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Kenaf (*Hibiscus cannabinus* L.) as a remedy to oxisol contaminated with different mercury (Hg^{2+}) concentrations

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Abstract

We evaluated *Hibiscus cannabinus* (kenaf) to remedy oxisol contaminated with Hg^{2+} potential. The study was conducted in a controlled environment in pots with soil contaminated with $HgCl_2$ solution, in a completely randomized design with 4 treatments: control (without Hg^{2+}) and treatments with 5, 24 and 36 mg Hg^{2+} kg^{-1} of soil and 5 replicates / treatment. The quantification of total Hg in plant and soil samples was performed by atomic absorption spectrometry. Kenaf grown in contaminated pots did not show visual symptoms of toxicity. Plant height did not differ among treatments, but the dry shoot phytomass was 21.65% higher in control than the average of treatments with Hg. Treatment with 24 mg kg^{-1} showed dry root phytomass greater than control and the others. In general, oxisol was responsible for retaining greater amount of Hg than plants. Hg accumulated in greater proportion in roots than in shoots. In pots that received 36 mg kg^{-1}, plants accumulated average of 2.57 mg kg^{-1} of Hg / pot, differing from the other treatments and the Hg transfer factor (TF) in plants was also calculated as the ratio of the concentration in shoots and in roots. The values were as follows: 3.11 for T1, 1.26 for T2, 0.05 for T3 and 0.02 for T4. Treatments showed no difference between T3 and T4 and TF decreased with increasing Hg dose. It could be concluded that Hg was more adsorbed by oxisol than by plants. Plants showed resistance to different soil Hg concentrations and can be considered as potential Hg^{2+} stabilizer.

Keywords: contamination; soil remediation; toxic metal.
Abbreviations: BF_bioaccumulation factor; TF_transfer factor; T1_treatment 1.

Introduction

The intensification of urbanization, modernization and industrial processes have significantly contributed to the increase in environmental pollution that negatively affects the quality of ecosystems and quality of life of human beings (Singh et al. 2019). For this reason, the demand for green technologies to recover areas contaminated by diverse contaminants has increased, as they are functional, sustainable and less costly methods.
Phytoremediation is a technique that uses the natural potential of certain plants to remedy the presence of potentially toxic metals in soils. It is an environmentally friendly and low-cost application method (Yan et al., 2019). Plants can act as extractors as they are able to absorb contaminants in roots and translocate them to shoots without affecting biomass production. Hyperaccumulators are plants that accumulate more contaminants in shoots than in roots, in less biomass. In this case, they are able to accumulate 100 to 1000 times more contaminant in leaves and stems than in roots. Although the remediation of contaminated areas with hyperaccumulative plants is attractive, this management can result in the entry of toxic elements in the food chain. This process can be reduced with the use of stabilizing plants that have less potential for translocating metals from roots to shoots (Alkorta et al. 2010; Burges et al. 2018; Gómez-Sagasti et al. 2012; Tangahu et al., 2011; Vijayaraghavan et al., 2018).
However, phytostabilization does not remove contaminants, but rather reduces their bioavailability, favoring their adsorption in the soil and complexation with humic substances, consequently, reducing the leaching of toxic elements in the environment. Stabilizing plants show rapid growth and a well-developed root system (Martínez-Martínez et al. 2018; Radziemska 2018; Vijayaraghavan et al., 2018).
Hibiscus cannabinus (kenaf) is an annual plant of economic interest, with height ranging from 2.5 to 6 m and extensive and deep roots, which provides it tolerance to drought and high cultivation density with up to 220,000.00 plants per ha (Alexopoulou et al. 2007; Asim et al. 2018). In addition, it has microbial diversity in roots and the symbiosis between plant and microorganisms benefits the remediation process

(Arbaoui et al., 2013; Chen et al., 2018; Santos et al. 2010). For these various reasons, the phytorremediation potential of kenaf has already been studied for several metals with high toxic potential. Kenaf acted as lead and mercury (Hg) extractor and as cadmium stabilizer in low fertility, acid and sandy soils. For lead, the extractor effect has also been reported in clayey soils. The extraction effect of kenaf was also observed for chromium in wastewater treatment (Abioye et al. 2012; Bada and Kalejaiye 2010; Catroga et al 2005; Chen et al. 2017; Fitria and Dhokhikah 2019). However, its potential for remediation of Hg-contaminated environments has not yet been studied.

