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Associations between microorganism and maize plant to remedy mercury-contaminated soil

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Abstract

Mercury (Hg) is one of the most toxic metals and is not essential for any organism. In this study, the potential of maize plants in association with bacteria to treat oxisol contaminated with Hg (II) was evaluated. The experiment was conducted in a controlled environment, and pots with 2 kg of oxisol were contaminated with $HgCl_2$ solution at a dose of 36 mg kg^{-1} of Hg in a 7x4 factorial scheme: control (soil without Hg(II) and microorganisms), T2= (soil with Hg(II) and without microorganisms), and T3= soil with Hg(II) + *Enterobacter cloacae*, T4= Hg(II) + *Bacillus subtilis*, T5= Hg(II) + *Enterobacter* sp., T6= Hg(II) + *Staphylococcus epidermidis*, and T7= Hg(II) + *Bacillus* sp. Total Hg quantification was performed by atomic absorption spectrophotometry. At the end of the experiment, the soil pH was significantly lower (0.3 to 0.4 pH unit) in the T2 (no inoculation), *Enterobacter cloacae*, *Enterobacter* sp. and *Bacillus* sp. treatments. Neither contamination of soil with Hg nor plant associations with bacteria led to differences in the root dry mass of maize plants. Maize plants associated with *Staphylococcus epidermidis* and *Bacillus* sp. bacteria had lower shoot biomass (71 and 50%) compared to the treatment 2. The best remedial effect was observed with the association of maize plants with *Bacillus* sp., which recovered 19.67% of Hg(II) in the soil when compared to control and treatment 2 and treatment with *B. subtilis*. The recommendation is the use of *B. subtilis* to decrease the toxicity caused by Hg(II).

Keywords: Heavy metals; Phytoremediation; Bioremediation; Bacteria.

Introduction

Mercury (Hg) is naturally found in igneous and sedimentary rocks (Adriano, 2001) and is often used in mining for the manufacture of lamps and batteries, in chlorine production, in dentistry, in the production of pharmaceuticals, pesticides, and insecticides and even in the manufacture of inks (Kabata-PendiasandMukherjee, 2007; Liet al., 2009).

Hg can be found in the oxidation states Hg^0 (elemental Hg), Hg(I) (mercurous) and Hg(II) (mercuric). In the atmosphere, Hg^0 is predominant, while in soil, water and sediments, most Hg is in the form of inorganic salts of Hg(II), and in the biota, most Hg in the organic form of methylmercury ($CH3Hg^+$) (Adriano, 2001; Beckers and Rinklebe, 2017).

In the environment, Hg cannot be eliminated; however, it can undergo transformations from the most toxic forms, which are Hg^{2+}, CH_3Hg^+ and dimethylmercury, to less toxic forms such as Hg^0 and HgS (Wagner-Döbler, 2013).

No organism uses Hg in its biosynthesis, and its presence in the environment has become a global concern due to its volatility, permanence in the environment and toxicity (Wanget al., 2003; Pacynaet al., 2016; Sundsethet al., 2017). For this reason, Hg was ranked by the Agency for Toxic Substances and Disease Registry as the third most dangerous substance, only behind arsenic and lead (ATSDR, 2016).

Several regions of Brazil, mainly in the state of Minas Gerais, are contaminated with Hg due to gold mining activity (Windmöller et al., 2015). In the Amazon region, it is estimated that 15 years of mining have caused contamination with approximately 4,000 t of Hg (Lacerda, 2003; Bastos et al., 2006).

In the period from 2010 to 2013, Brazil emitted 39,214,00 kg of Hg to the environment, most of it from gold mining (22,500 kg) (UNEP, 2018). The amalgamation process is responsible for 55 to 65% of Hg emissions into the atmosphere, and the remainder of the metal is directly released into water resources and soil (Bastos and Lacerda, 2004). Currently, industries apply techniques for the remediation of soil contaminated with Hg, which include vitrification (metal immobilization in a glass matrix), heat treatment (reduction of Hg(II) to Hg^0), physicochemical extraction of metal from the soil (decrease in bioavailability) and encapsulation of reactive forms (decreased mobility) (Mahbubet al., 2017). However, these measures are costly.

