

SELECTION OF SPECIES FOR PHYTOREMEDIATION OF ENVIRONMENTS CONTAMINATED BY BARIUM UNDER LOW REDOX POTENTIAL

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Abstract: The growing and intensive exploration activity of oil and gas has considerably increased the use of Barium Sulfate ($BaSO_4$) and consequently the amount of barium salts (Ba^{2+}) dispersed in the environment. Given this paradigm, the present research aimed to select plant species that have tolerance and ability to phytoremediate flooded environments contaminated by the heavy metal Barium (Ba). 10 Species were pre-selected: two rice varieties (*Oryza sativa*; IRGA 424 and IRGA Br. Tropical); junco (*Eleocharis interstincta*); braquiária (*Fuirena umbellata*); braquiarião (*Urochloa brizantha*); papiro (*Nephrolepsis cf. rivularis*), samambaia (*Nephrolepsis cf. rivulares*), junco (*Eleocharis acutangula* 1), junco (*Eleocharis acutangula* 2) and cattail (*Thypha domingensis*). The treatments presented six levels of ($BaCl_2$) and were kept under water depths. The *C. cf. papyrus* presented the best accumulation rates for leaf analysis, showing a general average of 12.34 mg of accumulated Ba^{2+} . As for the roots, the *T. domingensis* stood out with the best cumulative rates, reaching an Average of 45.48 mg of Ba^{2+} . In the plant as a whole, the species: *T. domingensis* was also the one that accumulated the most Ba^{2+} in its structure, with an average of 56.35 mg of accumulated Ba^{2+} . The species: *N. cf. rivulares* showed a high degree of sensitivity to ($BaCl_2$) levels, reaching death during the course of the phytoremediation assay. The Species that showed more tolerance and aptitude to extract and accumulate Ba^{2+} was the *T. domingensis*. The *C. cf. papyrus* ranked second, but showed a marked difference in relation to *T. domingensis*. The other Species reported relatively close values that ranged from 2.83 mg to 12.51 mg of extracted and accumulated Ba^{2+} . The Species: *N. cf. rivulares* showed high sensitivity to the contaminant, not being indicated for phytoremediation programs of this nature.

Keywords: Selection, phytoremediation, barium, redox potential.

INTRODUCTION

The growing industrial activity, which intensified mainly in the post-war period, has left an environmental liability that is getting worse every day with the emergence of increasingly toxic waste, whose consequences for the environment and public health are still in good shape. unknown parts (COUTINHO & BARBOSA, 2007).

Currently, there has been a growing problem in relation to environmental contamination by heavy metals, from the most diverse industrial, agro-industrial and urban segments. Smelting, mining, use of sewage as agricultural fertilizer (CHAOUÏ et al., 1997), metallurgical activities (KEFALA et al., 1999), petrochemical activities (ULRICH et al., 2003) and textile industries are the main sources of contamination. of soil and water by heavy metals (WAIHUNG et al., 1999).

Faced with this reality and the environmental paradigm that has been gaining strength in recent decades, accompanied with good eyes by society, leaders from all over the world are under pressure to seek and debate new forms of approaches that can be applied to the sanitation of contaminated areas (COUTINHO & BARBOSA, 2007).

In view of the demand for new technologies for environmental decontamination, Bioremediation has emerged as a promising technique, due to its lower cost, simplicity in execution, less time demanded by the process, less interference in the environment, favorable aesthetics and mainly for presenting efficiency in decontamination (PIRES et al., 2003). Bioremediation is a technique that uses microorganisms and plants that are resistant and/or tolerant to certain toxic elements. It is worth mentioning that the term Bioremediation is normally used to refer to the

use of microorganisms, such as bacteria and fungi (SANTOS et al., 2007 & PROCÓPIO et al., 2009). But when there is the use of plant organisms, the name used is Phytoremediation, being the aspect that most attracts research and also the most used, defined according to the Environmental Protection Agency - EPA (2000), North American acronym, as “the use of plants, and the microorganisms associated with them, as an instrument for containment, isolation, removal or reduction of concentrations of contaminants in solid, liquid or gaseous media”.

Taking into account the advantages described by Procópio et al., (2009), in a document by Embrapa Tabuleiros Costeiros, on the use of Phytoremediation, the following benefits can be cited: greater savings compared to other methods, especially those of the ex situ type, the contaminant can be transformed into a less or even non-toxic compound, promote biological, physical and chemical improvements in the soil, through the incorporation of organic matter and the fixation of atmospheric nitrogen, minimize the erosive process caused by rain and wind due to vegetation cover, the implantation process is less impactful, presents favorable aesthetics, makes use of solar energy and is well regarded by society.

