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Growth, tolerance and zinc accumulation in *Senna multijuga* and *Erythrina crista-galli* seedlings

Douglas L. Scheid[1], Rudinei D. Marco[2], André L. Grolli[2], Rodrigo F. da Silva[2], Clovis O. Da Ros[2] & Robson Andreazza[3]

[1] Universidade Federal de Santa Maria/Departamento de Solos. Santa Maria, RS. E-mail: douglasscheid@gmail.com (Corresponding author)
[2] Universidade Federal de Santa Maria/Campus de Frederico Westphalen/Departamento de Ciências Agronômicas e Ambientais. Frederico Westphalen, RS. E-mail: rudineidemarco@hotmail.com; andregrolli1990@hotmail.com; rodrigosilva@smail.ufsm.br; clovisdaros@gmail.com
[3] Universidade Federal de Pelotas/Centro de Engenharias. Pelotas, RS. E-mail: robsonandreazza@yahoo.com.br

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A B S T R A C T

Zinc (Zn) is a micronutrient that is reaching toxic levels in the soil, with the intensification of agricultural and industrial activities. The objective of this study was to evaluate the growth, accumulation and tolerance of *Erythrina crista-galli* and *Senna multijuga* seedlings in soil with addition of increasing Zn levels. The study was conducted in a greenhouse for 120 days, using a completely randomized design in a 2 x 6 factorial arrangement, corresponding to two tree species (*S. multijuga* and *E. crista-galli*) and six doses of zinc in the soil (0, 200, 400, 600, 800 and 1000 mg kg^{-1}) with six replicates. *E. crista-galli* and *S. multijuga* seedlings decreased root and shoot dry weight with increasing Zn doses. *E. crista-galli* and *S. multijuga* have low Zn translocation index and are capable to phytostabilize Zn in the roots. *E. crista-galli* had greater tolerance to Zn compared with *S. multijuga*. The species have potential for Zn phytostabilization programs in contaminated soil.

Palavras-chave:
espécies arbóreas nativas
metal pesado
contaminação do solo
fitoestabilização

Crescimento, tolerância e acúmulo de zinco em mudas de *Senna multijuga* e *Erythrina crista-galli*

R E S U M O

O zinco é um micronutriente que está atingindo níveis tóxicos no solo, com a intensificação das atividades agrícolas e industriais. O objetivo deste trabalho foi avaliar o crescimento, o acúmulo e a tolerância de mudas de *Erythrina crista-galli* e *Senna multijuga* em solo com adição de níveis crescentes de zinco. O trabalho foi conduzido em casa de vegetação por 120 dias, utilizando delineamento experimental inteiramente casualizado em arranjo fatorial 2 x 6 sendo duas espécies arbóreas (*S. multijuga* e *E. crista-galli*) e seis doses de zinco no solo (0, 200, 400, 600, 800 e 1000 mg kg^{-1}), com seis repetições. Houve redução na massa seca radicular e aérea com o aumento das doses de zinco nas mudas de *E. crista-galli* e a *S. multijuga*. As espécies apresentaram baixa translocação de zinco e têm capacidade de fitoestabilizar o metal no sistema radicular. A espécie *E. crista-galli* apresenta maior tolerância às doses de zinco que a *S. multijuga*. As espécies apresentam potencial de utilização em programas de fitoestabilização de Zn em solo contaminado.

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Introduction

Zinc (Zn) is a potentially polluting heavy metal, but it is a micronutrient that acts as enzymatic cofactor, in the maintenance of biomembrane integrity, metabolism of carbohydrates and in the synthesis of proteins (Broadley et al., 2007; Hooda, 2010). However, Zn contents between 100 and 500 ppm in the soil can be extremely phytotoxic (Kabata-Pendias, 2011).

Soil contamination by Zn results from inadequate application of agricultural pesticides, sewage sludge and animal waste, and from the intensification of industrial activities (Hooda, 2010; Kabata-Pendias, 2011). According to the National Environmental Council (CONAMA, 2009), in agricultural soils of Brazil, Zn contents above 450 mg kg^{-1} pose potential risks, direct or indirect, to human health. In this context, soils with high contents of this chemical element require remediation actions to avoid problems for human health.

