 0.16^{b}	4.82 ± 0.27^{c}	1.23 ± 0.17^{ef}	970 ± 34.7^{a}	83.8 ± 6.7^{a}	68.2 ± 5.2^{a}	166.5 ± 7.1^{c}
Stem								
T1	1.58 ± 0.20^{e}	0.78 ± 0.22^{f}	1.64 ± 0.19^{f}	1.37 ± 0.10^{e}	256 ± 26.6^{e}	5.9 ± 0.54^{f}	11.9 ± 1.2^{e}	23.5 ± 0.16^{d}
T2	3.36 ± 0.34^{d}	1.38 ± 0.15^{ef}	2.40 ± 0.24^{e}	1.54 ± 0.12^{de}	480 ± 15.8^{d}	45.6 ± 5.3^{d}	26.7 ± 2.6^{d}	188.6 ± 7.8^{b}
T3	4.84 ± 0.42^{c}	2.18 ± 0.32^{d}	2.75 ± 0.13^{de}	2.05 ± 0.14^{c}	608 ± 14.6^{c}	68.3 ± 7.1^{b}	38.5 ± 4.8^{c}	194.8 ± 6.4^{b}
Leaves								
T1	3.80 ± 0.21^{d}	1.75 ± 0.12^{de}	3.05 ± 0.12^{de}	1.85 ± 0.11^{cd}	380 ± 13.3^{d}	12.8 ± 2.3^{ef}	14.6 ± 1.4^{e}	25.7 ± 2.4^{d}
T2	9.48 ± 0.56^{a}	5.04 ± 0.17^{b}	6.12 ± 0.19^{b}	2.84 ± 0.13^{b}	629 ± 20.8^{c}	51.5 ± 4.2^{cd}	32.4 ± 1.6^{cd}	207.8 ± 8.7^{a}
T3	12.90 ± 0.63^{a}	6.90 ± 0.26^{a}	8.63 ± 0.26^{a}	3.73 ± 0.10^{a}	780 ± 27.2^{b}	79.7 ± 6.4^{a}	47.3 ± 4.3^{b}	216.2 ± 10.4^{a}

T1, canal water; T2, treated textile wastewater; T3, treated textile wastewater and *Burkholderia* sp. strain PsJN::*gusA*10.
Means in the same column followed by the same letter are not significantly different at a 5% level of significance, $n = 3$; results are given $\pm$ the standard error of three replicates.

Table 4. Accumulation of mineral nutrients in various parts of *E. camaldulensis*