Therefore, less costly strategies for the remediation of contaminated areas, such as phytoremediation, have aroused worldwide attention for the recovery of soils and water resources. Plants can act as phytoextractors, phytostabilizers, and phytovolatilizers and act on phytodegradation, rhizodegradation and rhizofiltration (Tangahuet al., 2011). In addition, they indirectly contribute to phytoremediation by supporting symbiotic microorganisms that live in roots and are responsible for the detoxification of contaminants (Kumaret al., 2017).

Several studies have demonstrated the potential of plants to accumulate Hg in their biomass (Xunet al., 2017; Qianet al., 2018) along with a tendency for Hg to accumulate in higher proportions in roots than in shoots (Pedron et al., 2013, Chauhan and Mathu, 2018; Debeljak et al., 2018; Cabrita et al., 2019).

Concomitant to phytoremediation, bioremediation is a promising technique that uses microorganisms such as fungi, bacteria and yeasts (Naguibet al., 2018) that are resistant to heavy metals and other contaminants and transforms them into less toxic and less mobile forms in the environment (Dixit et al., 2015).

Microorganisms have different resistance mechanisms and strategies to bioaccumulate, biomineralize, biotransform, bioleach and adsorb contaminants from the environment. Bioremediation is successful in using appropriate microorganisms for each contaminant and in understanding interactions between microorganisms and the environment (Dixit et al., 2015).

Satisfactory results for the remediation of contaminated soils can be obtained from the association between bacteria and plants. Maize (*Zea mays* L.) is easily cultivated, has a high degree of mycorrhization and was evaluated as a possible Hg phytoremediation agent, especially when associated with microorganisms (Kodreet al., 2017; Debeljaket al., 2018). However, knowledge of the interactions between plants and microorganisms to phytoremediate Hg in oxisol is still incipient.

The aim of this study was to evaluate the remediation potential of maize plants associated with bacteria in soils contaminated with Hg(II) at concentrations higher than the maximum content of this chemical legally allowed in soils destined for agricultural use.

Results and discussion

Regarding soil pH, there was no difference among treatments in the same evaluation period. However, the final pH was significantly lower (0.3 to 0.4 pH unit) than the initial pH in the control and in treatments that received *Enterobacter cloacae, Enterobacter* sp. and *Bacillus* sp.

Influence of the cultivation conditions on maize growth variables

Soil contamination with Hg (II) and the presence of microorganisms led to differences in the shoot dry mass (PA) of maize plants (Fig 1). The shoot dry mass of the treatment 2 was higher than that of treatments with *S. epidermidis* and *Bacillus* sp. The growth of maize roots in soil contaminated with Hg and inoculated with bacteria was homogeneous in all treatments, with production of approximately 8 g kg^{-1}.

Influence of the cultivation conditions on Hg (II) content in the different compartments (soil, roots, and shoots).

After 44 days of soil contamination, associations between maize plants and bacteria did not show any differences among treatments; however, in general, there was a 56% reduction in soil Hg content (Fig 2). The concentration of Hg in the roots of maize plants associated with bacteria varied between 583.30 and 763.10 mg kg^{-1} and was always higher than the concentration found in the positive control (0.99 mg kg^{-1}) (Fig 2). Plants associated with *Bacillus* sp. showed 41% greater Hg accumulation in roots compared to the treatment 2. Regarding shoot phytomass, Hg concentrations varied between 0.17 and 8.67 mg kg^{-1}. Treatments associated with *Enterobacter cloacae* and *Bacillus* sp. presented an effective increase in shoot Hg accumulation by 306 and 142%, respectively, compared to the treatment 2.

Remediation efficiency

In the control (no inoculated), most Hg (92%) initially present in soil (0.3 mg kg^{-1}) was removed by the end of the experiment, but only a small part (0.13%) was removed by plants (Table 3).