Phytoremediation is an alternative to recover contaminated areas, but there are only few studies involving tree species native to Brazil for phytoremediation of contaminated soils. According to Domínguez et al. (2009), the utilization of tree species is a strategy to recover areas contaminated with metals, because they have large biomass production along the growth cycle. According to Magalhães et al. (2011), another advantage of using tree species is that they are able to absorb and retain metals in the roots, restricting their transport to the leaves, which is an interesting feature for projects of reclamation of contaminated areas.

The long life cycle of tree species causes the studies with seedlings to be the fastest and easiest way to determine the ability of different tree species to tolerate and survive in contaminated soils (Pulford & Watson, 2003). In addition, young plants are more sensitive, compared to adult plants, to the adverse conditions imposed by metals (Souza et al., 2012). However, tree species have different responses to soil contamination (Souza et al., 2012; Gomes et al., 2013), reinforcing the need for studies with tree species in soils contaminated by Zn.

Regarding native tree species, 'pau-cigarra' (*Senna multijuga* (Rich.) H. S. Irwin & Barneby) and 'corticeira-do-banhado' (*Erythrina crista-galli* L.) are pioneer species of the Fabaceae family, with fast to moderate growth. Thus, these species may exhibit feature for Zn phytoremediation, such as tolerance and accumulation of the metal. Therefore, this study aimed to evaluate the initial growth, tolerance and Zn accumulation in *Senna multijuga* and *Erythrina crista-galli* seedlings.

Material and Methods

The experiment was carried out in a climate-controlled greenhouse (temperature of 28 °C, relative humidity of 60%) of the Frederico Westphalen Agricultural School – RS, Brazil. The soil used in the experiment was collected at the Federal University of Santa Maria, Campus Frederico Westphalen, classified as Rhodic Hapludox and its physical and chemical analyses are presented in Table 1, according to the methodology described by Tedesco et al. (1995).

Seeds of the studied tree species, *Senna multijuga* (Rich.) H. S. Irwin & Barneby and *Erythrina crista-galli* L., were provided by the Center of Forest Research of the State Foundation of Agricultural Research (FEPAGRO), unit of Santa Maria - RS. Tegument dormancy was overcome with immersion of the seeds, respectively, for 15 and 30 min in sulfuric acid, and washing in running water. Sowing was performed in trays and, when the seedlings showed one pair of true leaves, they were selected and transplanted to polyethylene plastic bags with volumetric capacity of 600 cm^3. Each plastic bag was considered as one experimental unit.

The experimental design was completely randomized in 2 x 6 factorial arrangement, corresponding to two tree species (*S. multijuga* and *E. crista-galli*) and six Zn doses added to the soil (0, 200, 400, 600, 800 and 1000 mg kg^{-1}), with six replicates. Zn doses were applied 30 days before seedlings transplantation for the stabilization of the chemical reactions between the soil and the contaminant, in the form of solution of Zn acetate dihydrate ($C_4H_6O_4Zn.2H_2O$). The contaminated soil was sampled for the determination of the pseudo-total Zn contents, according to the methodology 3050b described by USEPA (1996).

The experiment was carried out for 120 days after transplantation of the seedlings. Along this period, irrigations were applied based on the weight of the experimental units, maintaining soil moisture at approximately 80% of field capacity.

Fertilizations were applied before sowing using urea, triple phosphate and potassium chloride and applying 150 mg of N, 700 mg of P_2O_5 and 100 mg of K_2O per dm^3 of soil and, as top-dressing, using 20 mg of N and 15 mg of K_2O, diluted in water and applied as 50 mL $seedling^{-1}$. Three top-dressing fertilizations were applied: at 30 days after seedling transplantation, N and K: at 60 days, N, and at 90 days, N and K, following the recommendations of Gonçalves & Benedetti (2005).

At the end of the experiment, root dry matter (RDM) and shoot dry matter (SDM) were determined. The seedlings were washed with distilled water, separated in the basal region, dried in an oven at 60 ± 1 °C until constant weight, and then weighed on analytical scale with precision of 0.0001 g. Total dry matter (TDM) was obtained by the sum of RDM and SDM and the methodology of Tennant (1975) was used to estimate the root specific surface area (SSA), according to the formula: SSA = $2\pi \times R \times L$, (L: root length; R: radius). Radius was calculated using the formula: R = V/L, in which V: volume (Root system fresh matter).

