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Bioaugmentation-assisted phytoremediation of As, Cd, and Pb using *Sorghum bicolor* in a contaminated soil of an abandoned gold ore processing plant

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ABSTRACT: The two main bottlenecks for a successful phytoremediation program are the metal availability in soil and the metal uptake and transfer to shoots of high biomass plants. Several agronomical practices have been tested to boost the bioavailability of metals in soils and accumulation in plants. Here we assessed the feasibility of plant-growth-promoting bacteria (PGPB) isolated from a site contaminated by gold ore processing activities to assist the phytoremediation of As, Cd, and Pb by *Sorghum bicolor* and mitigate the metal toxicity in plants. The bacteria *Kluyvera intermedia*, *Klebsiella oxytoca*, and *Citrobacter murliniae* were evaluated in single, double, and triple inoculations. They are regarded as metal resistant and were isolated from the rhizosphere of species naturally growing on the metal contaminated site. The treatments comprised two soils (contaminated and non-contaminated) and single (*K. intermedia*, *K. oxytoca,* or *C. murliniae)* or multiple inoculations (*K. intermedia* + *K. oxytoca*; *K. intermedia* + *C. murliniae*; *K. oxytoca* + *C. murliniae*; *K. intermedia* + *K. oxytoca* + *C. murliniae*). Plants were grown for 42 days after inoculation. The results showed that the PGPB *K. oxytoca* and the combination of *K. intermedia* + *K. oxytoca* and *K. intermedia* + *C. murliniae* were able to mitigate the metal toxicity in the contaminated soil and hence increase the shoot biomass, with implications to the effectiveness of phytoextraction. The sorghum ability to translocate Cd to shoots in the contaminated soil was enhanced through the single inoculation with *K. oxytoca*, *C. murliniae*, and *K. oxytoca*, as well as by the joint-inoculation with *K. oxytoca* + *C. murliniae*, and *K. intermedia* + *K. oxytoca* + *C. murliniae*. Higher accumulation of metals in shoots is a crucial factor in successful phytoextraction. Arsenic and Pb, on the other hand, had their uptake and concentration in roots stimulated by the inoculation. Therefore, regarding these two metals, phytostabilization programs could benefit from the use of the bacteria studied here.

Keywords: phytoextraction, heavy metals, trace elements, soil remediation, soil pollution.

INTRODUCTION

Phytoremediation is a soil remediation technology that uses plants to transfer metals from contaminated soils to harvestable parts (Cappa and Pilon-Smits, 2014; Chaney and Baklanov, 2017). However, despite some successful field experiments, several bottlenecks still exist for optimum phytoextraction (Robinson et al., 2015). Therefore, several agronomic techniques such as soil pH correction, fertilization, application of chelators to the soil, and microbial inoculation have been used to improve the efficiency of plants to remove metals from contaminated soils (Phieler et al., 2015; Boechat et al., 2016a; Sheoran et al., 2016; Álvarez-López et al., 2016; Nascimento et al., 2020). Microbes can improve the ability of plants to deal with the toxicity of heavy metals in anthropogenically contaminated or metalliferous sites (Phieler et al., 2015). The plant-microbe interaction in the rhizospheric has been investigated due to the microorganisms' ability to accumulate metals from polluted environments. Besides, the so-called plant growth-promoting bacteria (PGPB) can increase metal uptake and hence improve phytoextraction (Chen et al., 2019; Kong et al., 2019).

Bioaugmentation-assisted phytoremediation relies on the use of plants (e.g., rhizosphere associated process), efficient microorganisms, and plant-growth-promoting bacteria (PGPB) to optimize the synergistic effect of both and enhance metal bioavailability in soil and plant metal uptake and biomass (Muratova et al., 2015; Phieler et al., 2015; Agnello et al., 2016; Ma et al., 2016a; Irshad et al., 2019; Kong et al., 2019;). Some bacteria are known as plant growth-promoting because they promote a wide range of benefits to the plant, including nitrogen fixation, phosphate solubilization, and exudation of siderophores; also, they act as biocontrol agents and produce indole-3-acetic acid that increases plant biomass, nutrition, and health (Ahemad and Kibret, 2014; Irshad et al., 2019).

Several studies showed that bacteria inoculation boosts the bioavailability of metals in soils and their uptake by plants. For instance, inoculating a metal resistant bacteria significantly enhanced the biomass and absorption of Pb, Cd, and Zn by *Sedum plumbizincicola* (Ma et al., 2016b). Likewise, the introduction of the multiple tolerant bacterium *Brevibacterium casei* into a contaminated soil increased by 208, 86, and 39 % the accumulation of Cd, Zn, and Cu, respectively, by white mustard (Plociniczak et al., 2016). However, the inoculation effects on the phytoextraction efficiency are inconsistent (Sessitsch et al., 2013), and more studies are needed to make bioaugmentation-assisted phytoremediation a feasible technique to improve phytoextraction.

We assessed the ability of sorghum [*Sorghum bicolor* L. (Moench.)] inoculated with three metal-resistant bacteria species (*Kluyvera intermedia*, *Klebsiella oxytoca*, and *Citrobacter murliniae*) to accumulate As, Cd, and Pb from contaminated soil. Sorghum was chosen owing to high biomass, rusticity, and tolerance to heat, drought, and saline soils (Muratova et al., 2015). Sorghum was previously tested for remediation of soil and nutrient solution contaminated with metals (Al Chami et al., 2014; Schütze et al., 2014; Soudek et al., 2014; Muratova et al., 2015; Phieler et al., 2015). The bioaugmentation-assisted phytoremediation was assessed by the biomass yield and nutrient uptake of sorghum, while phytoextraction capacity was measured by the metal contents in roots and shoots and root-to-shoot translocation.

