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BASIC AREA
Article

Plant growth-promoting rhizobacteria effect on maize growth and microbial biomass in a chromium-contaminated soil

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ABSTRACT: Chromium contamination in soils affects plant growth and this metal can accumulate in plants tissues. In addition, Cr can affect soil microbial biomass and activity. On the other hand, plant growth-promoting rhizobacteria (PGPR) can protect plants against metals and, at the same time, promote plant growth and could alleviate adverse effects on microbial biomass. This study evaluated five PGPR on maize growth, Cr accumulation and soil microbial biomass in a Cr-contaminated soil. Five PGPR (LCC04, LCC41, LCC69, LCC81 and IPA403), isolated from soil under permanent application of composted tannery sludge and contaminated with Cr, were inoculated in maize plants grown in soils with (+Cr) and without (–Cr) Cr. In Cr-contaminated soil, LCC41 promoted the highest growth of maize, while LCC04 contributed with the highest N accumulation. The shoots of maize accumulated less Cr with LCC81, while LCC41 contributed to the highest Cr accumulation in roots. The translocation of Cr was highest with IPA403, while LCC81 contributed to reduce Cr translocation. In conclusion, LCC41 and LCC81 could be effective as PGPR inoculants to promote plant growth and reduce Cr accumulation in maize, respectively, in Cr contaminated soil.

Key words: metals, tannery sludge, soil contamination, PGPR.

INTRODUCTION

The accumulation of metals in soils has been an important environmental issue, since these elements are not naturally degraded, persist in the ecosystem and are translocated to different parts of plants (Ogundiran and Osibanjo 2009). Particularly, chromium (Cr) shows high accumulation in soils, especially on those with a history of application of Cr-contaminated sludge, such as tannery sludge (Araújo et al. 2013, 2018).

Indeed, the long-term application of Cr-contaminated tannery sludge has increased the accumulation of Cr in soils and promoted changes in soil chemical (Araújo et al. 2013), physical (Araújo et al. 2016) and biological properties (Sousa et al. 2017). In addition, Cr accumulated in soil is translocated and bioaccumulated in plants (Sousa et al. 2018). On the other hand, some studies have found different microbes that showed tolerance to high Cr concentration (Miranda et al. 2018, 2019), including plant growth-promoting rhizobacteria (PGPR) (Rocha et al. 2019).

Some PGPR have been recognized to be tolerant to metal-contaminated soils, since they present different strategies to withstand these elements (Hao et al. 2014; Ojuederie and Babalola 2017). Some strategies include the secretion of enzymes and bioactive metabolites that could protect plants against metals and, at the same time, promoting plant growth (Hao et al. 2014). For instance, PGPR produce and release enzymes and exopolysaccharide (EPS) that can assist in metal detoxification and, indirectly, improve plant growth (Lal et al. 2018).

The PGPR tolerance to Cr contamination could be associated with their ability to produce and release enzymes that enable them to thrive under high Cr concentrations (Tirry et al. 2018). In a previous study in a Cr-contaminated soil, Rocha et al. (2019) selected some PGPR with high biochemical capability, i.e., urease, catalase and phosphate solubilization activity, and tolerance to high Cr concentrations. Therefore, the use of these Cr-tolerant PGPR may present potential in Cr-contaminated soil by acting on plant growth promotion (Anyanwu and Ezaka 2011). Plant growth-promoting rhizobacteria could also alleviate the effect of Cr on soil microbial biomass and activity by stimulating root growth and enhancing the rhizosphere effect (Souza et al. 2015). In addition, the production of extracellular enzymes by PGPR contributes to decompose organic residues and increase microbial biomass and activity (Moraes et al. 2018).

The hypothesis of this study is that the inoculation of selected PGPR could stimulate plant growth and improve soil microbial biomass in Cr-contaminated soils. Therefore, five potential PGPR, that present high biochemical capability, Cr tolerance and production of EPS (Rocha et al. 2019) were evaluated in a pot experiment. The features presented by these PGPR could contribute to ameliorate Cr stress, avoid Cr translocation on plants and promote plant growth. Thus, the aim of this study was to evaluate the inoculation effect of five PGPR on maize growth and microbial biomass in both noncontaminated and Cr-contaminated soil.