Table 1. Physical-chemical analysis of the soil used in the cultivation of *Erythrina crista-galli* and *Senna multijuga* seedlings

pH_{water}	Ca + Mg[(1)]	Al[(1)]	H + AL	P[(2)]	K[(2)]	$Zn_{soluble}$[(3)]	OM	Clay[(4)]
1:1	cmol$_c$ kg^{-1}			mg dm^{-3}			%	
5.2	4.23	0.3	5.34	2.2	61.5	1.45	1.2	65.0

pH: Determined in water (1:1); [(1)]Extractor: 1 mol L^{-1} KCl; H+Al determined by the SMP index; [(2)]Extractor: Mehlich-1. OM: Sulfochromic with external heat; [3] Extractor: 0.005 mol L^{-1} KCl; [4]Clay: Determined using a hydrometer after soil dispersion with sodium hydroxide

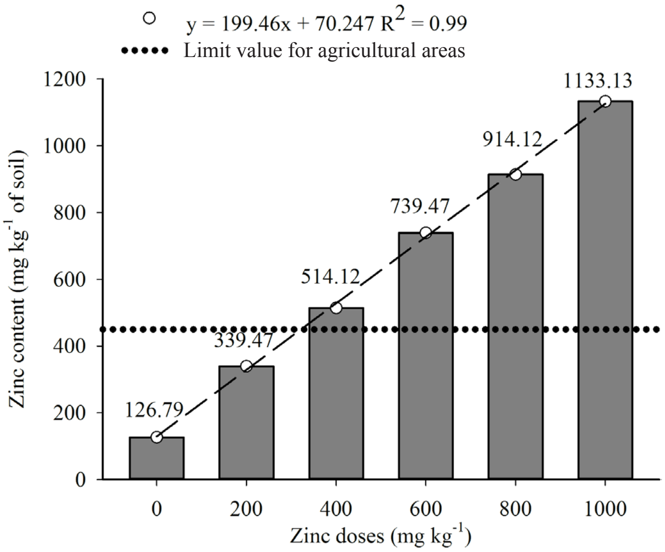
Root and shoot dry matters were ground in a Wiley-type mill (10-mesh sieve) to determine the Zn contents in the plant tissue, through nitric-perchloric digestion (3:1) and determined through atomic absorption spectrophotometry, as described by Tedesco et al. (1995).

TDM, Zn contents (mg kg^{-1}) in the root system (ZnR) and shoots (ZnS), accumulated amounts of Zn (μg plant^{-1}) in the root system (ZnAR), shoots (ZnAS) and total of the seedlings (ZnAT), at the dose of zero Zn (d_0) and the doses of 200 to 1000 mg kg^{-1} (d_n) were used to calculate the indices of tolerance (Itol = [TDMdn/TDMd0]×100) and translocation (Itra = [ZnASdn/ZnATdn]×100). Itol measures the ability of the seedlings to grow under high concentration of the metal (Wilkins, 1978), while Itra corresponds to the total absorbed percentage of Zn that was transported to the shoots (Abichequer & Bohnen, 1998).

The results were subjected to analysis of variance and, when the interaction was significant, they were subjected to regression analysis of the quantitative factor at each level of the qualitative factor, using the program SISVAR (Ferreira, 2011).

