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EDTA-induced phytoextraction of lead and barium by brachiaria (*B. decumbens* cv. Basilisk) in soil contaminated by oil exploration drilling waste

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ABSTRACT. The phytoextraction of heavy metals using chelating agents has been widely studied for the remediation of contaminated soils. To evaluate the efficiency of EDTA-induced phytoextraction of Ba and Pb using *Brachiaria decumbens* for the remediation of soil contaminated by oil well drilling and exploration waste, an experiment was conducted by applying a single dose (6 mmol EDTA kg^{-1} soil) and split doses of EDTA (three applications of 2 mmol EDTA kg^{-1} soil). The samples were subjected to sequential extractions using the method proposed by Ure et al. (1993) as modified by Rauret et al. (1999).The application of EDTA did not influence the distribution of Ba in various chemical fractions of the soil. The dry matter production did not differ significantly between the treatments and the control, thereby demonstrating the tolerance of plants to the experimental conditions. The absorption of Pb by plants was influenced by the application of EDTA. The application of a single dose of EDTA influenced the absorption of Pb and its translocation to the aerial plant parts. The application of split doses favoured higher accumulation of Pb in roots. Because of its tolerance to heavy metals and EDTA, *B. decumbens* has the potential to be used in phytostabilisation.

Keywords: heavy metals, phytoremediation, chelating compounds.

Uso de EDTA na fitoextração induzida de chumbo e bário por braquiária (*B. decumbens* cv. Basilisk) em solo contaminado por resíduos de perfuração de poços de petróleo

RESUMO. A fitoextração de metais pesados usando agentes quelantes tem sido amplamente estudado para a remediação de solos contaminados. Para avaliar a eficiência do EDTA na fitoextração induzida de Ba e Pb, usando *Brachiaria decumbens* para a remediação de solos contaminados por perfuração poço de petróleo e resíduos de exploração, foi conduzido um experimento, aplicando uma dose única (6 mmol EDTA kg^{-1} solo) e divisão de doses de EDTA (três aplicações de 2 mmol EDTA kg^{-1} solo). As amostras foram submetidas a extrações sequenciais com o método proposto por Ure et al. (1993), conforme modificado por Rauret et al. (1999). A aplicação de EDTA não influenciou a distribuição do Ba nas diferentes frações geoquímicas. A produção de matéria seca não diferiu significativamente entre os tratamentos e o controle, demonstrando assim a tolerância das plantas com as condições experimentais. Houve maior absorção de Pb pelas plantas com a aplicação de EDTA. A aplicação de uma dose única de EDTA influenciou a absorção de Pb e sua translocação para a parte aérea das plantas. A aplicação dividida das doses favoreceu maior acúmulo de Pb nas raízes. Devido à sua tolerância para os metais pesados EDTA, *B. decumbens* tem o potencial para ser utilizado em fitoestabilização.

Palavras-chave: metais pesados, fitorremediação, compostos quelantes.

Introduction

Exploration for oil generates waste that can potentially cause pollution. This waste arises from the drilling of prospecting wells and primarily consists of fragmented rock mixed with drilling fluid. This waste also contains hydrocarbons and heavy metals, such as Ba and Pb (DEUEL JR.; HOLLIDAY, 1997).

Contamination by heavy metals and other elements considered toxic to humans and the environment has occurred since the late eighteenth century, thereby raising concerns and warranting studies to search for effective low-cost technologies to reduce the toxic effects of such contamination (GRATÃO et al., 2005).

Phytoremediation has been considered a promising and relatively cost-effective method compared to other techniques for the remediation of areas contaminated with heavy metals (SAIFULLAH et al., 2009; SALT et al., 1998). Phytoextraction can be classified as natural or induced. Natural phytoextraction involves the use of plants known as hyperaccumulators of heavy metals. Induced phytoextraction involves the use of plants with a high biomass production that can accumulate elevated heavy metal concentrations in their tissues when grown in soils treated chemically with chelating agents that increase the bioavailability of metals and absorption by the plant (MEERS et al., 2005).

Among the many chelating agents used to enhance phytoextraction, ethylene-diamine-tetraacetic acid (EDTA) has been shown to be very efficient in forming soluble complexes with metals, especially for the removal of Pb (DOUMET et al., 2008; KIM et al., 2003; LESTAN et al., 2007; XIA et al., 2009).