Although it is a new area of research in Brazil, the country has been showing potential, presenting an increasing number of studies and phytoremediation programs. It is not known for sure how much is spent on depollution measures in Brazil, but it is certain that the country has been evolving in relation to investments in the treatment of industrial, agricultural and urban waste, and this progress is closely linked to the emergence of laws stricter, more comprehensive inspections, both national and international and mainly due to the demands of an increasingly conscious society (PROCÓPIO

et al., 2009). In addition, Brazil has a great natural potential to be explored, due to the fact that it has the greatest existing plant biodiversity, with more than 55 thousand Species cataloged, equivalent to 22% of the world total (BRASIL, 2002). The country still has a tropical climate (hot and humid) that favors the development of microbiological activities that occur in the rhizosphere and that optimize the phytoremediation process (MARQUES et al., 2011).

As in the world, national research has had as its main target the recovery of areas contaminated by heavy metals, such as barium, which according to the National Environmental Council - CONAMA (2006) is one of the newest elements included in the list of metals that have great potential for soil and water contamination. Barium is represented by the symbol Ba, belonging to the class of Alkaline Earth Metals, included in family 2A, has 56 as atomic number and 137u as atomic mass and is found in solid form under ambient conditions. The same is naturally present in igneous and sedimentary rocks, but is not found in the free form, ionic form, but in the form of barite or barite, which is a natural mineral form of barium sulfate (BaSO_4) (ULRICH et al, 2003 & LIMA et al., 2012).

Barium sulfate is widely used as one of the components of drilling and prospecting fluids for oil and gas wells in the petrochemical industry. Some attributes such as low chemical mobility, its high density (4.2 g cm^{-3}), relative abundance and low extraction and processing costs make baryte the main source of barium and barium salts (LIMA et al., 2012). Also according to the author, the growing and intensive exploratory activity of oil and gas has considerably increased the use of barite and consequently the amount of barium salts (Ba^{2+}) dispersed in the environment.

Although barium is quite immobile and has

low availability, due to its low solubility in water (2.47 mg L⁻¹ at 25 °C), reducing conditions (-200 mV), alter the natural balance of the soil, which leads to a series of biological, physical, chemical and electrochemical transformations (PHILLIPS et al., 2003). Flooding conditions favor a decrease in the redox potential of the soil, increasing electrochemical exchanges, allowing for a greater release of (Ba²⁺) cations in the environment, enhancing its bioavailability (ULRICH et al., 2003).

However, studies directly related to Phytoremediation of environments contaminated by the chemical element barium are still very scarce, especially with regard to Brazilian ecosystems, a marked deficit when the condition of flooding is added to the investigative process. Due to the premise and the scarcity of research that uses Phytoremediation in the recovery of areas contaminated by barium and that have a high electrochemical potential, there is a real need to implement specific and judicious studies that seek to make use of this biotechnological tool in the recovery of environments. that exhibit these characteristics. Therefore, the present research aimed to select plant species that have tolerance and ability to phytoremediate flooded environments contaminated by the heavy metal Barium (Ba).

MATERIAL AND METHODS

The experiment was installed in a greenhouse, located at the Experimental Farm of the Centro Universitário Norte do Espírito Santo, Federal University of Espírito Santo – CEUNES – UFES. Located in the municipality of São Mateus, in the extreme north of the State of Espírito Santo, on the geographic coordinates: 18° 40' 19" S and 39° 51' 13" W, at 35 m altitude. The experimental assay was developed under the factorial scheme of 10 x 6 x 3, being represented by 10 plant species, followed by a control and 05 increasing

doses of barium chloride (BaCl₂), with 03 repetitions, totaling 180 treatments, carried out in a entirely randomized – DIC.

Species with natural adaptation to flooded sites were pre-selected, in order to verify the tolerance of the Species to barium, under flooding conditions, in order to infer about their potential to be used in phytoremediation programs. The previously chosen Species were based on information obtained in the scientific literature, natural occurrence in places contaminated by barium and natural adaptation to flooded places.

The pre-elected Species were two rice varieties (*Oryza sativa*; IRGA 424 and IRGA Br. Tropical); rush: (*Eleocharis interstincta*); braquiária (*Fuirena umbellata*); braquiaraão (*Urochloa brizantha*); papyrus (*Nephrolepis cf. rivularis*) and fern (*Nephrolepis cf. rivulares*). The reed (*Eleocharis acutangula*) represented two treatments in the same experiment, being classified as: *E. acutangula* 1 and *E. acutangula* 2, differentiated by the origin of the Species collection. The first was collected on the banks of the Cricaré River and the BR 101 north, São Mateus - ES, and the second was collected in a site contaminated by Barium, from where the cattail was also removed. (*Thypha domingensis*).