MATERIALS AND METHODS

Soil sampling and chemical and physical analyses

The soil utilized in the experiment was collected in a site contaminated by gold ore processing activities (Boechat et al., 2016b). The site is located in Lavras do Sul, southern Brazil (30° 81' 58" S and 53° 92' 05" W). A non-contaminated soil was also collected in the vicinity of the site and used as a reference. The soil in the area is an Entisol Orthent

(Soil Survey Staff, 2014), which corresponds to a *Neossolo litólico* in accordance with the Brazilian Soil Classification System (Santos et al., 2018).

Soil physical and chemical analyses (Table 1) were performed on air-dried soil (<2.0 mm) according to the standard procedures (Silva, 2009). The particle size analysis was carried out by using NaOH 1 mol L^{-1} as a dispersant under slow stirring, with the clay content being obtained by the pipette method. Soil pH was measured in water (1:2.5). Available contents of Na^+, K^+, and P were extracted with Mehlich-1 and determined by flame photometry and photocolorimetry, respectively. Exchangeable Ca, Mg, and Al were extracted by KCl 1 mol L^{-1} and obtained through titration. Total acidity (H+Al) was obtained by calcium acetate extraction and titration. Soil organic carbon (SOC) was determined by the Walkley-Black method (Tedesco et al., 1995). The content of As, Cd, Cr, Pb, and Zn in soil was determined by ICP-OES after sample digestion using the 3050b method (Usepa, 1998). Quality control of analyses used an internal soil standard, and recovery rates were satisfactory, i.e., between 93 and 105 %.

Bacteria isolation and identification

The indigenous bacteria *Kluyvera intermedia*, *Klebsiella oxytoca,* and *Citrobacter murliniae* were isolated of the rhizosphere of plants spontaneously growing on the metal-contaminated site as described in Boechat et al. (2016a). These species are identified in the GenBank by the accession numbers of NR028803.1, NR028802.1, and NR028688.1, respectively, and are regarded as metal-resistant and plant-growth promoters (Arunakumara et al., 2015; Anaukwu et al., 2016). The metal resistant-rhizobacteria were grown in Luria-Bertani liquid medium with an addition of of $PbCl_2$ 300 mg L^{-1}, as the bacteria were Pb-resistant, and an initial pH of 6.5 at a controlled temperature of 31 °C. Lead was added to the growth medium aiming the bacteria maintain their resistance to metal.

Table 1. Chemical properties, metals, and clay contents of the soils used in the experiment

Soil properties	Contaminated soil	Non-contaminated soil
pH(H_2O)	6.0	6.0
P (mg kg^{-1})	30.0	7.9
K^+ ($cmol_c$ dm^{-3})	0.33	0.42
Ca^{2+}+Mg^{2+} ($cmol_c$ dm^{-3})	7.7	5.0
H+Al ($cmol_c$ dm^{-3})	2.8	2.2
SB ($cmol_c$ dm^{-3})	8.0	5.4
CEC ($cmol_c$ dm^{-3})	10.7	7.5
SOM (%)	2.7	1.4
Clay (g kg^{-1})	140.0	120.0
Zn (mg kg^{-1})	167.0	42.0
Cu (mg kg^{-1})	61.0	4.7
Mn (mg kg^{-1})	13.0	4.0
Cd (mg kg^{-1})	2.0	0.4
Ni (mg kg^{-1})	8.0	5.0
Cr (mg kg^{-1})	12.0	9.0
Pb (mg kg^{-1})	599.0	46.0
Ba (mg kg^{-1})	109.0	84.0
As (mg kg^{-1})	42.0	<2.0

Clay: determined by the pippete method, using NaOH 1 mol L^{-1}; exchangeable Ca and Mg: extracted with KCl 1 mol L^{-1}; P and K^+: estimated by Mehlich-1; H+Al: extracted with calcium acetate 0.5 mol L^{-1}, pH 7.0; SOM (soil organic matter): determined by the Walkley-Black combustion method (Tedesco et al., 1995). The content of As, Cd, Cr, Pb, and Zn in soil was determined by ICP-OES after sample digestion using the 3050b method (Usepa, 1998). H+Al: potential acidity; SB: sum of the bases; CEC: total cation-exchange capacity; SOM: soil organic matter.

Pot experiment

The treatments comprised two soils (contaminated and non-contaminated) and single (*K. intermedia*, *K. oxytoca*, or *C. murliniae)* or multiple inoculations (*K. intermedia* + *K. oxytoca*; *K. intermedia* + *C. murliniae*; *K. oxytoca* + *C. murliniae*; *K. intermedia* + *K. oxytoca* + *C. murliniae*), arranged in a randomized block design with five replicates.