The objective of the present study was to evaluate the use of EDTA in the induced phytoextraction of Ba and Pb using *Brachiaria decumbens* for the remediation of soil contaminated by waste from oil exploration drilling.

Material and methods

The contaminated soil used in the experiment consisted of a mixture of waste from oil prospecting wells; this waste was deposited more than 20 years ago in an area containing Latossolo Vermelho Distrófico típico.

The chemical (Table 1) and physical characteristics of soil samples were determined according to Embrapa (1997). A soil particle size analysis showed a silt content of 62%, a sand content of 38% and a negligible percentage of clay.

Contaminated soils were used to assess the ability of *Brachiaria decumbens* cv. Basilisk to phytoextract Ba and Pb in the presence of EDTA. The experiment was assembled and conducted in a greenhouse at the Federal Rural University of Rio de Janeiro ('Universidade Federal Rural do Rio de Janeiro'), and the contaminated soil samples were collected in Santa Maria do Oeste in the Brazilian State of Paraná.

Pots were filled with 4 kg of contaminated soil, and 24 seeds were placed in each pot. After sprouting, the number of plants was thinned to four per pot. The pots were maintained at a moisture content equivalent to 80% the field capacity by the addition of deionised water. After the first month, approximately 100 mL of a one-quarter-strength Hoagland solution (HOAGLAND; ARNON, 1950) were added every three days. Two treatments plus the control were used; one treatment received a split application of EDTA, and the other received a single dose of EDTA. Each condition had four replicates for a total of 12 experimental units.

Table 1. Chemical characterisation of the soil used in the experiment.

	Soil
pH H_2O	8.3
Ca^{2+} ($cmol_c\ dm^{-3}$)	9.5
Mg^{2+} ($cmol_c\ dm^{-3}$)	2.0
H + Al ($cmol_c\ dm^{-3}$)	1.3
Al^{3+} ($cmol_c\ dm^{-3}$)	0.0
S (%)	11.53
T (%)	12.83
V (%)	90
P (mg kg^{-1})	3
K (mg kg^{-1})	5
C (g kg^{-1})	1.4
Ba (mg kg^{-1})	6.700
Pb (mg kg^{-1})	350.4

pH in water (1:2.5); Ca and Mg, H+Al – extracted in 1 mol L^{-1} KCl; Al - extracted in 1 mol.L^{-1} calcium acetate at pH 7.0; P - extracted with North Carolina solution; K - extracted with 0.5 mol.L^{-1} potassium dichromate; C – Walkley – Black; Ba and Pb - aqua regia digestion (pseudototal) (ISO 11466, 1995).

The treatment with a split application of EDTA, applied via the surface pot, consisted of three doses of 2 mmol EDTA kg^{-1} soil (0.6 mg EDTA g^{-1} soil). The first dose was administered at 120 days after sowing the plants followed by two additional applications at intervals of seven days. The single EDTA dose was administered by the addition of 6 mmol EDTA kg^{-1} (1.8 mg EDTA g^{-1} soil) on the day of the last dose in the split application treatment.

Soil samples were collected at five days after the first application of 2 mmol EDTA kg^{-1} soil in the split application treatment and after the application of 6 mmol EDTA kg^{-1} soil in the single-dose treatment. The samples were subjected to sequential extractions using the method proposed by Ure et al. (1993) as modified by Rauret et al. (1999).

The plants were collected one month after the final application of EDTA, which completed a cycle of 164 days. After collection, the roots and leaves were separated, weighed, rinsed in deionised water and placed in an oven at 70 °C until a constant weight was obtained. The samples were ground and then digested using a 6:1 nitryl perchlorate solution according to the method of Tedesco et al. (1995).