The origin of Hg in the environment can be natural (geogenic) or associated with anthropogenic processes (e.g. gold mining, burning fossil fuels) (Kim et al. 2016). While Hg concentration in uncontaminated soils is usually <1 mg kg^{-1}, concentrations of thousands of mg can be found in areas contaminated by human activities (Higueras et al., 2003; Teixeira et al., 2018).

Global Hg contamination, especially in Brazil due to anthropic contribution, is not a recent issue and ecosystems suffer consequences until today. Mining gold for decades has impacted the soils of Alta Floresta, Mato Grosso, Brazil (4.10 mg kg^{-1} Hg) (Rodrigues Filho and Maddock 1997), and in Tartarugalzinho, Amapá, Brazil, mining has caused significant contamination of soil by Hg (> 300 mg kg^{-1} total Hg) (Oliveira et al. 2001). The authors identified that the superficial contamination of soils (95% of total Hg) in this region is significantly anthropogenic. Concentrations between 0.022 to 0.16 mg kg^{-1} of total Hg in the soil are still found in the same region (Miserendino et al. 2018) and total Hg concentrations ranging from 0.0371 to 161 mg kg^{-1} in soil with gold mining activities in Descoberto, Minas Gerais, Brazil (Durão Jr. et al. 2009). The contamination of Hg by atmospheric deposition resulting from gold exploitation from other regions and from pedogenesis, may also have contributed to Hg enrichment in soils of the Tapajos National Forest, Pará, Brazil, (Figueiredo et al. 2018.)

The toxicity degree of this metal varies according to its speciation (i.e., oxidation state, organic / inorganic form) (Schaefer 2016). Hg has three oxidation states: elemental mercury (Hg^0), mercurous (Hg^{+}) and mercuric ions (Hg^{2+}) (Adriano 2001). In the environment, it can be found in elementary, inorganic and organic forms (methyl mercury and dimethyl mercury). One of its organic forms, methyl mercury (CH_3Hg^+), the most toxic, can be bioaccumulated by organisms and biomagnified in the food chain.

No organism uses Hg in its biosynthesis and its presence in the environment has become a global concern due to its volatility and toxicity (Pacyna et al. 2016; Sundseth et al. 2017; Wang et al. 2003). For this reason, it was classified by the Agency for Toxic Substances and Disease Registry as the third most dangerous substance, only behind arsenic and lead (ATSDR 2016). In general, exposure to this metal, in its different forms, can cause damage, with varying degrees of severity to the physical and mental health of humans and animals (UNEP 2020).

In view of the need to recover contaminated areas to minimize environmental impacts, the remedial effect of kenaf grown in oxisol (according to the Embrapa classification (Embrapa, 2018), contaminated with $HgCl_2$ at different Hg^{2+} concentrations was evaluated, quantifying the total Hg content accumulated in roots and shoots (leaves and stem).

Results

Influence of cultivation conditions on the growth variables of Hibiscus cannabinus

Table 1 depicts the chemical analysis of soil. Regarding soil pH, no difference among treatments was observed in the same evaluation period (beginning or end of cultivation), but for all treatments, the final pH was significantly lower (0.6 to 0.9 pH units) than pH at the beginning of the experiment, which ranged from 5.3 to 5.4.

For the same growing period, plants did not show any height difference among the different treatments. However, at the end of the experiment, plant height was significantly higher (1.57 to 2.05 m) than at the beginning of the experiment (0.51 to 0.60 m). Although plant height at the end of the experiment did not differ among treatments, the dry stem phytomass was significantly higher in control pots (T1) than in all treatments with Hg (Table 2). On the other hand, soil contamination with Hg did not affect leaf phytomass.