MATERIAL AND METHODS

The study was conducted in a greenhouse located at the Department of Soil and Agricultural Engineering (DEAS), Federal University of Piauí, Brazil (05°05'S, 42°48'W; 75 m). Pots (2.8 L) were filled with soil (Fluvic Neosol) collected from a long-term experimental field with a history of application of composted tannery sludge (CTS). In this study, soil samples were collected from plots with application of 20 ton·ha^{-1} CTS (highest rate). This soil presents an accumulation of about 300 mg·kg^{-1} Cr. As a control without CTS, soil samples were collected from an adjacent site (without CTS application) that does not present Cr.

The Cr was extracted from the soil by the DTPA-TEA method and measured using the USEPA-3050 method (EPA 1986). Thus, two soil conditions were used in this experiment, according to Cr concentration: soil without Cr (–Cr) and soil with Cr (+Cr). Soil chemical analysis were conducted using air dried and sieved (2 mm) samples. Soil pH, exchangeable Ca^{2+}, Mg^{2+}, K^{+}, and the available P were estimated according to EMBRAPA (1997). Soil organic C (TOC) was determined by wet combustion using a mixture of 5 mL of 0.167 mol·L^{-1} potassium dichromate and 7.5 mL of concentrated sulfuric acid under heating (170 °C for 30 min) (Yeomans and Bremner 1988). The chemical characteristics of the soils are shown in Table 1.

Table 1. Chemical characteristics of soils.

Soil	pH	OM	P	K	Ca	Mg	Cr
	H_2O	G·kg^{-1}	--------- mg·kg^{-1}---------		--------- cmol$_c$·kg^{-1}---------		mg·kg^{-1}
–Cr	6.0	5.3	4.3	38.2	1.8	0.5	0.0
+Cr	6.6	12.1	7.6	70.3	3.3	1.2	320

–Cr: noncontaminated soil; +Cr: Cr-contaminated soil; OM: organic matter.

This study evaluated four potential PGPR that presented high biochemical capability (urease, catalase and phosphate solubilization activity), tolerance to Cr and production of EPS (Rocha et al. 2019) in the presence of Cr, being: LCC04, LCC41, LCC69 and LCC81. A description on the origin of these PGPR is provided by Rocha et al. (2019). Another potential PGPR (IPA403), with high production of EPS (Antunes et al. 2017) was also evaluated. A treatment without inoculation was used as control. The experiment design was completely randomized in a factorial scheme with two Cr concentrations (0 and 300 mg·kg^{-1}) and six treatments (LCC04, LCC41, LCC69, LCC81, IPA403 and control), in four replicates.

All rhizobacteria were grown in nutrient agar solid medium containing 3.0 $g \cdot L^{-1}$ of yeast extract, 5 $g \cdot L^{-1}$ of peptone and 20 $g \cdot L^{-1}$ of agar for 48 h at 32 °C. Bacterial colonies were transferred to 100 mL of sterile saline water supplied with 0.01 $mol \cdot mL^{-1}$ $MgSO_4$ and the suspension was stirred in vortex for bacterial dispersion. Bacterial inoculant concentration was adjusted to 1.0×10^8 $cells \cdot mL^{-1}$. These aqueous suspensions containing each isolate were used as bacterial inoculants. Maize (*Zea mays* L., AG1061) seeds were surface-disinfested (5% sodium hypochlorite for 3 min) and rinsed with sterile distilled water. These disinfested seeds were used in the experimental procedures. The bacterial aqueous suspension was applied directly onto the seeds during planting in the amount of 1 mL per seed. Eight days after germination, plants were thinned, leaving one plant per pot. Each pot received 600 mg ammonium sulphate, 410 mg super single phosphate and 225 mg potassium chloride. The rates of ammonium sulphate and potassium chloride were divided into two applications (at 15 and 40 days after emergence). Pots were irrigated daily with sterilized water to maintain soil moisture at 70% of field capacity.

At harvest (60 days after plant emergence at pre-flowering stage), soil and plants were collected. Soil microbial biomass C (MBC) was estimated using the chloroform fumigation-extraction method according to Vance et al. (1987). The extraction efficiency coefficients of 0.38 was used to convert the difference in C between fumigated and nonfumigated soils in MBC. Soil respiration was monitored with a daily measurement of CO_2 evolution under aerobic incubation at 25 °C for 7 days (Alef and Nannipieri 1995). The respiratory quotient (qCO_2) was calculated from the ratio between respiration and MBC and is expressed as mg $CO_2 \cdot kg^{-1} \cdot MBC \cdot day^{-1}$.