In contaminated soils, Hg removal ranged from 45.67 to 50.46%, and no difference was observed among treatments. Nevertheless, only 5.91 to 9.34% of Hg initially present in contaminated soil was accumulated in plants, which accounted for 11.61 to 19.67% of Hg removed from the soil. When compared to the control, only cultivation in association with *Bacillus* sp. showed better efficiency in bioaccumulating Hg and therefore removing Hg from the soil. This treatment was able to remove 42.74% more Hg than the treatment 2 and 69.42% more Hg than the treatment with *Bacillus subtilis.*

The Hg content in the soil did not show a high correlation ($p < 0.01$) with the root Hg content and an average correlation ($p < 0.05$) with total shoot Hg content. On the other hand, the Hg contents in the root and shoot phytomass showed an average correlation with each other. The other correlation values were low and not significant (Table 3).

pH and soil organic matter content (SOM) are the most important factors that directly affect the availability of heavy metals in the environment. Hg(II) has a higher affinity for SOM and its sulfur compounds than for inorganic complexes (Adriano, 2001; Zenget et al., 2011; Letermeand Jacques, 2015).

In general, higher pH and CTC values in soil directly favor the available negative charge sites and consequently increase the soil Hg adsorption (Soares et al., 2015). For this reason, liming was not performed in order to favor Hg availability.

The metal bioavailability in soil tends to be low and to accumulate in roots, as the endoderm acts as a barrier, decreasing Hg absorption by plants. As a defense mechanism, less Hg is translocated to the xylem, and the metal is accumulated in roots (Adriano, 2001; Debeljak et al., 2013). In our study, this finding was confirmed, as greater accumulation of the metal in roots than in the shoot phytomass was observed (Fig 2), corroborating the results obtained by Debeljak et al. (2013). The accumulation in maize roots grown in Hg-contaminated soil can also be enhanced with the association of arbuscular mycorrhizae (Debeljaket al., 2018).

The chemical speciation of Hg, as well as the vegetal species used in phytoremediation, has a direct influence on its translocation (Adriano, 2001). The content translocated by maize plants tends to accumulate more in the leaves and

Table 1. Mean total mercury content in different treatments.

Treatments	Hg in soil Beginning (mmSi)	Hg in soil Final (mmSf)	Hg roots (mmR)	Hg shoots (mmPA)	Total Hg at the end of Ti (mmSf+mmR+mmPA)
	---mg kg^{-1} Hg x 2 kg---		-mg kg^{-1} Hg x dry mass-		---mg kg^{-1}---
Positive control	11.12	0.60	0.007	0.003	0.61
Negative control	74.86	40.35	4.57	0.068	44.99
Enterobacter cloacae	74.86	40.68	5.15	0.25	46.1
Bacillus subtilis	74.86	37.1	4.29	0.13	41.51
Enterobacter sp.	74.86	38.64	5.66	0.046	44.09
Staphylococcus epidermidis	74.86	40.18	4.94	0.030	45.14
Bacillus sp.	74.86	39.28	6.84	0.152	46.28

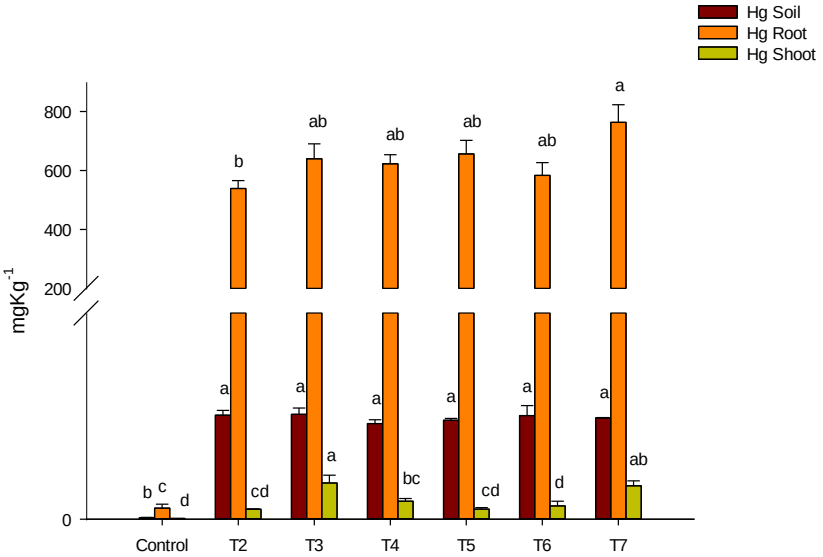

Fig 2. Total Hg concentration in different compartments (soil, root and leaf) after maize cultivation in soil that was contaminated with mercury and inoculated with different bacteria. Averages followed by the same letters do not differ statistically from each other by the Duncan test at the 5% probability level.