As the definitive substrate for the establishment of Species in a greenhouse, soil samples from horizonta A, 0-20 cm deep, were used, which later went through a process of sieving in a mesh of 04 mm. A small part of the substrate was sieved through a 1.0 cm mesh to obtain Terra Fina Air Dry (TFSA), used in the physicochemical characterization. The soil was packed in polyethylene pots, 15 kg of soil were used in each container, in total 180 pots with a capacity of 20 L were used. according to Species, levels and repetitions.

The barium chloride solution (BaCl₂) was prepared in the Laboratory of Soil and Leaf Analysis – LAGRO, located in the Postgraduate

Building in Tropical Agriculture – CEUNES – UFES. 10,000 mg/L of Ba²⁺ (17786.4 mg/L of BaCl₂·2H₂O) were prepared by dissolving 17786.4 g, molecular weight of the BaCl₂ salt, in a 1000 mL volumetric flask. Table 01 shows the concentrations of (BaCl₂) added to the different treatments from the aforementioned solution.

As one of the research objectives was to analyze the effects of barium associated with flooded environments, a water depth of 1.0 cm was maintained under each experimental unit, in order to simulate a flooded area. Water replacement was performed whenever necessary, in order to keep the water volume constant.

At the end of the period established for the selective assay, the area part was cut and the roots were extracted for each treatment. To establish the amount of dry matter for shoots and roots, they were taken to a forced air circulation oven (65 ± 2 °C), Fanen model 320, for 72 hours, followed by a precision balance, Bioprecisa. model JH2102, to determine the biomass. Each treatment was submitted separately to the mechanical grinding process, using a Willye macro mill, model TE-650, and later stored in properly identified polyethylene bags. Aliquots were taken from the stored samples and analytical quantification of barium levels was performed according to USEPA 3051 using ICP OES. With the results in hand, the Barium Averages found in plant tissues were subjected to analysis of variance and the Average Scott Knott test, at 5% significance, using the SISVAR 5.3 Build 77 softwares.

RESULTS AND DISCUSSION

The species evaluated differed in terms of the rate of barium extracted and accumulated in different parts of the plant organism. The Species: *N. cf. rivulares* showed a high degree of sensitivity to (BaCl₂) levels, even dying

during the course of the phytoremediation assay, so it was not possible to evaluate it analytically.

According to Suwa et al. (2008), high levels of barium have a negative influence on the photosynthetic apparatus, stomatal conductance, intercellular concentration of carbon dioxide and transpiration rate, evidencing the toxicological effect of the element. In one of the pioneering studies carried out with the presence of barium, the death of the *Phaseolus vulgaris* after 96 hours of exposure to concentrations of 100 mmol L⁻¹ of Ba²⁺ (WALLACE & ROMNEY, 1971). In a more recent study using the same culture, it was reported that in addition to the decrease in bean growth, there was an inhibition of potassium absorption at concentrations of 500 mmol L⁻¹ of Ba²⁺ (LLUGANY et al., 2000).

The other Species were systematically evaluated, showing differences in the rates of barium accumulated in the leaves. Although (BaCl₂) was not added to the control treatment, level zero, it showed an amount of Ba²⁺ when analyzed. This is due to the natural content of Ba²⁺ present in the soil, and the only Species that showed a statistical difference was the *T. domingensis*, with a much higher value compared to other Species, this is explained by the fact that the *T. domingensis* have been collected at an oil extraction site, flooded and contaminated by barium sulfate (BaSO₄), thus presenting naturally higher rates of Ba²⁺. At dose D2, corresponding to 2.5 mg of barium, the *C. vf. Papyrus* and *T. domingensis* presented the highest cumulative rates, the other Species did not differ statistically. For the dose: D3, the *C. vf. Papyrus* stood out with 5.46 mg of accumulated Ba²⁺, followed by *T. domingensis*. In D4 and D5, the *C. vf. Papyrus* continued to show the highest cumulative levels, still followed by the *T. domingensis*, but at the last level the process was reversed, the *T. domingensis* accumulated more than

CONCENTRATIONS	TREATMENTS					
	0	1	2	3	4	5
mg of (BaCl ₂) / 13(mg/vaso)	0	2,5	5,0	15	30	65
mL of solution* (10.000 mg/L)	0	4,00	8,00	24,00	48,00	96,00

* A 500 mL beaker was used to apply the solution.

Table 01: (BaCl₂) concentrations added to different treatments.