The shoot was separated from roots and they were dried (65 °C; 72 h) and weighed to determine both shoot (SDW) and roots (RDW) dry weight. Total N content in the shoots was estimated by Kjedahl method. The chlorophyll was estimated by a portable chlorophyll meter (Falker ClorofiLOG CFL 1030), according to manufacturer's instruction (Falker 2008). The accumulation of N (AcN) in the shoot was estimated by the SDW and total N content. Chromium content in shoot and roots were estimated according to the method described in USEPA-3050 (EPA 1986). The translocation factor of Cr (TF) (Patel et al. 2016) was calculated as Eq. 2:

$$TF = \text{Cr content in the shoot } (mg \cdot kg^{-1}) / \text{Cr content in the roots } (mg \cdot kg^{-1}) \quad (2)$$

Shapiro–Wilk and Bartlett tests were used to test the normality and homogeneity of variance of data, respectively. Except for Cr accumulation and TF, all data were statistically analyzed using an analysis of variance (ANOVA) considering a factorial design with two soil conditions (–Cr and +Cr) and six treatments (isolates and control), and their interactions. The means were compared by Tukey's test. All data were analyzed using the R software (R Core Team 2014).

RESULTS AND DISCUSSION

The results showed differences between the responses to PGPR and soil conditions for all evaluated parameters. In noncontaminated soil, inoculation with LCC81 and LCC41 promoted the highest SDW, while in Cr-contaminated soil, LCC69 contributed to increased SDW (Fig. 1a). The PGPR showed no significant effects with respect to RDW, compared to the control, in noncontaminated soils; however, LCC41 showed a greater RDW than LCC81 (Fig. 1b). In Cr-contaminated soil, inoculation with LCC41 increased RDW as compared with LCC04, LCC81, IPA403 and the control. Inoculation with LCC41 and LCC81 contributed to the highest root:shoot ratio in Cr-contaminated soil (Fig. 1c). In noncontaminated soil, there were no differences between treatments to root:shoot ratio, except the treatment with LCC81, which showed the lowest value.

The results showed that, in Cr-contaminated soil, shoot biomass was significantly stimulated by inoculation with LCC04, LCC41 and LCC69, with a range of 50 to 100%, as compared to the uninoculated control. Previous studies have also reported some Cr-tolerant PGPR increasing the growth of sorghum (Bruno et al. 2020), wheat (Khan et al., 2013) and alfalfa (Tirry et al. 2018) in Cr-contaminated soils.

Plant growth-promoting rhizobacteria present the ability to stimulate plant growth directly, by production of phytohormones, and indirectly, by amelioration of metal stress (Mallick et al. 2018). According to Chiboub et al. (2016), several metal-tolerant PGPR are capable of producing phytohormones, such as indole-3-acetic acid (IAA), and enzymes even under metal stress conditions. Indeed, the selected PGPR used in this study presents high capability of producing IAA and enzymes in Cr-contaminated soils (Rocha et al. 2019). Particularly, Rocha et al. (2019) found that LCC04, LCC41 and LCC69 presented positive activity of catalase, urease, lipase and phosphate solubilization in Cr-contaminated soil. Thus, the inoculation with these isolates contributed to increased maize growth in Cr-contaminated soil. In noncontaminated soil, inoculation with LCC81 increased maize shoot growth, although this response was not observed in Cr-contaminated soil.