The concentrations of metals in soil extracts were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Perkin Elmer model OPTIMA 3000). The limit of detection (LD) and limit of quantitation (LQ) for Ba were 0.036 mg kg^{-1} and 0.36 mg kg^{-1}, respectively, and the LD and LQ for Pb were 0.020 mg kg^{-1} and 0.067 mg kg^{-1}, respectively, in the soil digestion extracts. The LD was calculated as the mean of the blanks plus three standard deviations of the blanks from all analyses (10 replicates). The National Institute of Standards and Technology (NIST) certified standard reference material (SRM) 22709a (San Joaquin soil; Ba and Pb concentrations of 979 ± 28 and 17.3 ± 0.1 mg kg^{-1}, respectively) was used to validate the determination of the pseudototal contents of Ba and Pb in the soil. NIST certified SRM 1573a (tomato leaves; Ba concentration of 63 ± 0.7 mg kg^{-1}) was used to validate the Ba contents of the plant material, and NIST certified SRM 1547 (peach leaves; Pb concentration of 0.87 ± 0.03 mg kg^{-1}) was used to validate the Pb contents of the plant materials. All analyses of the certified samples showed a range of recovery of 93-95%, which was within the ranges accepted as normal by NIST for soil and plant samples.

To evaluate the potential of Brachiaria to extract Pb and Ba, we determined the bioaccumulation factor (AF) and the index of translocation (IT) of each element in the plant according to Araújo et al. (2011); The phytoextraction efficiency (PE) was determined according to Vysloužilová et al. (2003), as described below:

AF = M (aerial part) / M (soil)

IT = (accumulation of the element in the aerial part / accumulation of the element in the plant) * 100.

PE = [M(plant) x Plant weight / M(soil) x pot weight] x 100, where M is the concentration of the metal contaminant.

The data were evaluated by analysis of variance using the F test ($p < 0.05$), and the means were compared using Tukey's test ($p < 0.05$). The analyses were performed using the statistical software SAEG statistical software package, version 9.0 (Arthur Bernardes Foundation at the Federal University of Viçosa, Viçosa State, Minas Gerais).

Results and discussion

The geochemical fractionation of contaminated soil samples (Table 2) showed that the Ba was almost entirely in the most recalcitrant fraction (F4 - residual), thus resulting in low concentrations in the other soil fractions. The presence of EDTA, both in the split application and single-dose treatments, did not produce significant differences between the different factions. Studies conducted by Smeda and Zyrnicki (2002) also found higher levels of Ba in the residual fraction. The occurrence of Ba in the soil, especially in the residual fraction, and the Ba^{2+} ion in the soil tends to be precipitated and adsorbed over time in the form of oxides and hydroxides (PICHTEL et al., 2000).

Table 2. Geochemical fractionation of Ba and Pb in contaminated soils and soils with different EDTA concentrations.

Metal	EDTA concentration	Geochemical fraction F1	F2	F3	F4
		mg kg^{-1}			
	0	118 (± 1.5)	252(±1.7)	358 (± 10.9)	6082 (±6.2)
Ba	3 x 2[1]	119(±6.2)	243(±4.0)	377(±12.4)	6091(±22.4)
	1 x 6[2]	115(±2.5)	257(±1.4)	374(±16.1)	6136(±130.8)
	0	53 (± 4.0)	156 (± 2.2)	26 (± 1.5)	335 (± 3.3)
Pb	3 x 2	95(±7.97)	151(±17.4)	27(±2.0)	303(±26.9)
	1 x 6	92(±5.9)	151(±7.4)	25(±2.2)	312(±15.0)

F1 - acid-soluble fraction; F2 - fraction bound to iron oxides and manganese oxides; F3 - fraction bound to organic matter and sulphides; F4 - Residual. The values correspond to an average of 4 treatments ± standard deviation. There was no significant difference (Tukey's test at 5% significance) between treatments. 1: Treatment with three applications of EDTA at 2 mmol kg^{-1} soil. 2: Treatment with the application of a single dose of EDTA at 6 mmol kg^{-1} soil.

In the control, approximately 10% of the total Pb content was detected in the exchangeable phase (F1), where electrostatic and carbonate bonds occur, and approximately 30% occurred in the fraction bound to oxides (F2). This binding of Pb to carbonates and oxides was also mentioned by Pichtel et al. (2000). The Pb has a high affinity for iron oxides and, in particular, manganese oxides (XIA et al., 2009).

The addition of EDTA caused a significant change in the distribution of Pb in the geochemical fractions of the soil. Significant increases in Pb concentrations were observed in the soluble acid fractions (F1). Studies by Grčman et al. (2001) and Saifullah et al. (2009) also showed a marked increase in Pb levels in the soil solution with the addition of 3 mmol L^{-1} EDTA.