Treatment	Na (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (mg kg^{-1})	Ni (mg kg^{-1})	Al (mg kg^{-1})	Cr (mg kg^{-1})
Roots								
T1	1.02 ± 0.12^{f}	1.26 ± 0.09^{e}	1.12 ± 0.16^{f}	1.36 ± 0.14^{bc}	368 ± 68.3^{f}	20 ± 1.36^{e}	9.2 ± 0.62^{f}	10.6 ± 0.64^{g}
T2	2.27 ± 0.10^{e}	2.24 ± 0.13^{d}	3.45 ± 0.18^{d}	1.48 ± 0.12^{bc}	798 ± 72.5^{b}	42 ± 4.9^{c}	38 ± 2.60^{d}	136.8 ± 5.78^{f}
T3	3.91 ± 0.15^{c}	3.38 ± 0.16^{c}	4.82 ± 0.12^{b}	1.74 ± 0.17^{b}	834 ± 56.6^{b}	58 ± 5.3^{b}	46 ± 2.46^{c}	165.3 ± 4.75^{e}
Stem								
T1	1.84 ± 0.21^{e}	1.08 ± 0.08^{e}	2.56 ± 0.07^{e}	1.14 ± 0.09^{c}	342 ± 37.8^{f}	29 ± 2.8^{d}	6.8 ± 0.48^{f}	19.4 ± 1.74^{g}
T2	4.02 ± 0.20^{c}	3.06 ± 0.17^{c}	4.15 ± 0.19^{c}	1.32 ± 0.14^{bc}	617 ± 65.3^{d}	55 ± 6.1^{b}	24 ± 2.63^{e}	231.6 ± 14.9^{d}
T3	5.12 ± 0.27^{b}	4.45 ± 0.19^{b}	5.08 ± 0.40^{b}	1.56 ± 0.16^{bc}	698 ± 36.5^{c}	69 ± 4.6^{a}	33 ± 4.82^{d}	278.5 ± 25.3^{c}
Leaves								
T1	3.12 ± 0.18^{d}	1.82 ± 0.17^{d}	3.02 ± 0.11^{de}	1.68 ± 0.08^{b}	470 ± 42.7^{e}	36 ± 1.32^{cd}	12 ± 2.19^{f}	28.5 ± 1.02^{g}
T2	5.36 ± 0.28^{a}	4.46 ± 0.15^{b}	5.88 ± 0.30^{a}	1.79 ± 0.14^{b}	860 ± 56.5^{ab}	59 ± 3.74^{b}	65 ± 2.57^{b}	335.2 ± 14.7^{b}
T3	6.20 ± 0.14^{a}	5.08 ± 0.22^{a}	6.12 ± 0.37^{a}	2.66 ± 0.20^{a}	920 ± 37.8^{a}	75 ± 4.46^{a}	84 ± 3.61^{a}	434.9 ± 15.3^{a}

T1, canal water; T2, treated textile wastewater; T3, treated textile wastewater and *Burkholderia* sp. strain PsJN::*gusA*10.
Means in the same column followed by the same letter are not significantly different at a 5% level of significance, $n = 3$; results are given $\pm$ the standard error of three replicates.

Table 5. Accumulation of mineral nutrients in various parts of *L. leucocephala*

Treatment	Na (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (mg kg^{-1})	Ni (mg kg^{-1})	Al (mg kg^{-1})	Cr (mg kg^{-1})
Roots								
T1	3.04 ± 0.14^{f}	1.48 ± 0.13^{e}	1.75 ± 0.14^{f}	1.08 ± 0.14^{c}	356 ± 19.6^{f}	15 ± 1.4^{f}	30 ± 3.2^{f}	10.5 ± 1.3^{c}
T2	5.11 ± 0.22^{d}	4.44 ± 0.16^{b}	3.46 ± 0.18^{bc}	1.63 ± 0.18^{b}	625 ± 36.2^{d}	48 ± 4.7^{c}	67 ± 4.7^{d}	135 ± 7.1^{b}
T3	5.78 ± 0.25^{c}	4.51 ± 0.17^{b}	4.04 ± 0.22^{a}	1.71 ± 0.12^{b}	643 ± 26.4^{cd}	56 ± 4.8^{c}	78 ± 5.1^{c}	138 ± 6.8^{b}
Stem								
T1	2.34 ± 0.11^{g}	1.26 ± 0.19^{f}	1.95 ± 0.11^{ef}	0.66 ± 0.15^{d}	330 ± 18.5^{f}	23 ± 3.8^{e}	17 ± 3.5^{g}	11.7 ± 0.94^{c}
T2	5.64 ± 0.20^{cd}	3.44 ± 0.24^{c}	3.07 ± 0.29^{d}	0.96 ± 0.16^{c}	670 ± 27.6^{bc}	76 ± 4.5^{b}	80 ± 5.4^{c}	141 ± 10.4^{b}
T3	5.48 ± 0.27^{d}	3.38 ± 0.29^{c}	3.60 ± 0.35^{b}	1.03 ± 0.12^{c}	684 ± 15.3^{b}	73 ± 5.2^{b}	76 ± 4.9^{bc}	148 ± 8.6^{b}
Leaves								
T1	4.64 ± 0.34^{e}	1.95 ± 0.14^{d}	2.12 ± 0.23^{e}	2.21 ± 0.12^{a}	457 ± 16.4^{e}	37 ± 3.4^{d}	46 ± 3.8^{e}	17.9 ± 1.16^{c}
T2	6.67 ± 0.28^{b}	5.93 ± 0.35^{a}	3.63 ± 0.24^{b}	2.29 ± 0.35^{a}	720 ± 24.9^{a}	85 ± 4.7^{a}	106 ± 6.9^{b}	207 ± 8.5^{a}
T3	6.86 ± 0.12^{a}	6.07 ± 0.25^{a}	3.27 ± 0.34^{cd}	2.14 ± 0.25^{a}	739 ± 34.5^{a}	92 ± 5.2^{a}	115 ± 7.4^{a}	201 ± 10.6^{a}