Species	Barium concentrations added to the soil (mg)						
	0	2,5	5	15	30	65	Average
<i>O. sativa</i> (IRGA 424)	0,19 B	0,31 B	0,52 D	0,72 D	1,42 E	2,06 G	0,87
<i>O. sativa</i> (IRGA Br. Tro.)	0,11 B	0,31 B	0,54 D	0,65 D	1,08 E	2,6 G	0,88
<i>U. brizantha</i>	0,08 B	0,31 B	0,33 D	0,47 D	1,47 E	4,44 F	1,18
<i>F. umbellata</i>	0,63 B	0,97 B	2,4 C	3,15 C	5,73 C	15,83 C	4,78
<i>E. acutangula</i> 2	0,24 B	0,64 B	0,72 D	1,66 D	4,79 C	6,27 E	2,39
<i>E. acutangula</i> 1	0,22 B	0,45 B	1,41 C	3,86 C	3,65 D	10,85 D	3,41
<i>E. interstincta</i>	0,33 B	0,53 B	1,12 D	1,25 D	7,43 B	11,08 D	3,62
<i>C. vf. papyrus</i>	0,91 B	3,16 A	5,46 A	8,71 A	19,92 A	35,91 B	12,34
<i>T. domingensis</i>	2,45 A	2,95 A	3,71 B	7,5 B	8,33 B	40,29 A	10,87
Average	0,57	1,07	1,80	3,11	5,98	14,37	4,48

CV = 16,07%

Averages not followed by the same letter, uppercase on the vertical and lowercase on the horizon, differ by the Scott Knott test at 5% probability.

Table 02: Barium concentrations in leaves after analytical quantification (mg).

C. cf. Papyrus. In general terms, the Species that accumulated the most Ba²⁺ in the leaves was *C. cf. Papyrus*, soon after was the *T. domingensis* followed in descending order of accumulation by: *F. umbellata*, *E. interstincta*, *E. acutangula* 1, *E. acutangula* 2, *U. brizantha*, *O. sativa* (IRGA Br. Tropical) and *O. sativa* (IRGA 424), as reported in (Table 02).

According to the National Environmental Council – CONOMA (2009), concentrations above 75 mg kg⁻¹ and mg L⁻¹, for soil and water respectively, are already considered contaminated. Figure 01 shows graphs with their respective regression equations, informing the concentrations of Ba²⁺ extracted and accumulated in the leaves in relation to each level of (BaCl₂) added to the soil. A *C. cf. papyrus* presented the best rates of accumulation for levels D2, D3, D4 and D5, presenting itself as a good alternative for the sanitation of contaminated areas, since its structure presents the highest rates of accumulation in the leaves, with the exception of level D6. According to Marques et al., (2011) it is preferable that the phytoremediating species present the highest cumulative rates in the area, as it facilitates their management and eradication, if necessary. At the last level, equivalent to 65 mg of (BaCl₂), a *T. domingensis* showed greater accumulation, 40,29 mg of Ba²⁺.

The concentrations of Ba²⁺ extracted and accumulated in the roots were markedly higher in the *T. domingensis*, showing an overall average of 45.48 mg of accumulated Ba²⁺. In terms of comparison, the Species that showed the second highest average of accumulation was *E. acutangula* 1, with 9.10 mg of Ba²⁺ and the one that extracted and accumulated the least was *U. brizantha*, with 1.65 mg of analytically quantified Ba²⁺, according to data from (Table 03).

Figure 02 shows the cumulative rates for the roots in each Species. *T. domingensis*

showed the highest cumulative values.

As a whole, using the sum of exposed values for accumulation in leaves and roots, the species that presented the best results in terms of accumulated Ba²⁺ rates was *T. domingensis*, which presented a general average of 56.35 mg of Ba²⁺ accumulated in the whole plant. Value well above the second place, which accumulated a total of 14.75 mg of Ba²⁺, this in parameter of Average inferred by the sum and division of the six levels of Barium present in each treatment. Overall, the lowest average accumulation, 2.83 mg of Ba²⁺ belongs to the *U. brizantha* e as Species *E. acutangula* 2, *O. sativa* (IRGA 424), *F. umbellata*, *O. sativa* (IRGA Br. Tropical), *E. interstincta* and *E. acutangula* 1, have cumulative rates of 5.11; 5.51; 9.62; 9.84; 11.14 and 12.51 mg of Ba²⁺ respectively, according to data from (Table 04).

Due to the lack of studies related to barium extraction and accumulation rates for all the species mentioned above, it is not possible to compare the data obtained in this work with research by other authors. But in an investigative study on the phytoremediation capacity of *O. sativa* towards barium sulfate (BaSO₄), it was reported that there was a cumulative content of 7.8 mg vase⁻¹ of Ba²⁺ in the whole plant, at the level of dose D1, equivalent to 100 mg kg⁻¹ of (BaSO₄). At the D2 level, corresponding to 300 mg kg⁻¹ of (BaSO₄), a total of 12.6 mg of Ba²⁺ vessel⁻¹ was accumulated and at the last level of 3000 mg kg⁻¹ of (BaSO₄), 22 were accumulated, 4 mg of Ba²⁺, and this study was carried out under two conditions: in one, *O. sativa* was implanted in a soil with 70% field capacity and in the second condition, the soil presented a water depth to simulate the effects resulting from reducing conditions, and the data presented above were from the reducing condition (LIMA et al., 2012).