Soil samples in pots (800 g) were fertilized with rates equivalent to 30 kg ha^{-1} N, 200 kg ha^{-1} P_2O_5, and 150 kg ha^{-1} K_2O applied as urea, triple superphosphate, and potassium chloride, respectively. Seeds of *S. bicolor* were sterilized with ethanol 70 %, $NaClO_4$ solution 10 % v/v, and then washed with ultrapure water (Milli-Q®). *S. bicolor* was seeded at a rate of ten seeds per pot and, after thinning, two plants were kept to the end of the experiment. Plants were grown for 45 days in a greenhouse with a temperature between 25-31 °C. Soil moisture was kept close to 70 % of the pot-holding capacity. The inoculum of each bacterium was prepared in a liquid medium containing Pb as previously described; after centrifugation and resuspension with 0.8 % NaCl solution, the inoculum was adjusted to a concentration of 1.8×10^8 CFU g^{-1} (optical density λ600). The inocula were applied to the pots twice (15 and 30 days after thinning, time needed to roots development) according to the single and multiple inoculations.

Plant biomass analysis and phytoremediation capacity

At the end of the experiment, plants were separated into shoots and roots. The roots were immersed in HCl 0.1 mol L^{-1} and washed to remove metals adhered to the cell walls; the shoots were washed in tap water to remove airborne particles. The plant materials were placed in paper bags and oven-dried at 65 ± 3 °C until constant weight and had the biomass recorded. The above ground shoot and root materials were digested in a hot nitro-perchloric acid solution (Silva, 2009). The contents of As, Cd, and Pb were determined by ICP-OES. The ICP-OES analysis results for the extracts were compared with certificate reference plants (certification program of the Brazilian Society of Soil Science). Bioconcentration ($BCF = C_{shoots}/C_{soil}$) and translocation ($TF = C_{shoots}/C_{roots}$) factors were calculated to estimate the *S. bicolor* ability to uptake metals from the contaminated soil and transfer them to the shoots.

Statistical analyses

The data obtained were analyzed with one-way ANOVA (F test) at 5 %; in case significance was observed, means were tested by Tukey's test ($p<0.05$) through the use of the Sisvar software statistical package (Ferreira, 2011). Pearson's correlation analysis was used to relate metal concentration in roots and shoots with biomass.

RESULTS

Biomass production

The shoots biomass was negatively correlated ($p<0.05$) with Cd, and Pb contents. Arsenic, Cd and Pb also diminished the biomass of roots (Table 2).

Table 2. Pearson's simple correlation between heavy metal(loid) and shoot and root dry mass of *Sorghum bicolor* grown in heavy metal contaminated soil samples

Metal(loid)	Correlation	
	Shoot dry mass	Root dry mass
As	-0.19^{ns}	-0.67*
Cd	-0.86*	-0.86*
Pb	-0.66**	-0.71*

* or **: significant at 1 or 5 % confidence limit, respectively; ns: no significant.

The roots and shoots biomass of sorghum plants grown on the soil was increased through isolated inoculation with *K. oxytoca*. The double inoculations *K. intermedia* + *K. oxytoca* and *K. intermedia* + *C. murliniae* also increased the shoots biomass but did not affect roots. On the other hand, no significant ($p<0.05$) differences were observed in the shoots biomass of plants grown on the non-contaminated soil, but roots biomass was increased by the treatments *C. murliniae*, *K. intermedia* + *K. oxytoca*, and *K. intermedia* + *C. murliniae* (Tables 2 and 3).

Content of As, Cd, and Pb in plants

The content of metals in the shoots of plants growing on the contaminated soil followed the order Cd > Pb > As, while roots accumulated Pb > Cd > As (Figure 1). For the non-contaminated soil, the orders were Cd > As > Pb for shoots, and Pb > Cd > As for roots. The contents of Cd and Pb in the shoots of plants cultivated in the contaminated soil were 5- and 1.5-fold higher, respectively, compared to the non-contaminated soil. On the other hand, Cd, Pb, and As contents in roots were roughly 2, 4.8, and 4.8 times higher in the contaminated soil.

It is important to point out that the co-inoculation with *K. intermedia* (KI) and *K. oxytoca* (KO) promoted the lowest Cd content in plants (Figure 1) on the contaminated soil. However, no inoculation treatment affected the Cd content in plants grown on the non-contaminated soil. The treatments with bacteria consortia KI + KO, KI + *C. murliniae* (CM), and KO + CM promoted an increase in the concentration of Cd in roots; such increase was not observed for the triple inoculation (KI + KO + CM). The Pb content in roots of plants grown on the contaminated soil was higher in the CM and KO treatments, but all inoculated treatments resulted in increased Pb content in roots in comparison

Table 3. Shoot and root dry mass of *Sorghum bicolor* grown on heavy metal (loid) contaminated soil and inoculated with heavy metal resistant-rhizobacteria

Treatment	Shoot dry mass	Root dry mass
	g	
	Contaminated soil	
KI	0.47 bcA	0.31 bB
CM	0.33 cB	0.27 bB
KO	0.74 abA	0.52 aA
KI + KO	0.77 aA	0.27 bB
KI + CM	0.75 aA	0.26 bB
KO + CM	0.47 bcB	0.27 bB
KI + KO + CM	0.38 cB	0.23 bB
Control	0.31 cB	0.21 bB
	Non-contaminated soil	
KI	0.63 aA	0.48 dA
CM	0.80 aA	0.65 abA
KO	0.66 aA	0.48 dA
KI + KO	0.69 aA	0.75 aA
KI + CM	0.72 aA	0.73 aA
KO + CM	0.76 aA	0.60 bcA
KI + KO + CM	0.68 aA	0.58 bcdA
Control	0.71 aA	0.52 cdA
CV (%)	16.42	9.58

KI: *Kluyvera intermedia*; KO: *Klebsiella oxytoca*; CM: *Citrobacter murliniae*. Means followed by the same lowercase letter do not differ between treatments in each soil and same capital letter do not differ between soils in each treatment by Tukey test ($p<0.05$); CV: coefficient of variation.