Table 2. Remediation potential of RED LATOSOL contaminated by mercury, with maize cultivation in association with different bacteria

Treatments	Accumulated in plant (%)	Removed from soil (%)	Removed by plant (%)
Positive control	0.12c	92.23a	0.13c
Negative control	6.19b	46.10b	13.78b
Enterobacter cloacae	7.21ab	45.67b	15.84ab
Bacillus subtilis	5.91b	50.46b	11.61b
Enterobacter sp.	7.62ab	48.72b	15.67ab
Staphylococcus epidermidis	6.63b	46.33b	14.94ab
Bacillus sp.	9.34a	47.54b	19.67a

Averages followed by the same letters, in columns, do not differ statistically from each other by the Duncan test at the 5% probability level.

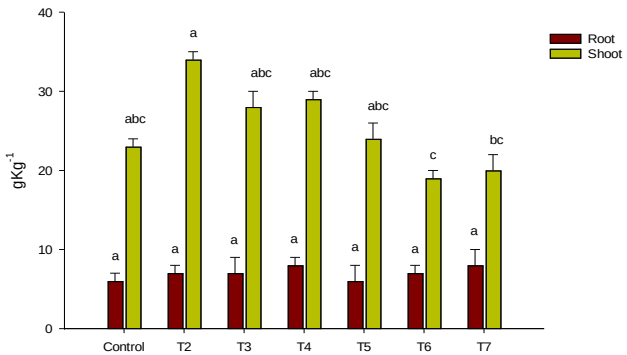

Figure 1. Root and shoot dry mass of maize plants grown in soil contaminated with mercury and inoculated with different bacteria. Averages followed by the same letters do not differ statistically from each other by the Duncan test at the 5% probability level.

Table 3. Correlation of variables in maize.

	Final soil pH	Root dry mass	Shoot dry mass	Total root Hg content	Total shoot Hg content
Final soil Hg content	0.27NS	0.34NS	0.05NS	0.82**	0.38*
Root Hg content	0.14NS	0,16NS	0.06NS	-	0.46*
Shoot Hg content	-0.12NS	-0.09NS	0.32NS	0.46*	_

NS = not significant; (*) = significance at 5% probability; (**) = significance at 1% probability.

stems than in the grains, and this mechanism is a detoxification strategy of maize plants (Fu et al., 2014).

The results of the mass balance (Table 2) showed a difference of 30 to 40% between the amount removed from the soil and the Hg stored in plants. Some plants can be considered phytovolatilizers, that is, they are able to absorb heavy metals, translocate them to shoots and volatilize them into the atmosphere (Tangahuet al., 2011). This process could therefore have favored the elimination of Hg initially present in the contaminated soil. Another hypothesis is that part of Hg has been lost by volatilization due to the reduction of Hg (II) to Hg^0.

Leaching losses can be disregarded since the soil was stored in plastic bags to minimize these losses. After contaminating the soil with $HgCl_2$ (2 mg kg^{-1} Hg(II)) and incubating for 2 months, Wang et al. (2003) found that only 0.14% of the metal was in the form of $HgCl_2$, 11.25% was converted into Hg^0 and the remainder to other forms. The gaseous form can be transferred from the soil to the atmosphere and then absorbed through the leaves via stomata.

The emission of Hg^0 by soil depends on several factors (soil properties, temperature, and light radiation) (Magarelliet al., 2005; Carpiet al., 2014). In this way, we suggest that part of the Hg found in the shoots of plants is due to the volatilization of the soil Hg.

Hg^0 is predominantly found deposited in the vegetation of the Antarctic tundra, with higher deposition at the beginning of spring, when vegetation grows and accumulates Hg^0 (Obristet al., 2017). The deposition of atmospheric Hg was also observed in the forests in the USA (Rischet al., 2017) and the Amazon region, which, due to their high leaf area and perennial characteristics, are able to trap atmospheric Hg. The cycle repeats when leaves fall and Hg deposited in shoots returns to the soil to be absorbed again (Ericksen et al., 2003; Fostier et al., 2015).