In another study developed by Magalhães

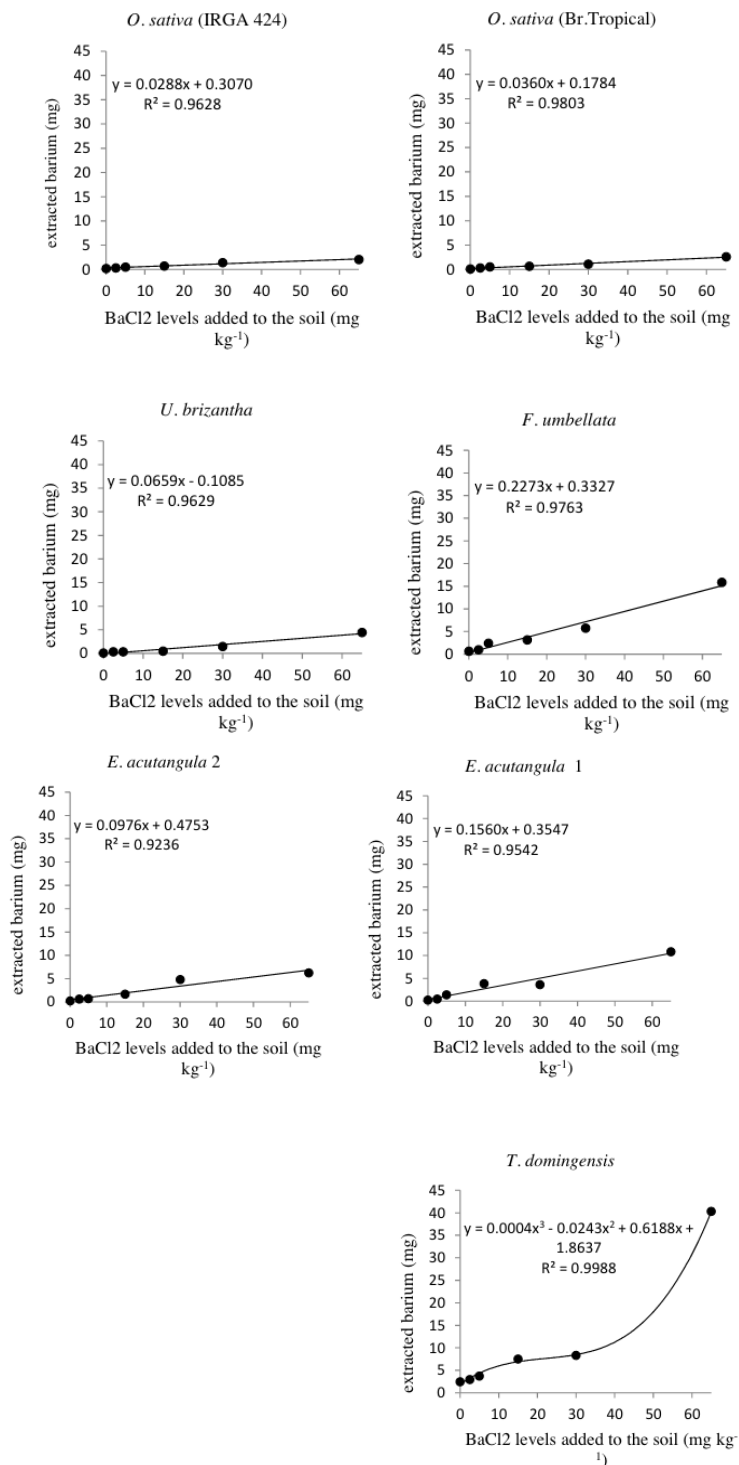


Figure 01: Amount of Ba²⁺ accumulated in leaves in relation to increasing levels of (BaCl₂) added.