T1, canal water; T2, treated textile wastewater; T3, treated textile wastewater and *Burkholderia* sp. strain PsJN::*gusA*10.
Means in the same column followed by the same letter are not significantly different at a 5% level of significance, $n = 3$; results are given $\pm$ the standard error of three replicates.

Table 6. Effect of secondary treated textile wastewater and *Burkholderia* sp. strain PsJN::*gusA*10 inoculation on soil chemistry

Treatment	pH	EC ($mS\,cm^{-1}$)	OM (%)	Na ($g\,kg^{-1}$)	Ca ($g\,kg^{-1}$)	Mg ($g\,kg^{-1}$)	Fe ($mg\,kg^{-1}$)	Ni ($mg\,kg^{-1}$)	Al ($mg\,kg^{-1}$)	Cr ($mg\,kg^{-1}$)
T0	$7.60 \pm 0.22)^{a}$	$4.58 \pm 0.35)^{a}$	$1.47 \pm 0.18)^{a}$	$4.86 \pm 0.25)^{a}$	$16.04 \pm 0.85)^{a}$	$0.72 \pm 0.052)^{a}$	$88.52 \pm 5.73)^{a}$	$25.80 \pm 3.57)^{a}$	$20.64 \pm 0.65)^{a}$	$140.82\ (6.83)^{a}$
A. ampliceps										
T1	7.20 ± 0.13^{a}	1.4 ± 0.06^{c}	0.40 ± 0.005^{cd}	0.71 ± 0.12^{ef}	13.81 ± 0.54^{b}	0.26 ± 0.052^{c}	10.73 ± 0.83^{f}	4.25 ± 0.25^{f}	2.72 ± 0.18^{de}	1.25 ± 0.12^{e}
T2	7.58 ± 0.32^{a}	3.77 ± 0.08^{b}	0.86 ± 0.01^{b}	2.60 ± 0.13^{c}	10.52 ± 0.68^{d}	0.19 ± 0.018^{cde}	28.96 ± 1.36^{b}	7.48 ± 0.18^{cd}	3.86 ± 0.23^{b}	20.28 ± 1.58^{bc}
T3	7.58 ± 0.28^{a}	3.65 ± 0.40^{b}	0.54 ± 0.007^{bcd}	1.84 ± 0.15^{d}	9.36 ± 0.82^{d}	0.17 ± 0.013^{de}	18.44 ± 1.08^{d}	4.86 ± 0.20^{ef}	2.94 ± 0.30^{cd}	17.85 ± 1.23^{d}
E. camaldulensis										
T1	7.30 ± 0.24^{a}	1.5 ± 0.03^{c}	0.52 ± 0.008^{bd}	0.97 ± 0.08^{e}	12.60 ± 0.86^{bc}	0.28 ± 0.014^{b}	10.62 ± 0.97^{f}	4.34 ± 0.35^{f}	2.19 ± 0.21^{e}	1.06 ± 0.13^{e}
T2	7.53 ± 0.13^{a}	3.85 ± 0.07^{b}	0.76 ± 0.01^{bc}	2.70 ± 0.30^{bc}	13.92 ± 1.06^{b}	0.25 ± 0.14^{bc}	22.59 ± 1.27^{c}	6.91 ± 0.31^{d}	3.71 ± 0.16^{b}	20.62 ± 1.74^{b}
T3	7.46 ± 0.14^{a}	3.72 ± 0.05^{b}	0.31 ± 0.006^{d}	1.85 ± 0.25^{d}	12.87 ± 0.83^{bc}	0.23 ± 0.010^{bc}	14.59 ± 0.85^{e}	5.40 ± 0.40^{e}	3.38 ± 0.27^{bc}	18.57 ± 1.69^{cd}
L. leucocephala										
T1	7.25 ± 0.18^{a}	1.3 ± 0.02^{c}	0.46 ± 0.005^{cd}	0.64 ± 0.08^{g}	12.15 ± 0.96^{c}	0.25 ± 0.015^{bc}	10.93 ± 0.75^{f}	4.58 ± 0.25^{f}	1.57 ± 0.18^{f}	1.73 ± 0.26^{e}
T2	7.64 ± 0.13^{a}	3.90 ± 0.06^{b}	0.62 ± 0.007^{bcd}	2.80 ± 0.34^{b}	12.58 ± 0.67^{bc}	0.14 ± 0.016^{e}	27.82 ± 1.17^{b}	8.35 ± 0.28^{b}	3.55 ± 0.26^{b}	20.2 ± 2.05^{bc}
T3	7.49 ± 0.15^{a}	3.83 ± 0.08^{b}	0.60 ± 0.009^{bcd}	2.78 ± 0.27^{b}	12.29 ± 0.92^{c}	0.13 ± 0.017^{e}	27.45 ± 1.83^{b}	8.14 ± 0.32^{bc}	3.50 ± 0.34^{b}	18.8 ± 1.73^{bcd}