Results and Discussion

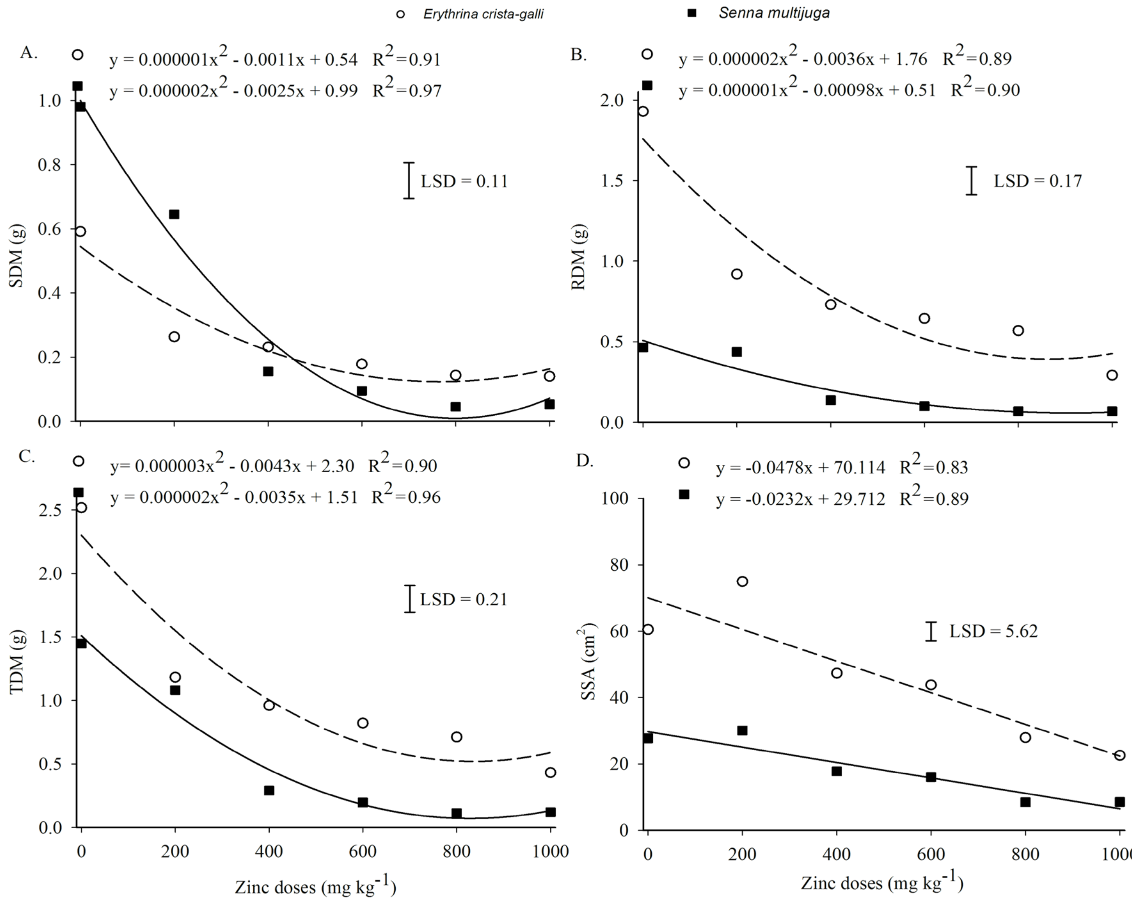
Zn doses in the soil increased the pseudo-total contents of the metal in the soil and, from 400 mg kg^{-1} on, these contents were higher than that established as maximum limit value allowed for agricultural soils by the resolution nº 420 (CONAMA, 2009), which is 450 mg kg^{-1} (Figure 1). Thus, the applied Zn doses were efficient to contaminate the soil, allowing the conduction of the experiment.



Limit value for agricultural area of 450 mg kg^{-1} of Zn in the soil, defined in the Resolution No. 420 (CONAMA, 2009)