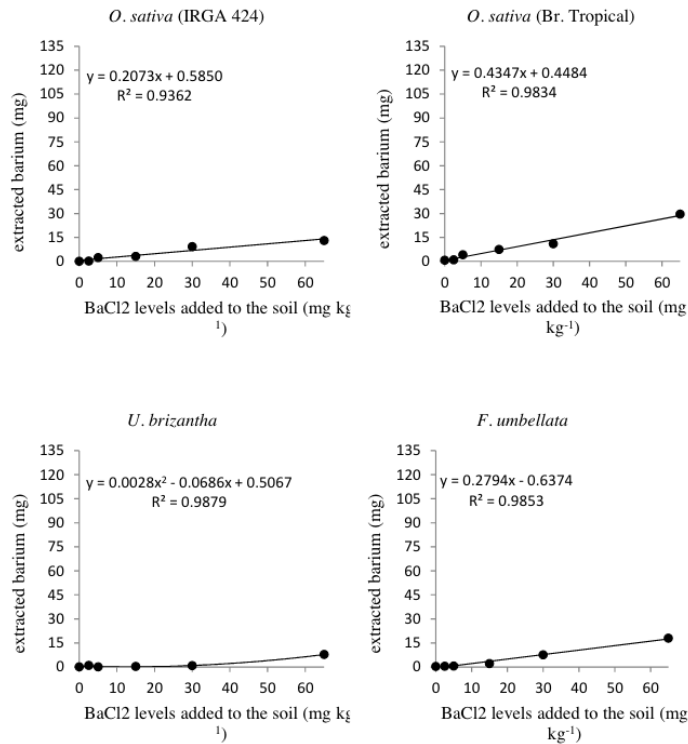
Concentrations of (BaCl₂) added to the soil (mg)

Species	0	2,5	5	15	30	65	Average
<i>O. sativa</i> (IRGA 424)	0,08 B	0,12 B	2,3 C	3,05 C	9,24 D	13,07 E	4,64
<i>O. sativa</i> (IRGA Br. Tro.)	0,62 B	0,9 B	4,15 B	7,42 B	11,04 C	29,64 B	8,96
<i>U. brizantha</i>	0,08 B	0,89 B	0,08 C	0,28 C	0,78 E	7,8 F	1,65
<i>F. umbellata</i>	0,36 B	0,48 B	0,61 C	2,09 C	7,51 D	17,97 D	4,83
<i>E. acutangula</i> 2	0,65 B	0,96 B	1,11 C	1,67 C	2,04 E	9,87 F	2,72
<i>E. acutangula</i> 1	1,3 B	3,65 B	6,29 B	4,62 B	15,76 B	22,98 C	9,10
<i>E. interstincta</i>	0,25 B	0,4 B	1,03 C	2,58 C	18,74 B	22,12 C	7,52
<i>C. vf. Papyrus</i>	1,04 B	1,85 B	2,95 C	2,35 C	3,64 E	2,59 G	2,40
<i>T. domingensis</i>	19,42 A	17,1 A	15,63 A	31,31 A	55,46 A	133,97 A	45,48
Average	2,65	2,93	3,79	6,15	13,80	28,89	9,70

CV = 24,22%

Averages not followed by the same letter, uppercase vertically and lowercase horizontally, differ by the Scott Knott test at 5% probability.

Table 03: Barium concentrations in roots after analytical quantification (mg).



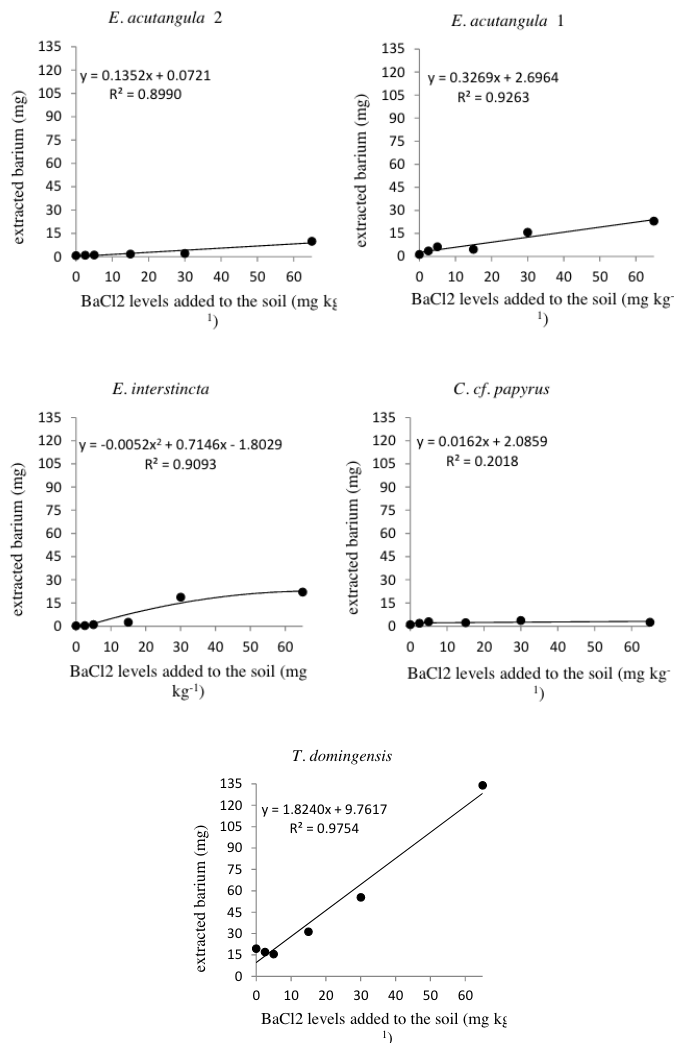


Figure 02: Amount of Ba+2 accumulated in the roots in relation to increasing levels of (BaCl2) added.