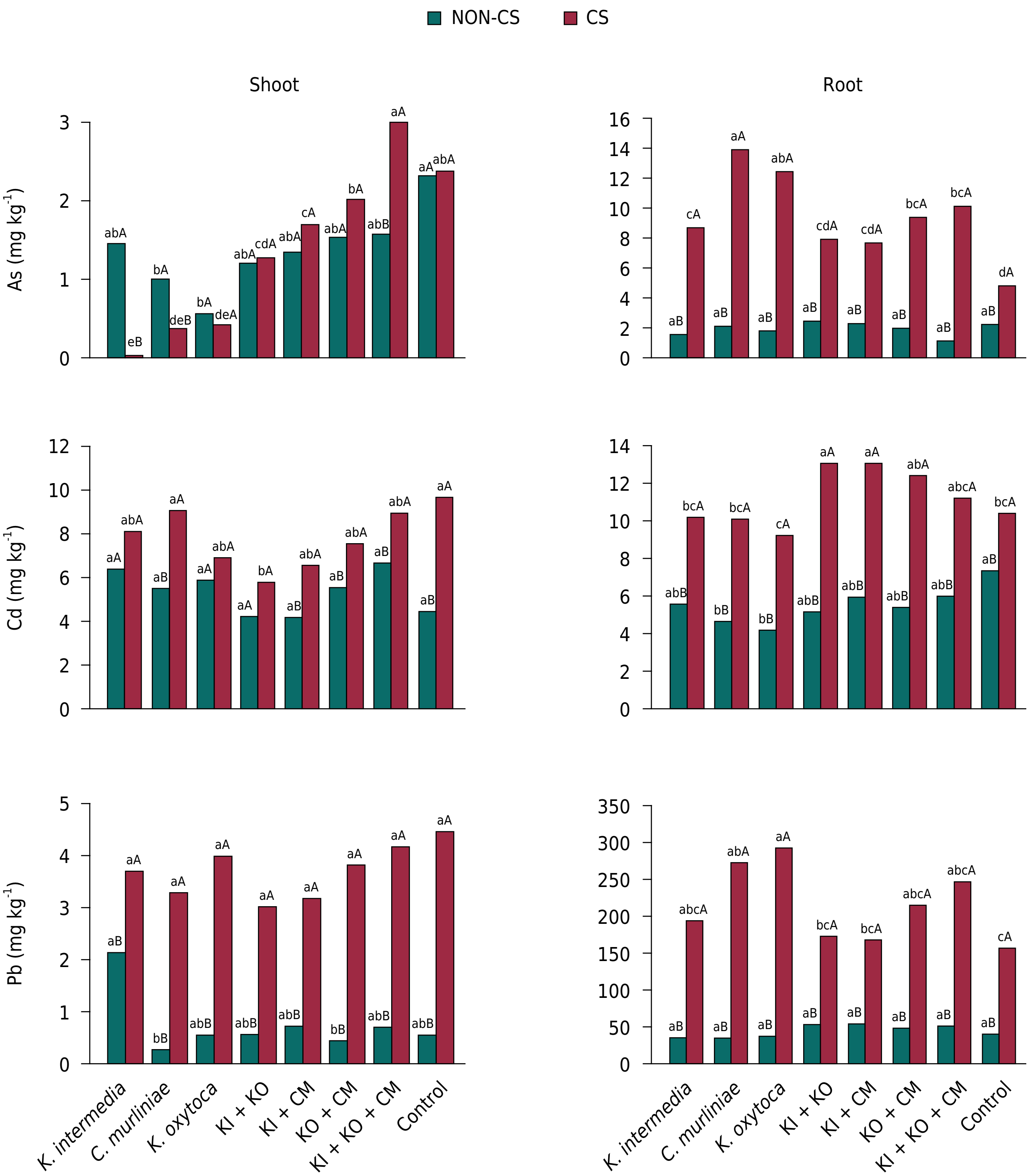

Figure 1. Heavy metals content in the shoot and root of *Sorghum bicolor* grown on heavy metals contaminated soil and inoculated with heavy metal resistant-rhizobacteria. KI: *Kluyvera intermedia*; KO: *Klebsiella oxytoca*; CM: *Citrobacter murliniae*. * Means followed by the same lowercase letter do not differ between treatments in each soil and same capital letter do not differ between soils in each treatment by Tukey test ($p<0.05$).

to the control. On the contrary, for the uncontaminated soil, no significant difference in the Pb content in roots was observed. But the treatments CM and KO + CM presented lower Pb content in the shoot (Figure 1).

We observed that As contents in shoots were not affected by inoculation regardless of the soil. Also, we found no difference in As contents in the roots of plants grown on the non-contaminated soil. Higher contents of As in roots were found in the treatments

CM and KO, while the lowest As content occurred in control. We found that the higher the As content in the soil, the higher the As content in the roots (Figure 1 and Table 4), but translocation factors were higher in the non-contaminated soil (Table 5).

Bioconcentration and translocation factors

Cadmium was the only metal posing BCF over 1 for both soils, although BCF in the non-contaminated soil roughly doubled the values found for the contaminated soil (Table 4). Such a great ability to concentrate Cd from the soil is independent of the inoculation and seems to be due to the known mobility of Cd in soils compared to Pb and As (Ahmadipour et al., 2014; Puga et al., 2015).