Although some of the Hg present in soil can be volatilized, other processes favor its retention. In a reducing environment, it precipitates in the form of mercury sulfide (HgS) (Mahbubet al., 2017), which has less mobility and reactivity as a function of adsorption to iron sulfate and pyrite (Steinet et al., 1996).

Hg adsorption to clay and iron and aluminum oxides also limits its mobility. The clay fraction of soil favors the retention of metal ions on the soil surface since it is the most reactive fraction due to its greater specific surface area (Soares et al., 2015).

In soils contaminated with Hg, as in Minamata Bay, Japan, *Bacillus* sp. was the microorganism most prevalent in sediments (Nakamura et al., 1988). This microorganism was also the most resistant in soil contaminated with Hg according to Figueiredo et al. (2016) and Purkan et al. (2017).

This bacterium has brown colonies with a circular outline, is gram-positive, and has the *merA* gene that is responsible for the detoxification mechanism by reducing Hg^{2+} into Hg^0, a less toxic form of the metal. *Mer* genes act in the coding of proteins associated with the transportation, regulation, reduction and decomposition of Hg compounds (Matsui and Endo, 2018) as a strategy for the survival of bacteria in contaminated environments.

The *mer* gene of *Bacillus* sp. encodes the mercury reductase enzyme (Giriet al., 2014; Amin and Latif, 2016; Dash et al., 2017) and shows optimal activity at pH 6 and 37°C (Purkan et al., 2017). These conditions are close to those of our study and the synergistic action of *Bacillus* sp. In addition, maize plants favor phytoremediation.

Several aerobic and anaerobic bacteria may exhibit resistance to heavy metals. *Enterobacter cloacae* previously isolated from soil contaminated with Hg (II) in the present study was also isolated by Amin and Latif (2016) and showed *in vitro* resistance to Hg in culture medium with 20 µg mL^{-1} of $HgCl_2$. According to the authors, this bacterium has high nitrogen fixation potential, similar to *Bacillus* sp., and significant AIA production (auxin).

In addition, the growth potential of *Enterobacter cloacae* and *Bacillus* sp. in soil contaminated with and without $HgCl_2$ and cultivated with *Cicer arietinum* L. (Amin and Latif, 2016) is known in the literature. The authors identified that the consortium inoculation of these bacteria resulted in increased germination of seeds, fresh root and shoot mass, and number of pods per plant when compared to control Hg-contaminated soil that did not receive bacteria.

In our study, it was observed that treatments inoculated separately with *E. cloacae* and *Bacillus* sp. showed faster germination than the control and treatment 2, suggesting that the use of these bacteria contributes to decreasing stress in maize plants grown in soils with high Hg concentrations. While there is no physical contact of roots with the soil, microorganisms are able to use molecular mechanisms to stimulate plant growth (Pérez-Floreset al., 2017) and to benefit the germination of treatments that were inoculated.

The literature has demonstrated the bioaccumulatory potential of *Enterobacter* sp. in reducing Hg^{2+} to Hg^0 and accumulating Hg in the cytoplasm (Sinha and Khare, 2012; Sinha et al., 2013; Amin and Latif, 2017).

Microorganisms have adaptation mechanisms to survive under contamination conditions; for example, an *S. epidermidis* isolate from soil in the city of Lanzhou, China, showed high resistance to the presence of Hg, with efficient Hg (II) reduction and the presence of the *mer A* gene. This bacterium showed optimal growth at 37°C and pH from 5.6 to 8.5 (Yuet al., 2014); however, when used in the present study, *S. epidermidis* did not express its full bioremediation potential, as *Bacillus subtilis* did. In our study, the faster germination of treatments that were contaminated with Hg (II) and received weekly bacteria inoculations may have been favored by the fact that microorganisms transformed contaminants into less toxic forms, which benefited the germination process by lowering contamination stresses and favoring initial growth.