T0, secondary treated textile wastewater (without plant and bacteria); T1, canal water; T2, secondary treated textile wastewater; T3, secondary treated textile wastewater and *Burkholderia* sp. strain PsJN::*gusA*10. OM, organic matter.
Means in the same column followed by the same letter are not significantly different at a 5% level of significance, $n = 3$; results are given ± the standard error of three replicates.

population and activity of beneficial microorganisms in the rhizosphere and 10- to 1000-fold greater numbers of bacteria were observed in the rhizosphere than the bulk soil, which may help to improve in situ degradation of organic pollutants [9, 40]. The concentrations of Na, K, Ca, Mg, Fe, Ni, Al, and Cr in soil which were significantly increased in T2 and T3 indicating that the nutrient removal efficiency of the plants is lower than the nutrient-input rate by wastewater irrigation (Tab. 6). Comparatively, low quantity of nutrients in the soil of T3 compared to those in the soil of T2 (although these received equal quantity of STTW) indicating that nutrients uptake by plants was enhanced by *Burkholderia* sp. strain PsJN::*gusA*10 inoculation. The maximum organic and inorganic contaminants removal was achieved with bacterial inoculation of *A. ampliceps*. It has been revealed earlier that bacterial inoculation can enhance metal transportation and accumulation in plants [25, 41, 42]. Organic compounds that are produced by bacteria, such as chelators, play an important role in tolerance, sequestration, and transportation of inorganic pollutants inside plant tissues [43].

4 Concluding remarks

This study explore the potential of use of plant growth-promoting bacteria for improving plant growth and biomass production in soil irrigated with secondary treated textile wastewater, although improving effects and their mechanisms are poorly understood. Furthermore, bacterial inoculation enhanced the removal of organic and inorganic pollutants from the soil. Application of plant growth-promoting bacteria is low cost and easy to apply, however, it is not realized that it may enhance plant biomass production and remediation of soil irrigated with industrial effluent. Further studies are needed in the future to understand the exact mechanisms that how microbial inocula improve plant growth and pollutants removal during industrial effluent application to plants for biomass production.

Acknowledgment

The authors would like to thank Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan for providing plants of different species.

The authors have declared no conflict of interest.

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