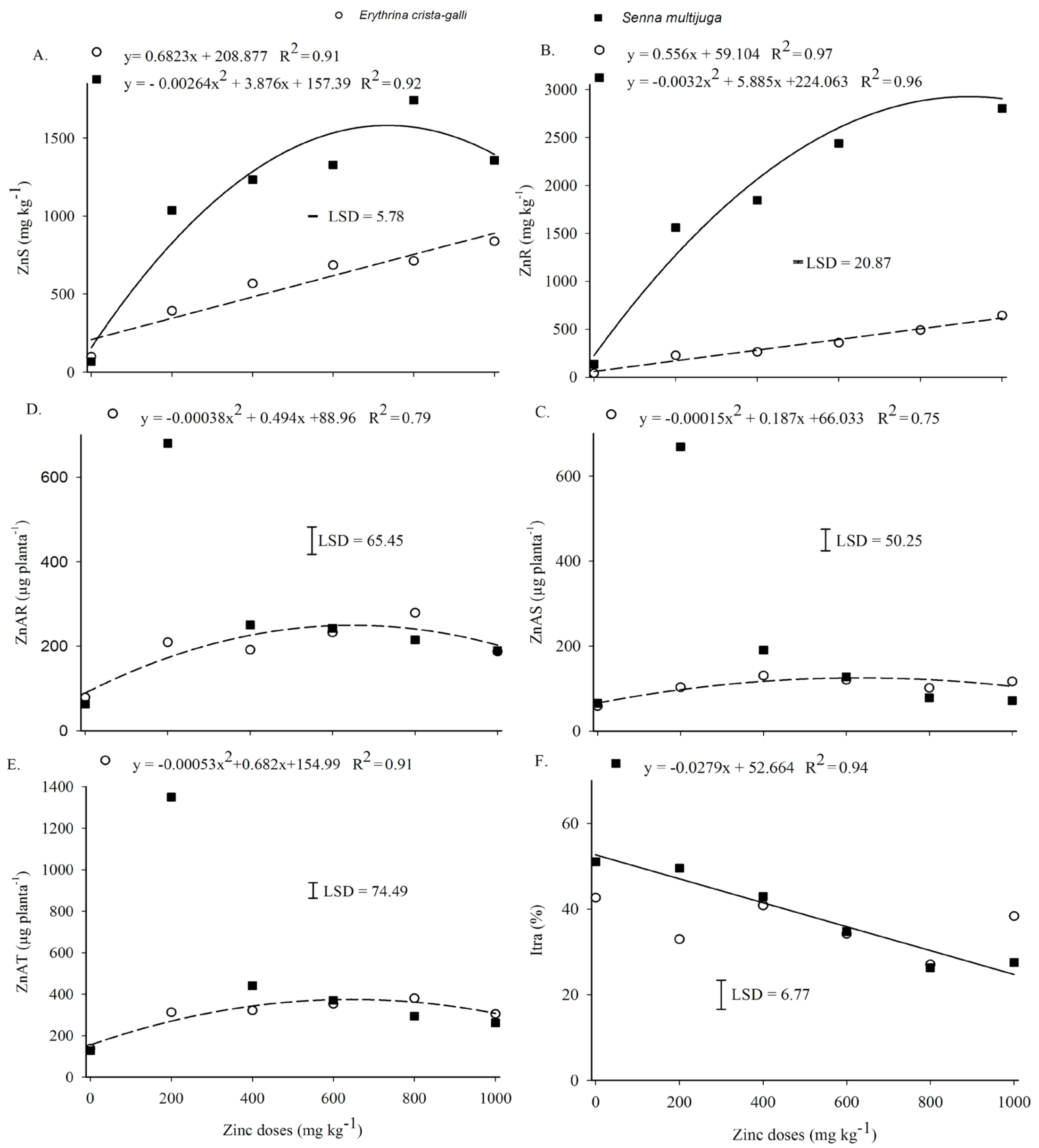
Figure 1. Pseudo-total contents of zinc as a function of zinc doses added to the soil, according to the methodology 3050b described by USEPA (1996)

The tree species and Zn doses applied in the soil showed significant interaction ($p \leq 0.05$) for all morphological and chemical parameters evaluated (Figures 2, 3 and 4). The Zn doses added to the soil reduced ($p \leq 0.05$) shoot dry matter



LSD: Least significant difference

Figure 2. Shoot dry matter - SDM (A), root dry matter - RDM (B), total dry matter - TDM (C) and specific superficial area - SSA (D) of *Erythrina crista-galli* and *Senna multijuga* seedlings cultivated in soil contaminated with zinc