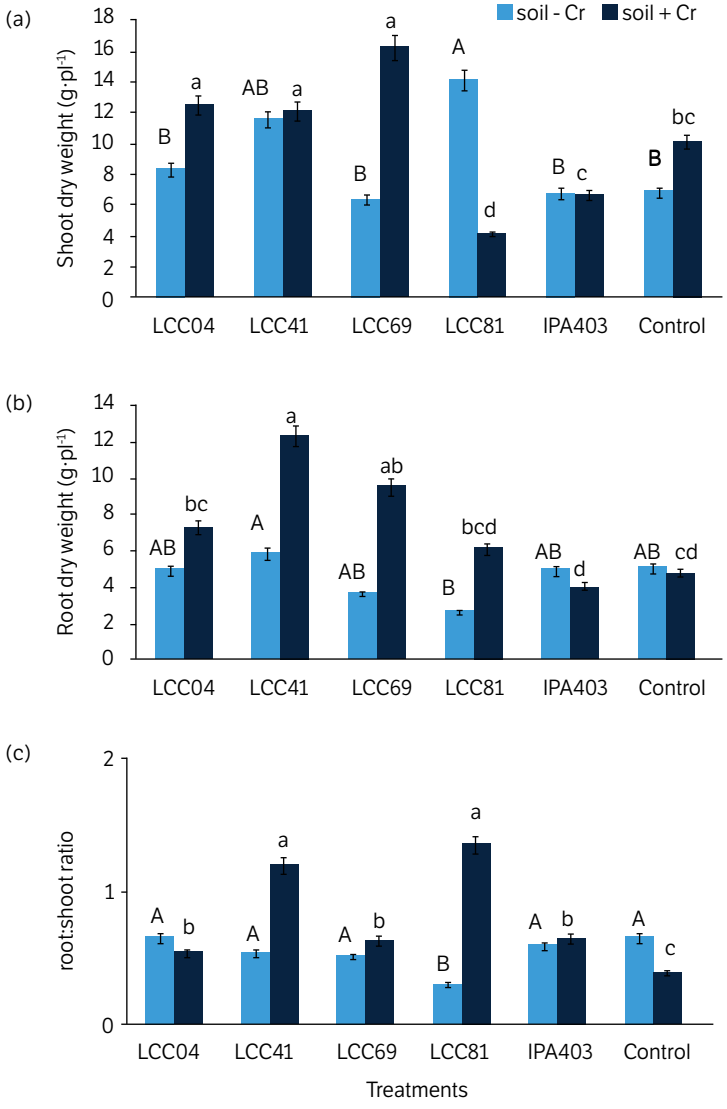

Figure 1. Shoot (a) and roots (b) dry weight, and root:shoot ratio (c) of maize inoculated with PGPR grown in soil with (+Cr) and without (–Cr) Cr.

Note: Different uppercase and lowercase letters indicate a significant difference ($p < 0.01$) between treatments in soil –Cr and +Cr, respectively. The bars represent the standard error of the mean ($n = 4$).

In regard to root growth, LCC41 promoted about three times greater root biomass than uninoculated plants. In a previous study, LCC41 was found to be a high producer of IAA in Cr-contaminated soil (70 µg IAA·mL^{-1} in soil under 100 mg Cr·kg^{-1}; Rocha et al. 2019). Indole-3-acetic acid is the most common plant hormone of the auxin class, being required for root

development (Tian et al. 2014). Thus, the increased root growth with LCC41 was likely influenced by its high capability in producing IAA. Since LCC41 stimulated more root growth in Cr-contaminated soil, it contributed to the highest root:shoot ratio found in this treatment. The higher root:shoot ratio found with LCC41 suggests that this isolate could ameliorate possible negative effects of Cr on root growth.

Under both soil conditions, maize accumulated more N with the inoculation of LCC04 (Fig. 2a). The LCC04 also contributed to the highest values of chlorophyll in noncontaminated soil (Fig. 2b), while in Cr-contaminated soil no significant differences were observed between treatments. These results suggest that LCC04 is able to contribute N to plants under both soil conditions. Since this PGPR was isolated from Cr-contaminated soil (Rocha et al. 2019), it may have been conferred the ability to fix or contribute N even under Cr stress. Therefore, the higher N accumulation observed in plants by inoculation with LCC04 reflects its Cr-tolerance and biochemical capability in Cr-contaminated soils. Compared to other PGPR, LCC04 is catalase, urease and phosphatase positive in soil with 200 mg $Cr \cdot kg^{-1}$ (Rocha et al. 2019). The urease catalyzes the hydrolysis of urea to ammonium and then it can be absorbed by plants (Nosheen and Bano 2014), while catalase protects plants against oxidative stress (Santos et al. 2018). Consequently, the higher N accumulation in plants, by inoculating with LCC04, increased chlorophyll content. Chlorophyll is a pigment related to photosynthesis, being important to confer plant growth (Kanwal et al. 2017) and some studies have reported PGPR increasing chlorophyll content in maize (Kifle and Laing 2016; Aquino et al. 2019). Interestingly, IPA403 is an isolate with efficiency to contribute N to maize, but it was isolated from a noncontaminated soil by Antunes et al. (2017). Thus, IPA403 was apparently not able to contribute N in the Cr-contaminated soil.