Species	Concentrations of (BaCl2) added to the soil (mg)						Average
	0	2,5	5	15	30	65	
<i>O. sativa</i> (IRGA 424)	0,27 B	0,43 B	2,82 C	3,78 C	10,66 D	15,13 D	5,51
<i>O. sativa</i> (IRGA Br. Tro.)	0,73 B	1,21 B	4,69 C	8,07 B	12,12 D	32,24 C	9,84
<i>U. brizantha</i>	0,16 B	1,2 B	0,41 C	0,75 C	2,25 F	12,24 D	2,83
<i>F. umbellata</i>	0,99 B	1,45 B	3,01 C	5,24 C	13,23 D	33,8 C	9,62
<i>E. acutangula 2</i>	0,9 B	1,6 B	1,84 C	3,33 C	6,84 E	16,14 D	5,11
<i>E. acutangula 1</i>	1,53 B	4,1 B	7,7 B	8,48 B	19,41 C	33,83 C	12,51
<i>E. interstincta</i>	0,59 B	0,93 B	2,15 C	3,83 C	26,17 B	33,19 C	11,14
<i>C. vf. Papyrus</i>	1,95 B	5,01 B	8,41 B	11,06 B	23,56 B	38,5 B	14,75
<i>T. domingensis</i>	21,87 A	20,05 A	19,34 A	38,81 A	63,79 A	174,26 A	56,35
Average	3,22	4,00	5,60	9,26	19,78	43,26	14,19

CV = 17,49%

Means not followed by the same letter, uppercase vertically and lowercase horizontally, differ by the Scott Knott test at 5% probability.

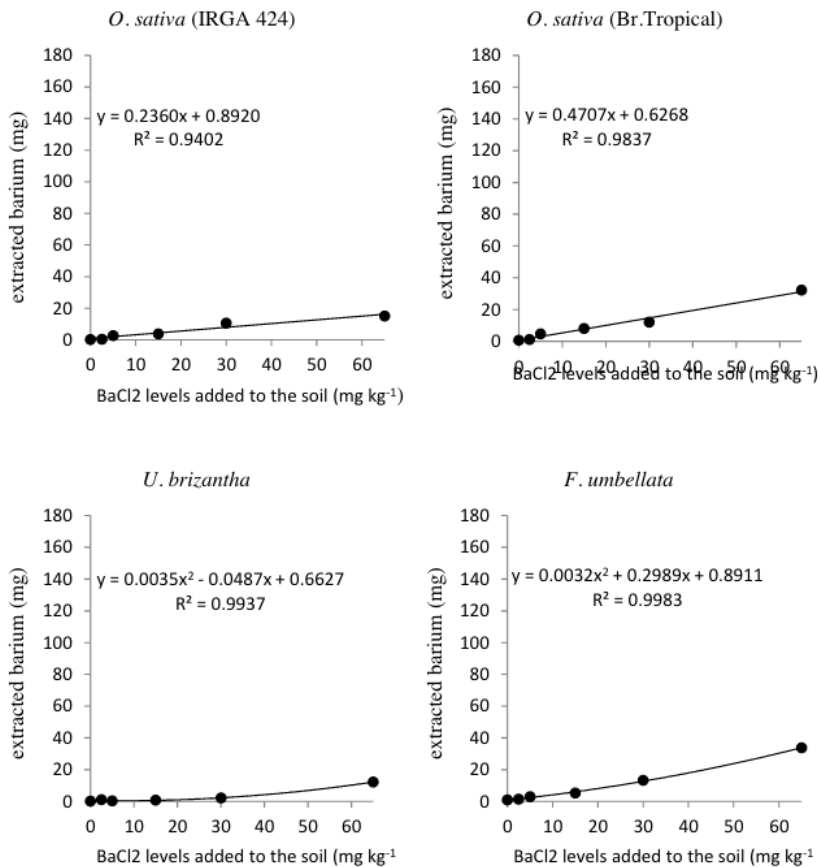
Table 04: Barium concentrations in plants after analytical quantification (mg).

et al., (2011), with the objective of evaluating the possible solubilization of barium sulfate (BaSO₄) in soils under reducing conditions and increased bioavailability of barium, a direct relationship was observed between the increase in uptake of Ba⁺² by plants and the decrease in redox potential, that is, the lower the redox potential, the more Ba⁺² was accumulated by *O. sativa*. A more recent study by Magalhães et al., (2014), showed that reducing soil conditions favor an increase in barium levels in the forms of greater lability and a decrease in the forms of greater stability. The highest levels of barium accumulation in leaves, roots, and grains were found in the highest dose and in the reduction condition, and these results showed that the reduction condition provided greater bioavailability of this element.