There was no difference in root size among treatments. However, thicker nodular roots were observed in treatments with the highest Hg concentrations (T3 and T4) compared to T1 and T2 treatments (Fig.1).

Total mercury concentrations in soil and total mercury accumulated in the dry root and shoot phytomass of Hibiscus cannabinus

The expected soil concentration of control pots (T1) at the beginning of planting (0.1 mg kg^{-1}) was calculated considering the Hg concentration initially present in the soil (0.099 mg kg^{-1}) plus the amount of Hg from fertilizers. Except for T1 pots, Hg concentrations in soil at the time of transplant were in accordance with dose applied at the beginning of the experiment (60 days before transplant), with recovery rate from 107 to 117% (Table 3). The relatively low CV values (<22%), calculated from concentrations found in the five pots of the same treatment demonstrate that there was adequate homogenization of soil contaminated with $HgCl_2$ solution (Table 3).

On the other hand, the average Hg concentration in control pots at the time of transplant was 1270% higher than at the beginning of the experiment. Considering the high Hg volatility (vapor pressure: 0.25 Pa at 25°C), this increase in concentration can be attributed to cross contamination due to the volatilization of part of Hg in contaminated pots followed by its deposition in control pots.

After 75 days of plant cultivation, total Hg concentrations in soil decreased significantly in all treatments, varying from 0.13 to 21.23 mg kg^{-1}, for T1 and T4 treatments, respectively (Table 4). Total Hg accumulation in the dry root phytomass varied from 0.003 to 2.52 mg kg^{-1} and was significantly higher in T4 treatment. Regarding the dry shoot phytomass, total Hg accumulation varied from 0.008 to 0.05 and was greater in pots that received the highest Hg dose (T4) (Table 4).

Bioaccumulation factors (BF) calculated as the ratio between total Hg concentration in plant and Hg concentration in soil at the end of planting (Tu and Ma, 2002) were: 0.09 for T1, 0.02 for T2, 0.03 for T3 and 0.12 for T4, with statistical difference between T4 and the other treatments. The Hg transfer factor

Table 1. Chemical analysis of soil.

Elements	Values
pH ($CaCl_2$ 0.01 mol L^{-1})	5.8
Organic matter	24 g dm^{-3}
P	27 mg dm^{-3}
S	6 mg dm^{-3}
Ca	36 $mmol_c$ dm^{-3}
Mg	14 $mmol_c$ dm^{-3}
K	8.1 $mmol_c$ dm^{-3}
Al	1 $mmol_c$ dm^{-3}
H+Al	23 $mmol_c$ dm^{-3}
Cation exchange capacity (CEC)	81 mmolc dm^{-3}
Base saturation	72%
B	0.28 mg dm^{-3}
Cu	8.5 mg dm^{-3}
Fe	26 mg dm^{-3}
Mn	31.1 mg dm^{-3}
Zn	5.9 mg dm^{-3}
Hg	0.099 mg kg^{-1}

Table 2. Average (5 replicates) of dry phytomass of *Hibiscus cannabinus* plants at the end of the experiment.

	Treatment	Root phytomass	Stem phytomass	Leaf phytomass	Shoot phytomass
		------------------------g--------------------------			
T1	0 mg kg^{-1} Hg^{2+}	16b ± 4.18	122a ± 17.54	103a ± 7.58	225a ± 10.61
T2	5 mg kg^{-1} Hg^{2+}	16b ± 4.18	83b ± 10.37	97a ± 5.7	179b ± 11.40
T3	24 mg kg^{-1} Hg^{2+}	21a ± 2.24	78b± 5.70	102a ± 4.47	180b ± 6.12
T4	36 mg kg^{-1} Hg^{2+}	18ab ± 2.45	92b ± 10.37	101a ± 7.42	195b ± 15.49