LSD: Least significant difference

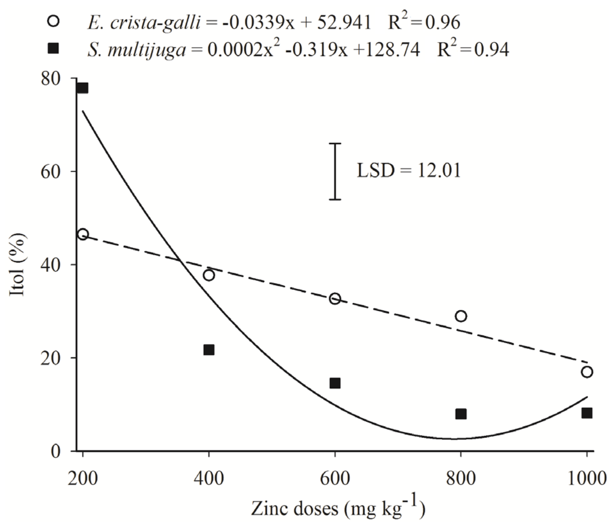
Figure 3. Regression equations for zinc contents in the shoots - ZnS (A) and roots - ZnR (B), zinc accumulated in the shoots - ZnAS (C), roots - ZnAR (D) and total - ZnAT (E) and index of translocation - Itra (F) in *Erythrina crista-galli* and *Senna multijuga* seedlings cultivated in soil contaminated with zinc

(SDM), root dry matter (RDM) and total dry matter (TDM) with minimum growth points of 550, 900 and 716.6 mg Zn kg^{-1} for *E. crista-galli* and 625, 490 and 875 mg Zn kg^{-1} for *S. multijuga*, respectively. *E. crista-galli* produced more RDM and TDM with the Zn doses applied to the soil, compared with *S. multijuga* (Figures 2A, B and C).

Zn excess causes phytotoxic effect on plants, resulting in growth inhibition, with occurrence of dwarfism, chlorosis and reduction in biomass yield (Kabata-Pendias, 2011). High Zn levels interfere with cell metabolism, induce injuries and physiological disorders, leading to reduction in the growth of the species, as reported by Luo et al. (2010). Although no symptoms of chlorosis were observed in *S. multijuga* and *E. crista-galli* seedlings, physiological alterations, such as iron deficiency with reduction in the synthesis of chlorophyll and degradation of the chloroplasts (Broadley et al., 2007), may have occurred, culminating in reduction of dry matter with the increase in the Zn doses added to the soil, and reduction in the amount of photoassimilates produced by the leaves, resulting in lower amount of biomass produced by the plants.

Biomass production may be related to the Zn contents in the root of each plant, since there may be damages to the roots with the reduction of growth (Li et al., 2012), thus decreasing the capacity of absorption of water and nutrients, as found by Kopittke et al. (2009) for copper in the species *Urochloa mosambicensis*.

Root specific superficial area linearly decreased with the increase of Zn doses in the soil, and *E. crista-galli* was significantly superior to *S. multijuga* at all Zn doses (Figure 2D). According to the estimated values, there were reductions of 68.2 and 78.1% in SSA at the dose of 1000 mg of Zn kg^{-1} of soil, in relation to the dose zero, for *E. crista-galli* and *S. multijuga*, respectively. In general, Zn excess in the soil



LSD: Least significant difference

Figure 4. Regression equation for the index of tolerance (Itol) of *Erythrina crista-galli* and *Senna multijuga* seedlings cultivated in soil contaminated with zinc

decreases root length (Hooda, 2010), which directly influences SSA. Shi et al. (2011) reported reduction of root length in the species *Vitex trifolia var. simplicifolia*, *Glochidion puberum*, *Broussonetia papyrifera* and *Styrax tonkinensis* cultivated in soil contaminated with Cu, Pb and Zn. In this context, the increase of Zn doses in the soil decreased root growth and SSA in plant species, possibly affecting their growth, for decreasing the capacity of absorption of water and nutrients.

Zn doses significantly influenced the contents of the metal in the shoots and roots of *S. multijuga* and *E. crista-galli*, and the contents were significantly superior in *S. multijuga* (Figures 3A, B). For *E. crista-galli*, Zn contents linearly increased in shoots and roots, while in *S. multijuga* they increased up to 734 and 919.5 mg kg^{-1} in the shoots and roots, respectively. Gomes et al. (2011) obtained increase of Zn contents in the shoots and roots with the increase in the amount of the metal in the soil with *Salix humboldtiana*. A similar result was found for Zn contents in the leaves of *Myracrodruon urundeuva* (Gomes et al., 2013). Hence, the results indicate that the species differ regarding the absorption and accumulation of Zn.

In the recovery of contaminated areas, it is interesting to use diversity of species but, since the studied species are pioneer, they can be used in the initial stage of the reclamation project. In addition, *E. crista-galli* and *S. multijuga* exhibit a characteristic of Zn phytoaccumulation in the roots.