Material and methods

Plant materials

Isolation of bacteria

The microorganisms used in the experiment were isolated from oxisol contaminated with $HgCl_2$ solution at concentrations of 5, 12 and 36 mg kg^{-1} of Hg (II) and cultivated with Kenaf (*Hibiscus cannabinus*) for 75 days in a parallel experiment. Of 180 microorganisms isolated from soil and plants, only 12 were grown *in vitro* in BHI cultivation medium with 54 mg kg^{-1} of Hg (II). Five microorganisms were chosen for the phytoremediation test of maize plants because they were easy to cultivate. DNA extraction from bacteria was performed by the adapted Kuramae-Izioka method (1997). Extracts were submitted to polymerase chain reaction (PCR) for 16S amplification.

At the Laboratory of Genomics and Expression (LGE) of Unicamp, SP (Brazil), samples were sequenced (Sanger) with a protocol for the Hitachi 4500 ABI and bioinformatics applications (Basecall, Alignment, Blastn) and identified as *Enterobacter cloacae* (95%), *Bacillus subtilis* (93%), *Enterobacter* spp. (96%), *Staphylococcus epidermidis* (95%) and *Bacillus* sp. (93%).

Experimental design

The experimental design was completely randomized with a 7x4 scheme: T1: control, without the inoculation of bacteria and without the addition of Hg(II); T2: addition of 36 mg kg^{-1} of Hg(II) no inoculation. T3: addition of 36 mg kg^{-1} of Hg (II) + inoculation with *Enterobacter cloacae*; T4: addition of 36 mg kg^{-1} of Hg(II) + inoculation with *Bacillus subtilis*; T5: addition of 36 mg kg^{-1} of Hg(II) + inoculation with *Enterobacter* sp. and; T6: addition of 36 mg kg^{-1} of Hg (II) + inoculation with *Staphylococcus epidermidis* and; T7: addition of 36 mg kg^{-1} of Hg(II) + inoculation with *Bacillus* sp.

Soil preparation

An oxisol with no history of Hg contamination was collected in the 0-0.20 m layer in Jaboticabal, SP. The sample was dried in air and shade, sieved using a 2 mm sieve and sent for chemical analysis.

The results were pH ($CaCl_2$) = 5.5, organic matter = 20 g dm^{-3}, phosphorus = 22 mg dm^{-3}, sulfur = 7 mg dm^{-3}, calcium = 30 $mmol_c$ dm^{-3}, magnesium = 12 $mmol_c$ dm^{-3}, potassium = 4.0 $mmol_c$ dm^{-3}, aluminum = 0 $mmol_c$ dm^{-3}, potential acidity (H+Al)= 19 $mmol_c$ dm^{-3}, cation exchange capacity= 65 $mmol_c$ dm^{-3}, base-cation saturation = 71, boron = 0.30 mg dm^{-3}, copper = 4.6 mg dm^{-3}, iron = 8 mg dm^{-3}, manganese= 9.5 mg dm^{-3}, zinc= 2.5 mg dm^{-3} and total Hg = 0.099 mg kg^{-1}.

For 3 days, soil was autoclaved for 30 minutes at 121°C and 1 atm. Dry soil was packed in pots with a capacity of 2 kg that were coated with plastic bags to avoid leaching losses.

Contamination was performed with manually homogenized solid $HgCl_2$. Throughout the experiment, sterile deionized water was used at 70% of the soil field capacity.

After contamination, soil was fertilized according to Melo et al. (1998). The total Hg content of the fertilizers used in the experiment had values of 0.38 mg kg^{-1} of ammonium sulfate, 0.056 mg kg^{-1} of superphosphate and 0.008 mg kg^{-1} of potassium chloride.

Maize planting

On the 8th day after soil contamination, samples were collected for pH determination. Then, maize seeds (2B710PW cultivar) were immersed for 10 minutes in sodium hypochlorite solution (10%), washed with sterile distilled water, and placed in pots (5 units per pot). Sowing fertilization (Melo et al., 1998) and bacteria were immediately applied. Thinning was performed when plants reached 0.10 m, maintaining only one plant per pot.

Preparation and application of microorganisms in mercury-contaminated soil

From a suspension of pure colonies that were individually cultured in BHI, inoculums were prepared at a concentration of 6 $x10^8$ CFU (Vivas et al., 2006) according to the MacFarland scale and 1 mL of inoculum per pot was applied at 7-day intervals.