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

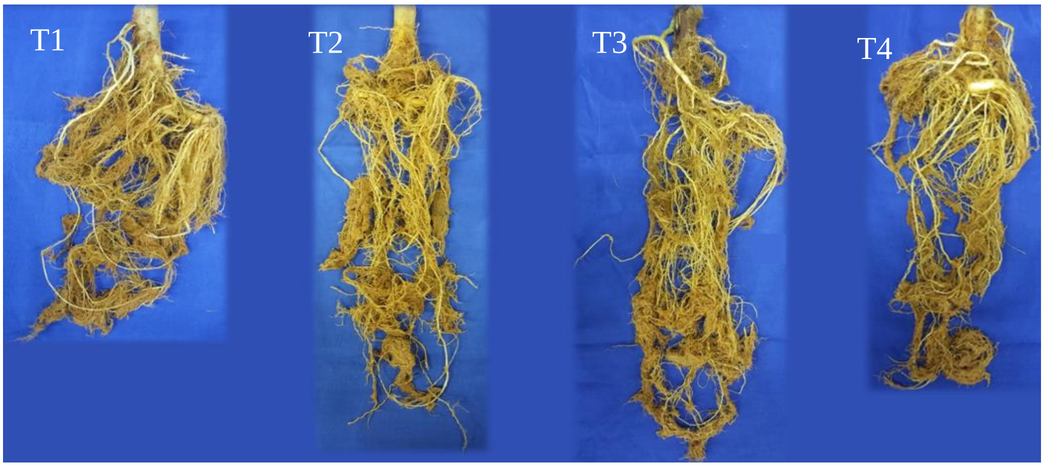

Figure 1. *Hibiscus cannabinus* roots development maintained in oxisol contaminated with different doses of Hg^{2+} mercury. Treatments: T1 = 0 mg Kg^{-1} Hg^{2+} (control); T2 = 5 mg Kg^{-1} Hg^{2+}; T3 = 24 mg Kg^{-1} Hg^{2+}; T4 = 36 mg Kg^{-1} Hg^{2+}.

Table 3. Expected mercury concentration found in soil at the beginning of planting. Mean, standard deviation and coefficient of variation (CV) calculated for five replicates of the same treatment.

Treatments	[Hg] expected in soil	[Hg] found in soil	Standard deviation	CV	Recovery
	----------------------mg kg^{-1}------------------------			-----------%---------	
T1	0.1	1.37	0.29	21.40	1370
T2	5.1	5.33	0.99	18.47	107
T3	24.1	26.39	5.75	21.77	110
T4	36.1	41.99	4.84	11.53	117

T1: control, T2: 5 mg kg^{-1} Hg^{2+}, T3: 24 mg kg^{-1} Hg^{2+} and T4: 36 mg kg^{-1} Hg^{2+}.

Table 4. Hg concentration in soil at the end of the experiment and Hg accumulation in dry root and shoot phytomass.

	Treatments	final [Hg] in soil	Accumulated Hg dry root phytomass	Accumulated Hg dry shoot phytomass	Total Hg in plant (root + shoot)
		mg kg^{-1}	-----mg kg^{-1}/pot-----		mg kg^{-1}/ pot
T1	0 mg kg^{-1} Hg^{2+}	0.13c ± 0.01	0.003b ± 0.01	0.008b ± 0.001	0.01b ± 0.001
T2	5 mg kg^{-1} de Hg^{2+}	0.84c ± 0.16	0.007b ± 0.003	0.008b ± 0.001	0.01b ± 0.003
T3	24 mg kg^{-1} Hg^{2+}	8.61b ± 0.96	0.27b ± 0.09	0.01b ± 0.004	0.28b ± 0.09
T4	36 mg kg^{-1} Hg^{2+}	21.23a ± 1.72	2.52a ± 0.69	0.05a ± 0.02	2.57a ± 0.71

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

(TF) in plants was also calculated as the ratio of the concentration in shoots and in roots. The values were as follows: 3.11 for T1, 1.26 for T2, 0.05 for T3 and 0.02 for T4. Treatments showed no difference between T3 and T4 and TF decreased with increasing Hg dose.