Arsenic and Pb presented BCF much lower than the unit in the contaminated soil. In the non-contaminated, Pb BCF >1 was found in the treatments KI + KO, KI + CM, KO + CM, and KI + KO + CM; for As, BCF >1 were found in the treatments Cm, KI + KO, and KI + CM. However, BCF values for both metals were very close to the unit and similar to the control (Table 4).

Translocation factors of plants grown on the contaminated soil were always below the unit for all metals, although most Cd TFs were close to 1. Cadmium presented BCF >1 for the treatments KI, CM, KO, KO + CM, and KI + KO + CM in the non-contaminated soil; such figures were 2-fold higher than the Cd TF of the control. Arsenic and Pb TFs, on the other hand, were very low in the contaminated soil (Table 5).

DISCUSSION

Our data showed that bacteria inoculation alleviated metal toxicity in the plants grown on the contaminated soil, which caused the increase in the sorghum's shoot biomass (Table 3). In the conditions of the low metal toxicity stress of the non-contaminated soil, such an effect on biomass was only observed to roots. Therefore, the role that

Table 4. Bioconcentration factor (BCF) in *Sorghum bicolor* grown on heavy metal (loid) contaminated soil and inoculated with heavy metal resistant-rhizobacteria

Treatment	As	Cd	Pb
		Contaminated soil	
KI	0.2	**5.1**	0.3
CM	0.3	**5.0**	0.5
KO	0.3	**4.6**	0.5
KI + KO	0.2	**6.5**	0.3
KI + CM	0.2	**6.8**	0.3
KO + CM	0.2	**6.2**	0.4
KI+ KO + CM	0.2	**5.6**	0.4
Control	0.1	**5.2**	0.3
		Non-contaminated soil	
KI	0.8	**13.9**	0.8
CM	**1.1**	**11.6**	0.8
KO	0.9	**10.4**	0.8
KI + KO	**1.2**	**12.9**	**1.2**
KI + CM	**1.2**	**14.8**	**1.2**
KO + CM	1.0	**13.5**	**1.1**
KI+ KO + CM	0.6	**15.0**	**1.1**
Control	**1.1**	**18.3**	0.9

KI: *Kluyvera intermedia*; KO: *Klebsiella oxytoca*; CM: *Citrobacter murliniae*. Values >1 are in bold.

Table 5. Translocation factors (TF) in *Sorghum bicolor* grown on heavy metal(loid) contaminated soil and inoculated with heavy metal resistant-rhizobacteria

Treatment	As	Cd	Pb
		Contaminated soil	
KI	0.01	0.8	0.03
CM	0.03	0.9	0.02
KO	0.04	0.8	0.01
KI + KO	0.2	0.5	0.02
KI + CM	0.3	0.5	0.02
KO + CM	0.2	0.6	0.02
KI+ KO + CM	0.4	0.8	0.02
Control	0.6	0.9	0.03
		Non-contaminated soil	
KI	1.0	**1.2**	0.06
CM	0.6	**1.2**	0.01
KO	0.4	**1.4**	0.02
KI + KO	**2.7**	0.8	0.01
KI + CM	0.6	0.7	0.01
KO + CM	0.8	**1.1**	0.01
KI+ KO + CM	**1.7**	**1.1**	0.01
Control	**1.1**	0.6	0.01

KI: *Kluyvera intermedia*; KO: *Klebsiella oxytoca*; CM: *Citrobacter murliniae*. Values >1 are in bold.

metal resistant bacteria play in metal toxicity amelioration is more relevant in stressful environments. Previous studies have shown that PGPB significantly improved biomass yield and development of plants submitted to heavy metal stress (Ma et al., 2011; Arunakumara et al., 2015).

Plant growth-promoting bacteria, including the ones tested in our study, enhance plant development mainly by producing phytohormones such as indole-3-acetic acid from auxin groups and stimulating the production of phytohormones, suppressing stress ethylene production (due to ACC deaminase activity), and improving plant nutrition (Sessitsch et al., 2013). High biomass is crucial for phytoremediation programs as the net metal removal from the soil is dependent on both metal accumulation in shots and biomass production (Jiang et al., 2015; Wood et al., 2016).

Regardless of the soil contamination level or inoculation, Cd was the metal most translocated from the soil to the aerial parts of the sorghum plants (Figure 1). The BCF for Cd is up to 2.5 orders of magnitude greater than those for As and Pb (Table 4). Various studies have shown the high availability of Cd in soils as compared to other metals (Cunha et al., 2008; Ahmadipour et al., 2014; Puga et al., 2015).

We found that the sorghum ability to translocate Cd to shoots in the contaminated soil was enhanced through the single inoculation with KI, CM, and KO, as well as by the inoculation consortia with KO + CM, and KI + KO + CM. Therefore, bacteria inoculums changed the Cd translocation rate to shoots. In general, Cd accumulation in the roots is much higher than in shoots (El-Beltagi et al., 2010; Izadiyar and Yargholi, 2010; Melo et al., 2014; Soudek et al., 2014), which can be a drawback for efficient phytoextraction. Thus, the use of microorganisms capable of stimulating Cd translocation to the aerial part of plants aids in phytoextraction's feasibility.