The plants developed without any symptoms of stress until the addition of EDTA. The treatment that received the first of three doses of 2 mmol EDTA kg^{-1} soil began to show leaves with a more intense green colour at 125 days after sowing.

In the treatment that received a single dose of EDTA (6 mmol EDTA kg^{-1} soil), the plants showed a yellowing of the leaves at 140 days after sowing; this yellowing continued until the time of collection. These symptoms could be attributed to the solubilisation of metals in the soil at levels greater than the phytotoxicity limit in the region of the rhizosphere as well as the phytotoxicity of the EDTA itself (EVANGELOU et al., 2007).

Despite these symptoms, the dry matter production of *Brachiaria* was statistically similar between treatments (Table 3), thereby indicating that the plants were tolerant of the metals and

EDTA under the experimental conditions. In an evaluation of the effect of EDTA on the induced phytoextraction of Zn and Cd by *Brachiaria decumbens*, Santos et al. (2006) also found that this plant had a tolerance to high concentrations of Zn and Cd in the soil.

Table 3. Dry matter production in the different vegetative parts of *Brachiaria decumbens* grown in contaminated soil with varied concentrations of EDTA.

Treatments	Dry matter (g per pot)		
EDTA kg^{-1} soil	Aerial parts	Roots	Total
0	16.04 ^{NS} (±1.21)	6.43 ^{NS} (±0.34)	22.47 ^{NS} (±1.25)
3 x 2[1]	19.70 ^{NS} (±4.02)	4.78 ^{NS} (±1.28)	24.48 ^{NS} (±4.95)
1 x 6[2]	15.90 ^{NS} (±2.47)	4.19 ^{NS} (±0.18)	20.09 ^{NS} (±2.63)

Values correspond to the means of four treatments ± standard deviation. NS - no significant difference (Tukey's test at 5% significance) in column. 1: Treatment with 3 applications of EDTA at 2 mmol kg^{-1} soil. 2: Treatment with the application of a single dose of EDTA at 6 mmol kg^{-1} soil.

Neugschwandtner et al. (2008) studied the application of split and single doses of EDTA and showed that signs of phytotoxicity in corn plants were more visible after the application of a single dose; the single dose also led to the death of the plants before harvest in some cases. According to Barocsi et al. (2003), the addition of the same amount of EDTA in split doses can increase the plants' resistance to damage caused by heavy metals and EDTA compared to a single dose.

There was no significant difference in the concentration of Ba among the treatments (Table 4), which may have been caused by the low bioavailability of this element in the soil (Table 1).

Table 4. Concentrations of Ba and Pb in the leaves and roots of *Brachiaria decumbens* (mg kg^{-1}) grown in contaminated soil and under different concentrations of EDTA.

	Leaf			Root		
Metal	0	3 x 2[1]	1 x 6[2]	0	3 x 2	1 x 6
Ba	26.9 ^{NS} (±5.8)	27.3 ^{NS} (±2.8)	24.3 ^{NS} (±1.28)	17.6 ^{NS} (±1.03)	18.5 ^{NS} (±2.56)	20.9 ^{NS} (±4.7)
Pb	0.5 C (±0.0)	1.41 B (±0.07)	9.02 A (±3.18)	1.41 B (±0.18)	5.3 A (±1.11)	4.2 A (±0.90)

The values correspond to the means of four treatments ± standard deviation. Different letters along the rows indicate statistically significant differences (Tukey's test at 5% significance or less) between the plant parts. NS = Not significant. 1: Treatment with three applications of EDTA at 2 mmol kg^{-1} soil. 2: Treatment with the application of a single dose of EDTA at 6 mmol kg^{-1} soil.

However, there was a marked increase in the Pb concentrations in the leaves relative to the control plants, especially in the treatment with the application of a single dose of EDTA at 6 mmol kg^{-1} soil. The addition of EDTA also led to an increase in the concentration of Pb in the roots. The induction of Pb uptake by plant roots has also been observed by other authors (DOUMETT et al., 2008; KIM et al., 2003; LESTAN et al., 2007) and is in agreement with the geochemical fractionation results (Table 2). Although no significant difference was found in the geochemical fractionation of Pb among the different treatments (Table 2), increased Pb absorption may have occurred due to the acidification of the medium in the region of the rhizosphere, which would increase the formation of soluble complexes around the roots.