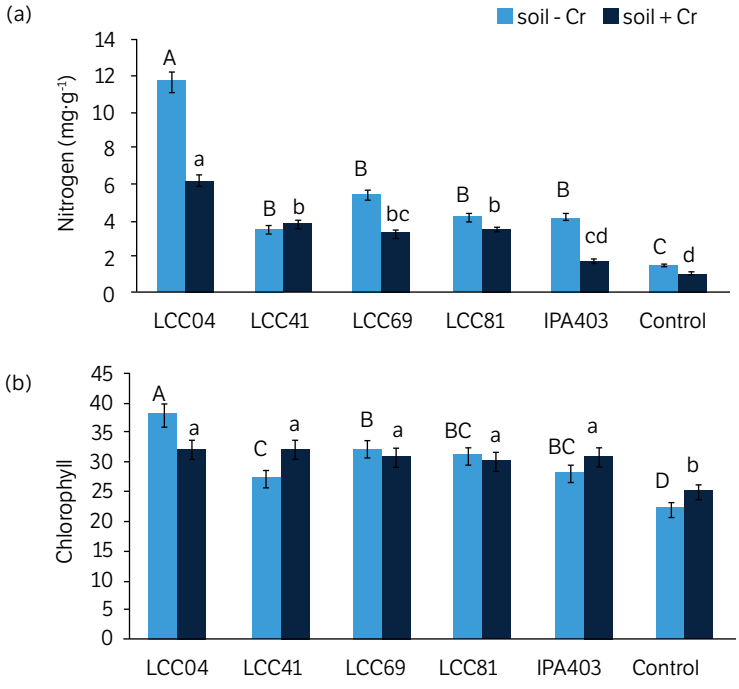

Figure 2. Nitrogen (a) and chlorophyll (b) content in maize leaves inoculated with PGPB grown in soil with (+Cr) and without (–Cr) Cr.

Note: Different uppercase and lowercase letters indicate a significant difference ($p < 0.01$) between treatments in soil –Cr and +Cr, respectively. The bars represent the standard error of the mean ($n = 4$).

The accumulation of Cr was more pronounced in roots than in shoots (Fig. 3a). In Cr-contaminated soil, the shoots accumulated less Cr with inoculation with LCC81. In contrast, plants inoculated with IPA403 and those not inoculated accumulated more Cr in shoot. Roots accumulated more Cr in plants inoculated with LCC41. The inoculation with IPA403 and LCC81 contributed to the highest and lowest TF, respectively (Fig. 3b).

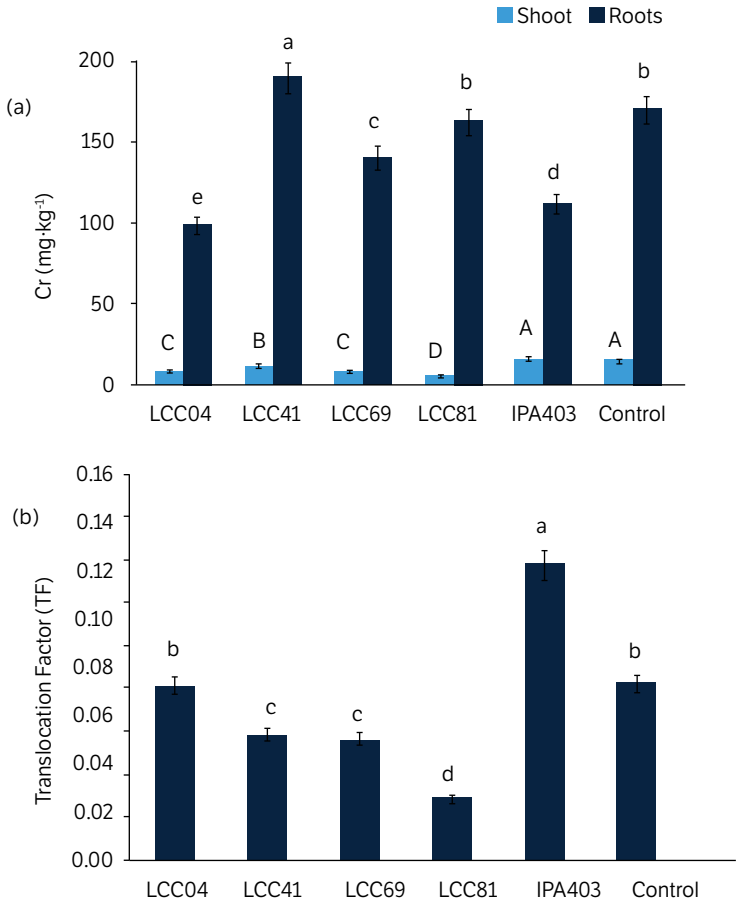

Figure 3. Chromium accumulation in plants (a) and the translocation factor (b) in maize inoculated with PGPB grown in soil with (+Cr) and without (–Cr) Cr.