The Zn amounts accumulated in shoots, roots and total differed between the species only at the dose of 200 mg kg^{-1} (Figures 3C, D and E). *E. crista-galli* showed maximum accumulation at 623.3 and 650 mg kg^{-1} for ZnS and ZnR, respectively. The regression for *S. multijuga* showed fit with $R^2 \leq 0.6$ (ZnAS = $-0.000595x^2 + 0.337x + 250.036$ $R^2 = 0.25$; ZnAR = $-0.00072x^2 + 0.607x + 232.68$ $R^2 = 0.18$; ZnAT = $-0.00132x^2 + 0.944x + 482.722$ $R^2 = 0.20$). The dose of 200 mg kg^{-1} showed higher values, because the accumulated amount of Zn is related to the quantity of dry matter produced by the plants and to the contents of the metal in their tissues. Roots of *Sesbania virgata*, Fabaceae family, accumulated larger amount of Zn compared to the shoots, even with reduction in the amount of biomass (Branzini et al., 2012).

The index of translocation was linearly reduced in *S. multijuga* with the increase of Zn doses in the soil (Figure 3F) and *E. crista-galli* showed Itra = $0.00002x^2 - 0.0265x + 41.969$ and $R^2 = 0.32$. On average, only 38.7 and 36% of the total absorbed of Zn were translocated to the shoots, respectively, for *S. multijuga* and *E. crista-galli*. However, the species did not show translocation index higher than 100, a value that characterizes species as fit for phytostabilization (Mendez & Maier, 2008), since most of the metal accumulated in the plant is retained in the roots. According to Gomes et al. (2011), the reduction in Zn translocation to the shoots in *Salix humboldtiana* is a survival strategy in soils with high contents of this metal, as observed by the author in soil contaminated with Zn. Thus, low translocation can be related to the resistance or to the survival strategy of the species in soil contaminated with Zn.

The tolerance index reduced linearly with the increase of Zn doses in the soil for *E. crista-galli* seedlings and there was a quadratic reduction with minimum point at 785.71 mg of Zn kg^{-1} of soil for *S. multijuga* (Figure 4). With 200 mg of Zn kg^{-1} of soil, *S. multijuga* was more tolerant than *E. crista-galli*; however, at doses of 400, 600 and 800 mg of Zn kg^{-1} of soil, *E. crista-galli* was significantly more tolerant. According to Lux et al. (2004), a species has high tolerance when Itol is higher than 60%, moderate when it is between 60 and 35%, and is sensitive when Itol is below 35%. Hence, *S. multijuga* can be classified as moderately tolerant to Zn up to the dose of 388.47 mg kg^{-1}, while *E. crista-galli* up to 529.23 mg kg^{-1}.

Sesbania virgata has capacity to tolerate and stabilize high Zn contents (Branzini et al., 2012), while *Erythrina speciosa* showed tolerance of 65% with addition of 1,000 mg kg^{-1} of Pb to the soil (Souza et al., 2012). Thus, species of the same family and botanical genus have different responses to the contamination with heavy metals.

E. crista-galli and *S. multijuga* have potential in programs of reclamation of Zn-contaminated areas that aim to phytostabilize the metal in the soil, through phytoaccumulation in the root system, because the species showed moderate tolerance up to the dose of 388.47 mg kg^{-1} of Zn in the soil (Figure 4). *E. crista-galli* has moderate tolerance above the investigation value established by the CONAMA Resolution No. 420 of 450 mg kg^{-1}, a characteristic that *S. multijuga* exhibited at contents lower than that defined by the Resolution No. 420. Thus, *E. crista-galli* has potential to be used in future studies in Zn-contaminated soils.

Conclusions

1. High zinc doses in the soil reduce the initial growth of *Senna multijuga* and *Erythrina crista-galli* seedlings.

2. The species *Erythrina crista-galli* has higher tolerance to zinc in comparison to *Senna multijuga* and both species have low zinc translocation.

3. The species *E. crista-galli* and *S. multijuga* can be used in programs of reclamation of zinc-contaminated areas that aim to phytostabilize the zinc of the soil, through its phytoaccumulation in the roots.

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