Given the high TF, the contents of Cd in roots and shoots were similar (Figure 1 and Table 5). In contrast, the content of As and Pb were much higher in roots for both soils. Several studies showed that Pb is mainly accumulated in roots rather than shoots in plants exposed to toxic levels of the metal (Romeiro et al., 2006; Costa et al., 2012; Ma et al., 2016b; Nascimento and Marques, 2018). Likewise, preferential accumulation of As in the roots as compared to shoots have been reported (Melo et al., 2012; Silva et al., 2017). The preferential retention of As and Pb in the sorghum roots is probably due to the defense strategy by preventing the translocation of these metals and hence damages to the aerial parts. This is also a relevant advantage regarding the phytostabilization of arsenic- and lead-contaminated sites.

We did not find inoculation effects on the Pb content in shoots, but KI, CM, and KO promote a high concentration of Pb in roots compared to the control (Figure 1). Inoculation with the endophytic bacteria *Achromobacter piechaudii* also increased the rhizoaccumulation of Pb by *Sedum plumbizincola* (Ma et al., 2016b). Lead uptake by plants depends on soil properties such as soil organic matter and texture, cation-exchange capacity, and pH (Sillanpää and Jansson, 1992). Given the low contents of clay and organic matter in the studied soil and hence low Pb sorption (Table 1), it is likely that the increased content of Pb in roots is due to enhanced solubility of Pb compounds in soil driven by the PGPB exudates in the rhizosphere (Yoon et al., 2006; Ma et al., 2011; Tangahu et al., 2011).

The triple inoculation (KI + KO + CM) promoted the highest As content in shoots in the contaminated soil, but results did not differ from the control treatment (Figure 1). Accordingly, these two treatments posed the highest TF for the contaminated soil. The inoculations with KI and KO showed the highest abilities to improve uptake and content of As in roots. For the non-contaminated soil, the inoculations with KI + KO and the triple inoculation were the only treatments to pose TF > 1, i.e., 2.7 and 1.7, respectively. More studies are needed to assess these species' potential to mobilize As in different soil conditions and As contents. As discussed to Pb, the general preferential As accumulation in roots is part of a tolerance mechanism to restrict the transfer of As to shoots (Lux et al., 2004; Wójcik et al., 2005; Pongrac et al., 2010, Kabata-Pendias, 2011).

CONCLUSION

We assessed the feasibility of using plant-growth-promoting bacteria to assist the phytoremediation of As, Cd, and Pb by *Sorghum bicolor* grown on a soil contaminated by gold ore processing activities. Our results showed that sorghum associated with the adequate metal resistant microorganisms could boost Cd phytoextraction and phytostabilization of Pb and As in the studied soils. The PGPB *K. oxytoca* and the combination of *K. intermedia* + *K. oxytoca* and *K. intermedia* + *C. murliniae* were able to mitigate the metal toxicity in the contaminated soil and hence increase the shoot biomass, with implications to the effectiveness of phytoextraction. The sorghum ability to translocate Cd to shoots in the contaminated soil was enhanced through the single inoculation with *K. oxytoca*, *C. murliniae*, and *K. oxytoca*, as well as by the joint-inoculation with *K. oxytoca* + *C. murliniae*, and *K. intermedia* + *K. oxytoca* + *C. murliniae*. Arsenic and Pb, on the other hand, had their uptake and content in roots stimulated by the inoculation. Therefore, regarding these two metals, phytostabilization programs could benefit from the use of the bacteria tested here.

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REFERENCES

Agnello AC, Bagard M, Hullebusch EDV, Esposito G, Huguenot D. Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. Sci Total Environ. 2016;563-64:693-703. https://doi.org/10.1016/j.scitotenv.2015.10.061

Ahmadipour F, Bahramifar N, Ghasempouri SM. Fractionation and mobility of cadmium and lead in soils of Amol area in Iran, using the modified BCR sequential extraction method. Chem Spec Bioavailab. 2014;26:31-6. https://doi.org/10.3184/095422914X13884321932037

Al Chami Z, Amer N, Smets K, Yperman J, Carleer R, Dumontet S, Vangronsveld J. Evaluation of flash and slow pyrolysis applied on heavy metal contaminated *Sorghum bicolor* shoots resulting from phytoremediation. Biomass Bioenerg. 2014;63:268-79. https://doi.org/10.1016/j.biombioe.2014.02.027

Álvarez-López V, Prieto-Fernández Á, Cabello-Conejo M, Kidd PS. Organic amendments for improving biomass production and metal yield of Ni-hyperaccumulating plants. Sci Total Environ. 2016;548-49:370-9. https://doi.org/10.1016/j.scitotenv.2015.12.147

Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci. 2014;26:1-20. https://doi.org/10.1016/j.jksus.2013.05.001

Anaukwu CG, Ezemba CC, Anakwenze VN, Agu KC, Okeke BC, Awah NS, Ekwealor IA. Effect of biosurfactant produced by *Citrobacter murliniae* AF025369 and a synthetic surfactant on degradation of crude oil. Edorium J Microbiol. 2016;2:1-6. https://doi.org/10.5348/M08-2016-3-OA-1

Arunakumara KKIU, Walpola BC, Yoon M. Bioaugmentation-assisted phytoextraction of Co, Pb and Zn: an assessment with a phosphate-solubilizing bacterium isolated from metal-contaminated mines of Boryeong Area in South Korea. Biotechnol Agron Soc. 2015;19:143-52.