Discussion

Comparing the Hg concentrations in soil between the beginning and end of planting, decreases of 90.5, 84.2, 67.4 and 49.4% were observed for T1, T2, T3 and T4 treatments, respectively. Although the results showed that part of Hg was accumulated in plants, the mass balance also showed that most of added Hg was lost during the experiment. These losses may be due to leaching and Hg volatilization processes. Volatilization losses should be considered because environmental factors such as solar radiation, in the different seasons of the year, can increase Hg emission into the atmosphere (Carpi et al., 2014; Choi and Holsen, 2009).

The BF values indicate the relationship of the metal concentration in soil with the plant and BF <1 indicates that plants have mechanisms to tolerate the presence of high Hg doses without bioaccumulation (Marrugo-Negrete et al. 2016; Xun et al. 2017).

In the present study, these values show that only a small part of the metal was accumulated in plant phytomass (root and shoot) and only T4 treatment differed from the others, with greater total Hg accumulation in the plant dry phytomass (Table 4) and consequently with higher BF (0.12), differing from results found by Xun et al. (2017), who identified decrease in BF values in *Cyrtomium macrophyllus* plant with increase in Hg concentration in soil. However, plants are more attractive for phytoremediation when capable of translocating Hg to shoots and presenting TF> 1 (Marrugo-Negrete et al., 2016; Xun et al., 2017), as they are thus considered phytoextractors.

In our study, it was observed that the highest Hg doses (T3 and T4) negatively influenced TF, since only control treatment (T1) and treatment with the lowest Hg dose (T2) had TF> 1, corroborating results found by Xun et al. (2017), who found lower TF in the *Cyrtomium macrophyllus* plants, with TF of 2.64 in soil contaminated with Hg at concentration of 5 mg kg^{-1} and 0.36 in soil that received 1000 mg kg^{-1}. In general, the authors identified decrease in TF with increase in Hg concentration in soil and suggest that higher TF values do not necessarily indicate that the plant is a potential Hg translocator, as the deposition of atmospheric Hg in leaves must be considered. Similar results were also identified by Wang et al. (2017), who observed low TF (~ 0.3) and FB (<0.1) values in mustard plant grown in soil with high total Hg concentration (90 mg kg^{-1}).

In our study, the initial soil pH varied from 5.3 to 5.4 and had significantly lower values at the end of the experiment, varying from 4.5 to 4.7. These pH values may thus have favored mercury adsorption in the soil, making it less available to plants.

The reduction in dry shoot phytomass verified in all treatments when compared to control can be related to damages in the cell format caused by the metal toxicity. Exposure to high Hg concentrations also reduces the intracellular spaces of the vascular bundle, the amount of chloroplasts and chlorophyll. All these effects interfere with plant physiology and compromise photosynthesis, which can lead to the death of leaf tissues (Ahammad et al. 2018; Chen et al. 2009; Rellán-Álvarez et al. 2006).

Given the above, Hg adsorption in soil, losses due to volatilization and proportion of metal accumulation in roots and shoots can vary from one location to another due to the intrinsic characteristics of the soil of each region and each plant species.

Materials and Methods

Treatments

The experiment was conducted in greenhouse with clay oxisol (clay = 55.6%, silt = 38.1% and sand = 6.3%, unpublished data from Silva, 2019). This class of soil is widely found in tropical regions, including Brazil (Lima et al. 2019a). Soil without history of Hg contamination was collected at 0-0.20 m layer in the Teaching, Research and Extension Farm of Unesp, campus of Jaboticabal, São Paulo. Chemical characterization was carried out according to standard methods proposed by Raij et al. (2001).