Collection of roots and shoots of maize plants and soil at the end of the experiment

Collection was performed 30 days after thinning. For the preparation of roots, all adhered soil was carefully removed, followed by washing with aqueous neutral detergent solution (1 mL L^{-1}), running water, distilled water and deionized water.

Samples were oven dried at 67°C with forced air circulation until a constant weight was obtained, weighed to obtain dry phytomass, milled in a Willey mill with a 40 mesh sieve and stored in plastic bags.

The 2 kg of soil in each pot was sieved with a 5 mm mesh diameter. Then, an aliquot of 0.5 kg of each sample was collected and sent for quantification of total Hg and pH evaluation at the end of the experiment.

Mercury content in soil and plant samples

All samples were crushed in a mortar with the aid of liquid nitrogen to obtain a more homogeneous material and better analytical accuracy. The mercury content in solid samples was measured using the Direct Mercury Analyzer® (DMA-80 TRICELL; Milestone Inc., Italy). This method combines sample combustion (for thermal Hg reduction and vaporization) with atomic absorption spectroscopy (Melendez-Perez and Fostier, 2013).

Two analytical curves were constructed in linear ranges from 0.2 to 10 ng of Hg and from 150 to 1,000 ng of Hg. For this purpose, Hg standard solutions (10, 100 and 10,000 µg L^{-1}) were prepared by diluting Hg standard stock solution (1.000 ± 0.003 mg mL^{-1}, Tec-Lab® Hexis, Jundiaí, Brazil) in deionized water with 10% subdistilled HNO_3. The validation parameters of the analytical method included linearity and limit of quantification (LOQ). Accuracy was checked daily by analyzing standard reference materials of tomato leaves (SRM NIST 1573) and soil (Montana soil SRM NIST 2711). Accuracy was assessed by the relative standard deviation of all analytical SRM replicates. Each experimental sample was also analyzed in duplicate. For each replicate, samples weighing between 10 and 200 mg were analyzed, depending on the expected concentration.

Validation of the analytical method

The correlation coefficients of the 0.2 to 10 ng and 150 to 1000 ng calibration curves were 0.9941 and 0.9966, respectively. The recovery percentages were 105 and 106% for soil and SRM leaves, respectively. Accuracy (19 and 9 analytical replications performed on soil and SRM leaves, respectively) was lower than 4%. The variation coefficient for samples analyzed in duplicate was <10%.

Remediation efficiency

The Hg remediation efficiency by plant according to the type of treatment was calculated based on mass balance data. For each pot in each treatment (Ti), the following parameters were considered (Table 1): mmSi: Hg mass present in the soil at the beginning of Ti (mg) = Hg concentration in the soil at the beginning of Ti (mg kg^{-1}) x 2 kg (soil mass in the culture pot); mmSf: Hg mass present in the soil at the end of Ti (mg) = Hg concentration in the soil at the end of Ti (mg kg^{-1}) x 2 kg (soil mass in the culture pot); mmR: Hg mass accumulated in the roots of Ti (mg) = Hg concentration in the roots of Ti (mg kg^{-1}) x root mass of Ti (kg); mmPA: Hg mass accumulated in the shoots of Ti (mg) = Hg concentration in the shoots of Ti (mg kg^{-1}) x shoot mass of Ti (kg)
From these, four other parameters were calculated: % of accumulated Hg in plants treated with Ti = (mmR + mmPA) x100 / mm Si; % of Hg recovered from soil in treatment Ti = (mmSi - mmSf) x100 / mm Si: Direct contribution of the plant in the recovery of Hg from soil in treatment Ti = (mmR + mmPA) x100 / (mmSi-mmSf)

Statistical analysis

The results were submitted to statistical analysis using the AgroEstat software (2015), with Duncan's test for comparison between means at the 5% probability level.

Conclusions

Among the evaluated treatments, the association of maize plants with *Bacillus* sp. showed better performance in soil Hg recovery, with higher Hg remediation by the plant, and therefore can be considered a potential remediation agent of this metal. However, further studies are needed to identify potential long-term bioremediation options and the effects of their application on large contaminated areas.

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