Boechat CL, Carlos FS, Gianelo C, Camargo FAO. Heavy metals and nutrients uptake by medicinal plants cultivated on multi-metal contaminated soil samples from an abandoned gold ore processing site. Water Air Soil Poll. 2016a;227:392-403. https://doi.org/10.1007/s11270-016-3096-4

Boechat CL, Pistóia VC, Gianelo C, Camargo FAO. Accumulation and translocation of heavy metal by spontaneous plants growing on multi-metal-contaminated site in the Southeast of Rio Grande do Sul state, Brazil. Environ Sci Pollut R. 2016b;23:2371-80. https://doi.org/10.1007/s11356-015-5342-5

Cappa JJ, Pilon-Smits EAH. Evolutionary aspects of elemental hyperaccumulation. Planta. 2014;239:267-75. https://doi.org/10.1007/s00425-013-1983-0

Chaney RL, Baklanov IA. Phytoremediation and phytomining: status and promise. In: Cuypers A, Vangronsveld J, editors. Advances in botanical research. Cambridge: Academic Press; 2017. p. 189-221.

Chen C, Wang X, Wang J. Phytoremediation of cadmium-contaminated soil by *Sorghum bicolor* and the variation of microbial community. Chemosphere. 2019;235:985-94. https://doi.org/10.1016/j.chemosphere.2019.07.028

Costa ETS, Guilherme LRG, Melo EEC, Ribeiro BT, Inácio ESB, Severiano EC, Faquin V, Hale BA. Assessing the tolerance of castor bean to Cd and Pb for phytoremediation purposes. Biol Trace Elem Res. 2012;145:93-100. https://doi.org/10.1007/s12011-011-9164-0

Cunha KPV, Nascimento CWA, Silva AJ. Silicon alleviates the toxicity of cadmium and zinc for maize (*Zea mays* L.) grown on a contaminated soil. J Plant Nutr Soil Sc. 2008;171:849-53. https://doi.org/10.1002/jpln.200800147

El-Beltagi HS, Mohamed AA, Rashed MM. Response of antioxidative enzymes to cadmium stress in leaves and roots of radish (*Raphanus sativus* L.). Not Sci Biol. 2010;2:76-82. https://doi.org/10.15835/nsb245395

Ferreira DF. Sisvar: a computer statistical analysis system. Cienc Agrotec. 2011;35:1039-42. https://doi.org/10.1590/S1413-70542011000600001

Irshad S, Xie Z, Wang J, Nawaz A, Luo Y, Wang Y, Mehmood S, Faheem. Indigenous strain *Bacillus* XZM assisted phytoremediation and detoxification of arsenic in *Vallisneria denseserrulata*. J Hazard Mater. 2019;381:120903. https://doi.org/10.1016/j.jhazmat.2019.120903

Izadiyar MH, Yargholi B. Study of cadmium absorption and accumulation in different parts of four forages. Am Eurasian J Sustain Agric. 2010;9:231-8.

Jiang Y, Lei M, Duan L, Longhurst P. Integrating phytoremediation with biomass valorisation and critical element recovery: A UK contaminated land perspective. Biomass Bioenerg. 2015;83:328-39. https://doi.org/10.1016/j.biombioe.2015.10.013

Kabata-Pendias A. Trace elements in soils and plants. 4th ed. Boca Raton: Taylor & Francis Group; 2011.

Kong Z, Wu Z, Glick BR, He S, Huang C, Wu L. Co-occurrence patterns of microbial communities affected by inoculants of plant growth-promoting bacteria during phytoremediation of heavy metal contaminated soils. Ecotox Environ Safe. 2019;183:109504-14. https://doi.org/10.1016/j.ecoenv.2019.109504

Lux AA, Sottniková A, Opatrná J, Greger M. Differences in structure of adventitious roots in *Salix* clones with contrasting characteristics of cadmium accumulation and sensitivity. Physiol Plant. 2004;120:537-45. https://doi.org/10.1111/j.0031-9317.2004.0275.x

Ma Y, Prasad MNV, Rajkumar M, Freitas H. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv. 2011;29:248-58. https://doi.org/10.1016/j.biotechadv.2010.12.001

Ma Y, Rajkumar M, Zhang C, Freitas H. Inoculation of *Brassica oxyrrhina* with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. J Hazard Mater. 2016a;320:36-44. https://doi.org/10.1016/j.jhazmat.2016.08.009

Ma Y, Zhang C, Oliveira RS, Freitas H, Luo Y. Bioaugmentation with endophytic *bacterium* E6S homologous to *Achromobacter piechaudii* enhances metal rhizoaccumulation in host *Sedum plumbizincicola*. Front Plant Sci. 2016b;7:75. https://doi.org/10.3389/fpls.2016.00075

Melo EEC, Guilherme LRG, Nascimento CWA, Penha HGV. Availability and accumulation of arsenic in oilseeds grown in contaminated soils. Water Air Soil Pollut. 2012;223:233-40. https://doi.org/10.1007/s11270-011-0853-2

Melo LCA, Silva EB, Alleoni LRF. Transfer of cadmium and barium from soil to crops grown in tropical soils. Rev Bras Cienc Solo. 2014;38:1939-49. https://doi.org/10.1590/S0100-06832014000600028

Muratova A, Lyubun Y, German K, Turkovskaya O. Effect of cadmium stress and inoculation with a heavy-metal-resistant bacterium on the growth and enzyme activity of *Sorghum bicolor*. Environ Sci Pollut R. 2015;22:16098-109. https://doi.org/10.1007/s11356-015-4798-7