Note: Different uppercase and lowercase letters indicate a significant difference ($p < 0.01$) between treatments in shoot and roots, respectively. The bars represent the standard error of the mean ($n = 4$).

The higher accumulation of Cr, in roots than shoots, agrees with previous studies that have shown metals being preferentially accumulated in maize roots, such as Cr (Sousa et al. 2018) and Cu (Rizvi and Khan 2018). Recently, Sousa et al. (2018) assessed the accumulation of Cr in maize and cowpea and found higher accumulation in roots. The results showed roots accumulating more Cr when inoculated with LCC41 and could be related to the higher root growth and root:shoot ratio found in this treatment.

In Cr-contaminated soil, the inoculation with LCC81 reduced the translocation of Cr from roots to shoot. Recent studies have reported that metal-tolerant PGPR could present the ability to immobilize and reduce the bioavailability of metals and their consequent accumulation in plants and, at the same time, promote plant growth (Han et al. 2018; Wang et al. 2018). For instance, *Bacillus megaterium* promoted the growth of *Brassica juncea*, *Luffa cylindrica* and *Sorghum halepense* and contributed to decrease the translocation of Ni from roots to shoots (Rajkumar et al. 2013). Recently, Rizvi and Khan (2018) reported that inoculation with *Azotobacter chroococcum* lowered the Cu and Pb accumulation in maize and that this was due to metal chelation and immobilization. There is some speculation that some substances produced by PGPR, such as IAA, siderophores and EPS, could help plants to resist metal contamination. In addition, these PGPR also were shown to form biofilms, which restricted metal uptake in these plants (Das and Sarkar 2018; Rizvi and Khan 2018).

The inoculation with LCC41 increased soil respiration in both noncontaminated and Cr-contaminated soils (Fig. 4a). LCC69 also contributed to increase soil respiration in Cr-contaminated soil. In noncontaminated and Cr-contaminated soils, LCC04 and IPA403 decreased soil respiration, respectively. Microbial biomass C was higher in both noncontaminated and

Cr-contaminated soils with LCC69 inoculation (Fig. 4b). LCC04 and LCC81 also contributed to high MBC in Cr-contaminated soil. The lowest values of MBC in both soil conditions were found in the uninoculated soil. In noncontaminated soil, qCO_2 was higher and lower with the inoculation of LCC41 and uninoculated soil, respectively (Fig. 4c). In contrast, uninoculated soil had the highest qCO_2 in Cr-contaminated soil. On the other hand, inoculation with LCC04, LCC69, LCC81 and IPA403 contributed to decrease qCO_2.