Conduction of study

Soil collected was dried in air and shade and passed through 2 mm sieve. Then, 5 kg of soil were placed in pots and contaminated with $HgCl_2$ solution (Synth) (except for control pots in which no Hg was added), manually homogenized and incubated for 60 days. Throughout the experiment, soil moisture was preserved with the addition of deionized water in the proportion of 70% of the water holding capacity. At the end of the incubation period, soil was fertilized as proposed by Melo et al. (1998). The total Hg content of fertilizers used in the experiment was 0.38 mg kg^{-1} for ammonium sulfate, 0.056 mg kg^{-1} for simple superphosphate and 0.008 mg kg^{-1} for potassium chloride.

Experimental design

The experimental design was completely randomized, with 4 treatments and 5 replicates: T1: control, without addition of Hg, T2: addition of 5 mg Hg^{2+} kg^{-1} of soil, T3: addition of 24 mg kg^{-1} and T4: addition of 36 mg kg^{-1}. For all concentrations, soil mass was considered on the dry basis and adequate volume of $HgCl_2$ solution was added. The highest dose was established considering the residential standard value for Hg in soil based on CONAMA Resolution No. 420 of 2009. Seedlings, acquired in a commercial nursery, were transplanted to pots on the 60th day after their contamination and plants were kept in pots for 75 days. Soil samples were collected before transplant and at the end of the experiment to measure pH and determine the Hg concentration.

Traits measured

Plant size was measured at the time of transplant and at the end of the experiment. Root and shoot collections (Shoots = stem + leaves) were performed 75 days after seedling transplant. In the preparation of roots, all soil adhered to them was carefully removed and roots were washed with aqueous solution of neutral detergent (1 mL L^{-1}), running water, distilled water and deionized water. The same procedure was applied to shoots. Samples were dried in an oven at 67 ° C with forced air circulation until constant weight. All samples were ground in mortar with the aid of liquid nitrogen to obtain a more homogeneous material and better analytical precision.

Quantification of total mercury in solid samples was performed using the Direct Mercury Analyzer® equipment (DMA-80 TRICELL; Milestone Inc., Italy). This method combines the combustion of samples with atomic absorption spectrometry (Melendez-Perez and Fostier, 2013). Two analytical curves were constructed in the linear ranges from 0.2 to 10 ng Hg and from 150 to 1,000 ng Hg. For that, standard Hg solutions (10, 100 and 10,000 µg L^{-1}) were prepared by diluting standard Hg solution (1,000 ± 0.003 mg mL^{-1}, Tec-Lab® Hexis, Jundiaí, Brazil) in deionized water with 10% sub-distilled HNO_3.

For each analytical replica, sample mass between 10 and 200 mg were analyzed, depending on the expected concentration. Each experimental sample was analyzed in duplicate. The regression coefficients for calibration curves from 0.2 to 10 ng and 150 to 1000 ng were 0.9941 and 0.9966, respectively. Recovery percentages for standard reference samples of soil (Montana soil SRM NIST 2711) and leaves (Tomato leaves SRM NIST 1573) were 105 and 106%, respectively. Accuracy evaluated by the coefficient of variation calculated considering all SRM analytical replicates (19 and 9 analytical replicates for soil and leaves, respectively) was less than 4%. The coefficient of variation for samples analyzed in duplicate was <10%.

Statistical analysis

Results obtained were submitted to statistical analysis using the AgroEstat software (2015), with the application of the Duncan test to compare means at 5% probability level.

Conclusion

Due to its chemical characteristics, the oxisol used retained greater amounts of mercury in relation to plants. Losses due to volatilization of soil and plant Hg were also considered. Kenaf plants showed tolerance to different Hg^{2+} concentrations up to 42 mg kg-1 without showing visual symptoms of toxicity. Kenaf was able to accumulate greater proportion of Hg^{2+} in roots than in shoots. For this reason, kenaf can be considered as a potentially Hg^{2+} stabilizing plant. However, as it is a plant that has not yet been studied for this purpose, further studies should be carried out to assess field behavior and its effect on large, contaminated areas.

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The authors declare no conflicts of interest.

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