The treatment with the split addition of EDTA retained the greatest amount of Pb in the roots, thereby reducing the extent of Pb translocation to the aerial parts. This greater ability to retain Pb in the roots may be due to the limited mobility of Pb within the plant (PICHTEL et al., 2000) as well as the capacity of the plant to acquire mechanisms to adapt and overcome injury caused by EDTA at lower concentrations (VASSIL et al., 1998).

The accumulation of Ba in the roots and aerial plant parts (Table 5) was not significant. However, the accumulation of Pb in the leaves was significantly higher in the treatment with a single addition of EDTA. In the roots, the application of EDTA also significantly increased the accumulation of Pb relative to the control plants.

Table 5. Accumulation of metals in the leaves and roots of *Brachiaria decumbens* (mg per pot^{-1}) grown in contaminated soil and under different treatments with EDTA.

	Leaf			Root		
Metal	0	3 x 2[1]	1 x 6[2]	0	3 x 2	1 x 6
Ba	0.42 ^{NS} (±0.08)	0.56 ^{NS} (±0.14)	0.38 ^{NS} (±0.06)	0.11 ^{NS} (±0.01)	0.09 ^{NS} (±0.02)	0.09 ^{NS} (±0.02)
Pb	0.01 C (±0.00)	0.03 B (±0.01)	0.13 A (±0.05)	0.01 B (±0.00)	0.02 A (±0.00)	0.02 A (±0.00)

The values correspond to the mean of four replicates ± standard deviation. Different letters along the rows indicate significant differences (Tukey's test of 5% significance or less) in each plant part. 1: Treatment with three applications of EDTA at 2 mmol kg^{-1} soil. 2: Treatment with the application of a single dose of EDTA at 6 mmol kg^{-1} soil.

The IT and PE of Ba and Pb in *Brachiaria decumbens* in response to the application of EDTA are shown in Table 6.

Table 6. Index of translocation (IT) and phytoextraction efficiency (PE) in *Brachiaria decumbens* plants grown in contaminated soil and under different treatments with EDTA.

	IT		PE	
EDTA	Ba	Pb	Ba	Pb
(mmol kg^{-1}soil)	%			
0	77.0 (±3.69)	47.6 (±5.26)	0.0018	0.0009
3 x 2[1]	84.7 (±4.17)	53.4 (±8.40)	0.0021	0.003
1 x 6[2]	80.6 (±4.42)	86.9 (±1.77)	0.0016	0.005

The values correspond to the mean of four replicates ± standard deviation. 1: Treatment with three applications of EDTA at 2 mmol kg^{-1} soil. 2: Treatment with the application of a single dose of EDTA at 6 mmol kg^{-1} soil.

Pb demonstrated an IT greater than 85% in the treatment with the application of a single dose of EDTA. This finding contradicts that of Pulford and Watson (2003) who claim that there are two major limitations for the phytoextraction of Pb: 1) low bioavailability and 2) a low extent of translocation from roots to the leaves. Pichtel et al. (2000), working with various plant species, found limited mobility of Pb in the plants. However, Santos et al. (2006) report that the presence of EDTA favours an increase in the Pb content in the leaves.

Despite the increased efficiency of Pb phytoextraction with the addition of EDTA when compared to controls, especially in treatments with a single-dose application, the values obtained in the present study are much lower than those reported by Huang et al. (1997) who state that greater than 1% of the total metal in the soil should be absorbed by the aerial parts for phytoextraction to be economically feasible. Similar results were observed by Neugschwandtner et al. (2008) who studied EDTA-induced the phytoextraction of Pb in corn.

The results of the present study suggest that the application of a single dose of EDTA enhanced the efficiency of Pb accumulation and translocation to the aerial plant parts. Similar results were reported by Neugschwandtner et al. (2008).

Conclusion

The application of EDTA did not influence the absorption of Ba in plants.

The application of EDTA caused an increase in the concentration of Pb in plants.

The application of EDTA increased the rate of Pb translocation to the aerial parts.

The efficiency of Pb phytoextraction was low, and under the experimental conditions, *Brachiaria decumbens* does not have the potential to be used in phytoextraction programs.

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