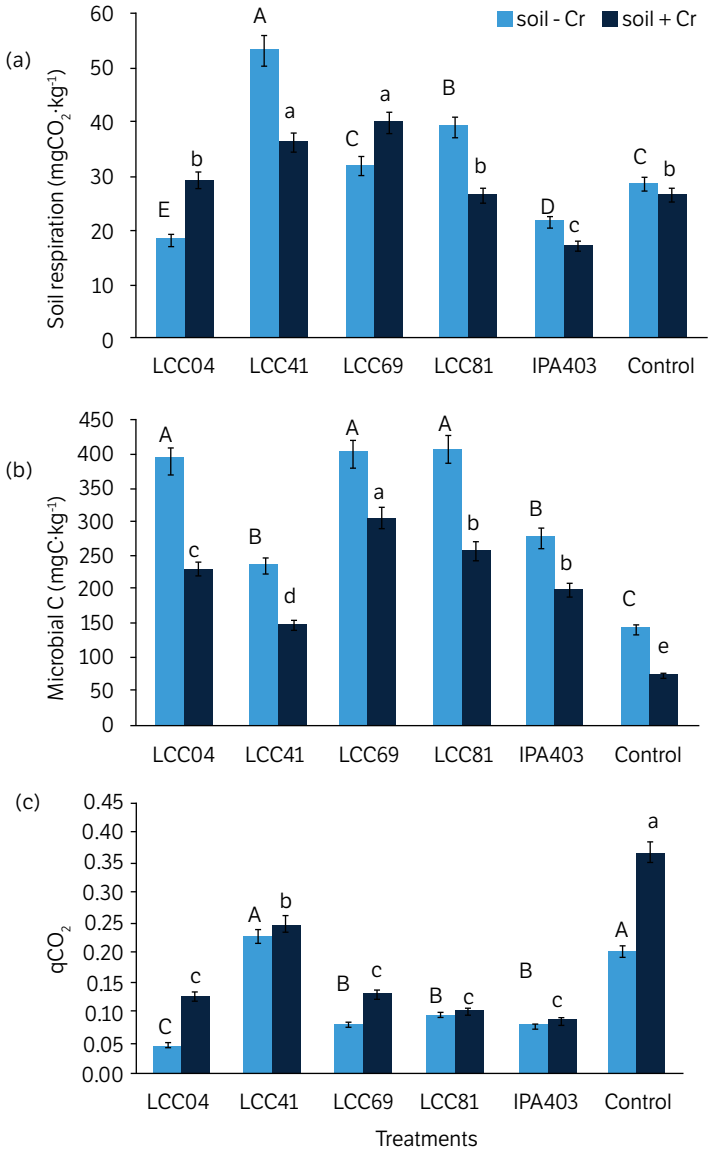

Figure 4. Soil respiration (a) MBC (b) and respiratory quotient (c) after inoculation of maize with PGPB in soil with (+Cr) and without (–Cr) Cr.

Note: Different uppercase and lowercase letters indicate a significant difference ($p < 0.01$) between treatments in soil –Cr and +Cr, respectively. The bars represent the standard error of the mean ($n = 4$).

The results showed, in general, a positive influence from inoculation on soil respiration and MBC. There are few reports about the effect of PGPR on soil microbial biomass and respiration, but these results agree with Sharma et al. (2013), who reported that inoculation with *Bradyrhizobium amyloliquefaciens* significantly increased these parameters compared to uninoculated controls. Thus, the results add more information about the effect of PGPR on soil microbial biomass and activity, mainly under Cr-contamination. Interestingly, inoculation with LCC69 contributed to higher soil respiration and MBC in Cr-contaminated soil. Since soil respiration is recognized as a useful indicator of

microbial activity (Alef and Nanipieri 1995), these results suggest that LCC69 could stimulate soil microbial biomass and activity in Cr-contaminated soil. In Cr-contaminated soil, microbial biomass decreased, while qCO2 increased in uninoculated soil. This suggests that high Cr contamination inhibits microbial biomass and causes soil microbial stress (Sousa et al. 2017).

Finally, the results of this study showed a differential response of several potential PGPR on maize growth and accumulation of Cr, and microbial biomass under noncontaminated and Cr-contaminated soils, in a pot-experiment. This study highlights the potential of these isolates to promote plant growth even in contaminated soils. Since this study was conducted in a controlled greenhouse experiment, further studies under field conditions, different soil types and plant species are necessary to potentially recommend these isolates as inoculants to be used by farmers. However, research under controlled conditions is one of the first steps required to identify potential strains prior to evaluation under field conditions. For example, Antunes et al. (2011) evaluated the responses of rhizobia on growth of lima bean, under a greenhouse, and identified potential isolates to be used under field conditions. Later, Costa et al. (2020) assessed these isolates on lima bean yield, under field conditions and two locations, and found two strains to be recommended as effective inoculants for this crop.

CONCLUSION

Plant growth-promoting rhizobacteria isolated from Cr-contaminated soils present the potential to promote plant growth, reduce translocation of Cr within the plant and improve soil microbial biomass and activity. Particularly, LCC41 and LCC81 could potentially promote maize growth and reduce Cr accumulation in shoots, respectively, in Cr-contaminated soil. In addition, LCC69 stimulated microbial biomass and activity. Further studies should be done aiming to verify the performance of these PGPR on Cr degradation.

AUTHORS' CONTRIBUTION

Conceptualization: Araujo, A. S. F.; **Methodology:** Araujo, A. S. F. and Melo, W. Y.; **Investigation:** Silva, R. S., Antunes, J. E. L. and Aquino, J. P. A.; **Writing – Original Draft:** Silva, R. S., Araujo, A. S. F., Sousa, R. S. and Antunes, J. E. L.; **Writing – Review and Editing:** Araujo, A. S. F. and Melo, W. Y.; **Funding Acquisition:** Araujo, A. S. F.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

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