Figure 03 shows the graphs for the total accumulation of Ba⁺², once again *T. domingensis* stood out with the highest cumulative rates.

CONCLUSION

The species that showed the most tolerance and aptitude for extracting and accumulating Ba⁺² was *T. domingensis*. The *C. cf. papyrus* ranked second, but showed a marked difference in relation to *T. domingensis*. The other species reported relatively close values that ranged from 2.83 mg to 12.51 mg of extracted and accumulated Ba⁺². The species: *N. cf. rivulares* showed high sensitivity to the contaminant, not being indicated for phytoremediation programs of this nature.



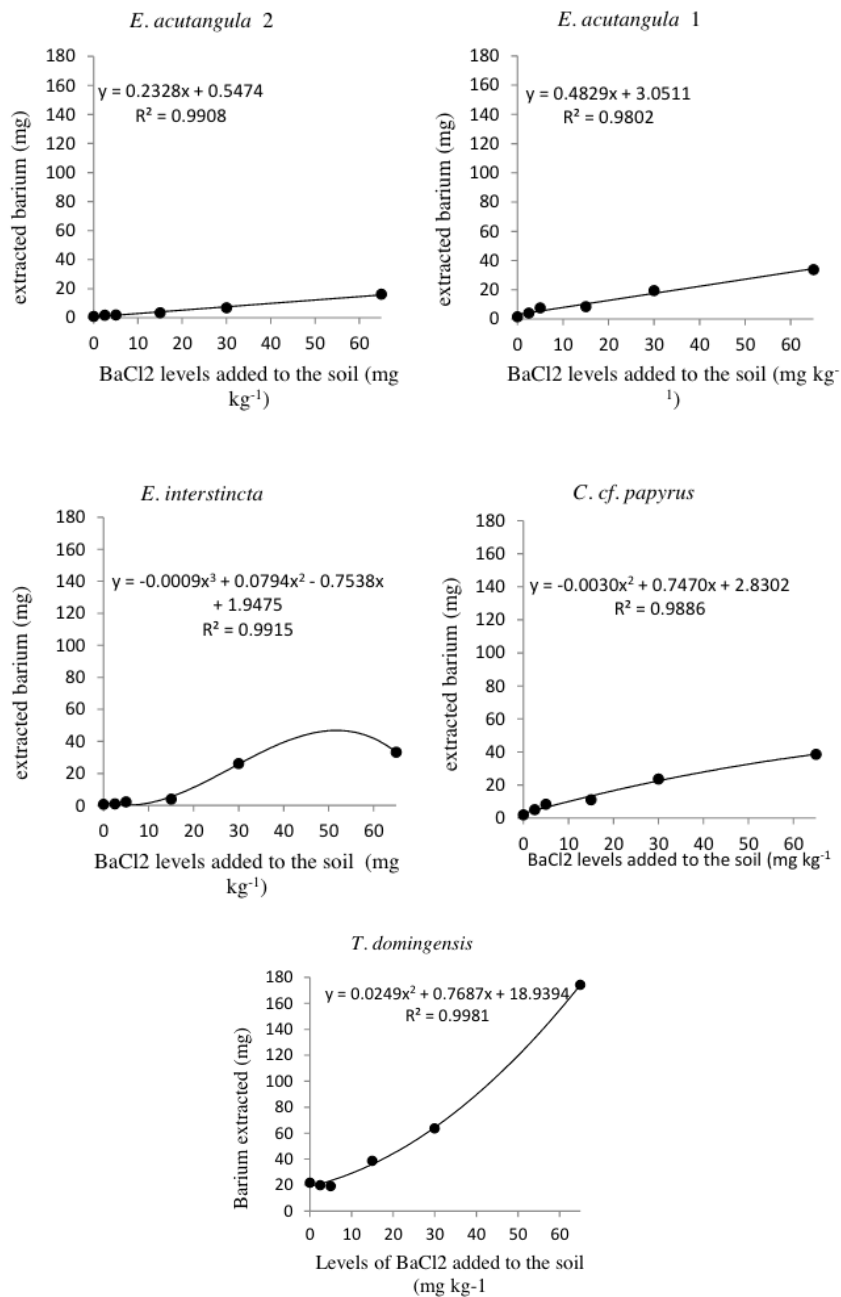


Figure 03: Amount of total Ba⁺² accumulated in leaves and roots in relation to increasing levels of (BaCl₂) added.

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