Nascimento CWA, Hesterberg D, Tappero R, Nicholas S, Silva FBV. Citric acid-assisted accumulation of Ni and other metals by *Odontarrhena muralis*: Implications for phytoextraction and metal foliar distribution assessed by µ-SXRF. Environ Pollut. 2020;245:114025. https://doi.org/10.1016/j.envpol.2020.114025

Nascimento CWA, Marques MC. Metabolic alterations and X-ray chlorophyll fluorescence for the early detection of lead stress in castor bean (*Ricinus communis*) plants. Acta Sci-Agron. 2018;40:e39392. https://doi.org/10.4025/actasciagron.v40i1.39392

Phieler R, Merten D, Roth M, Büchel G, Kothe R. Phytoremediation using microbially mediated metal accumulation in *Sorghum bicolor*. Environ Sci Pollut R. 2015;22:19408-16. https://doi.org/10.1007/s11356-015-4471-1

Plociniczak T, Sinkkonen A, Romantschuk M, Sułowicz S, Piotrowska-Seget Z. Rhizospheric bacterial strain *Brevibacterium casei* MH8a colonizes plant tissues and enhances Cd, Zn, Cu phytoextraction by white mustard. Front Plant Sci. 2016;7:101. https://doi.org/10.3389/fpls.2016.00101

Pongrac P, Tolrà R, Vogel-Mikuš K, Poschenrieder C, Barceló J, Regvar M. At the crossroads of metal hyperaccumulation and glucosinolates: is there anything out there? In: Sherameti I, Varma A, editors. Soil heavy metals. New York: Springer-Verlag; 2010. p. 272-5.

Puga AP, Abreu CA, Melo LCA, Paz-Ferreiro J, Beesley L. Cadmium, lead, and zinc mobility and plant uptake in a mine soil amended with sugarcane straw biochar. Environ Sci Pollut R. 2015;22:17606-14. https://doi.org/10.1007/s11356-015-4977-6

Robinson BH, Anderson CWN, Dickinson NM. Phytoextraction: where's the action? J Geochem Explor. 2015;151:34-40. https://doi.org/10.1016/j.gexplo.2015.01.001

Romeiro S, Lagôa AMMA, Furlani PR, Abreu CA, Abreu MF, Erismann NM. Lead uptake and tolerance of *Ricinus communis* L. Braz J Plant Physiol. 2006;18:483-9. https://doi.org/10.1590/S1677-04202006000400006

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Araújo Filho JC, Oliveira JB, Cunha TJF. Sistema brasileiro de classificação de solos. 5. ed. rev. ampl. Brasília, DF: Embrapa; 2018.

Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K. Puschenreiter M. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem. 2013;60:182-94. https://doi.org/10.1016/j.soilbio.2013.01.012

Schütze E, Klose M, Merten D, Nietzsche S, Senftleben D, Roth M, Kothe E. Growth of *Streptomycetes* in soil and their impact on bioremediation. J Hazard Mater. 2014;267:128-35. https://doi.org/10.1016/j.jhazmat.2013.12.055

Sheoran V, Sheoran AS, Poonia P. Factors affecting phytoextraction: a review. Pedosphere. 2016;26:148-66. https://doi.org/10.1016/S1002-0160(15)60032-7

Sillanpää M, Jansson H. Status of cadmium, lead, cobalt and selenium in soils and plants of thirty Countries. Rome: FAO; 1992. (FAO Soil Bull 65).

Silva FC. Manual de análises químicas de solos, plantas e fertilizantes. 2. ed. Brasília, DF: Embrapa; 2009.

Silva WR, Silva FBV, Araújo PRM, Nascimento CWA. Assessing human health risks and strategies for phytoremediation in soils contaminated with As, Cd, Pb, and Zn by slag disposal. Ecotox Environ Safe. 2017;144:522-30. https://doi.org/10.10.16/j.ecoenv.2017.06.068

Soil Survey Staff. Keys to soil taxonomy. 12th ed. Washington, DC: United States Department of Agriculture, Natural Resources Conservation Service; 2014.

Soudek P, Petrová S, Vanková R, Song J, Vanek T. Accumulation of heavy metals using *Sorghum* sp. Chemosphere. 2014;104:15-24. https://doi.org/10.1016/j.chemosphere.2013.09.079

Tangahu BV, Abdullah SRS, Basri H, Idris M, Anuar N, Mukhlisin M. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng. 2011;2011:939161. https://doi.org/10.1155/2011/939161

Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ. Análises de solo, plantas e outros materiais. 2. ed. Porto Alegre: Universidade Federal do Rio Grande do Sul; 1995. (Boletim técnico 5).

United States Environmental Protection Agency - Usepa. Method 3050B [internet]. Washington, DC: United States Environmental Protection Agency; 1998 [cited 2019 Ouc 09]. Available from: http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/3050b.pdf

Wood JL, Tang C, Franks AE. Microbial associated plant growth and heavy metal accumulation to improve phytoextraction of contaminated soils. Soil Biol Biochem. 2016;103:131-7. https://doi.org/10.1016/j.soilbio.2016.08.021

Wójcik M, Vangronsveld J, D´Haen J, Tukiendorf A. Cadmium tolerance in *Thlaspi caerulescens*. Environ Exp Bot. 2005;53:163-71. https://doi.org/10.1016/j.envexpbot.2004.03.011

Yoon J, Cao X, Zhou Q, Ma LQ. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ. 2006;368:456-64. https://doi.org/10.1016/j.scitotenv.2006.01.016