

Original Article

A Physiological behavior and tolerance of *Lactuca sativa* to lead nitrate and silver nitrate heavy metals

Comportamento fisiológico e tolerância de *Lactuca sativa* aos metais pesados nitrato de chumbo e nitrato de prata

J. D. O. Santos^a , E. S. Silva^a , P. Silva^a , M. A. C. Silva^a , L. A. Santos^a , A. F. Santos^{b,c} , T. J. Matos-Rocha^{b,d}  and J. M. S. J. Pavão^b 

^a Universidade Federal de Alagoas, Programa de Pós-graduação em Agricultura e Meio Ambiente, Arapiraca, AL, Brasil

^b Centro Universitário Cesmac, Programa de Pós-graduação em Análise de Sistemas Ambientais, Maceió, AL, Brasil

^c Universidade Estadual de Alagoas – UNEAL, Arapiraca, AL, Brasil

^d Universidade Estadual de Ciências da Saúde de Alagoas – UNCISAL, Maceió, AL, Brasil

Abstract

This study goal to evaluate the effects of different concentrations of lead (Pb) and silver (Ag) on germination, initial growth and anatomical alterations of *Lactuca sativa* L. Plants use various mechanisms to reduce the impacts caused by anthropic action, such as xenobiotic elements of soils and water contaminated by heavy metals. These metals were supplied as lead nitrate and silver nitrate and the following treatments were established: control for both metals, maximum dose of heavy metals, for arable soils, allowed by the National Council of the Environment (Ag = 25 mg. Kg⁻¹, Pb = 180 mg. Kg⁻¹), double (Ag = 50 mg. Kg⁻¹, Pb = 360 mg. Kg⁻¹) and triple (Ag = 75 mg. Kg⁻¹, Pb = 540 mg. Kg⁻¹) of this dosage. Vigor and germination tests of the seeds and possible anatomical changes in the leaves and roots of lettuce plants were performed. The species showed a high capacity to germinate under Pb and Ag stress, and the germination was never completely inhibited; however, the germination decreased with increasing Pb concentrations, but not under Ag stress. The use of increasing doses of metals reduced seed vigor and increased chlorophyll content. An increase in biomass was also observed in plants from treatments submitted to Pb. The phytotoxic effects of metals were more pronounced at 15 days after sowing. Anatomically, *L. sativa* was influenced by metal concentrations, and had a reduction of up to 79.9% in root epidermis thickness at the highest Pb concentration, although some structures did not suffer significant changes. The results suggest that *L. sativa* presents tolerance to high concentrations of heavy metals, showing possible mechanisms to overcome the stress caused by these metals. In this research lettuce possibly used the mechanism of exclusion of metals retaining Pb and Ag in the roots preserving the photosynthetic apparatus in the aerial part of the plants. In general, the chemical element Pb was more toxic than Ag, in these experimental conditions.

Keywords: lettuce, heavy metals, soil contamination, plant growth, tissue plasticity.

Resumo

Este estudo teve como objetivo avaliar os efeitos de diferentes concentrações de chumbo (Pb) e prata (Ag) na germinação, crescimento inicial e alterações anatômicas de *Lactuca sativa* L. As plantas utilizam vários mecanismos para reduzir os impactos causados pela ação antrópica, como elementos xenobióticos de solos e água contaminada por metais pesados. Esses metais foram fornecidos como nitrato de chumbo e nitrato de prata e foram estabelecidos os seguintes tratamentos: controle para ambos os metais, dose máxima de metais pesados, para solos cultiváveis, permitida pelo Conselho Nacional do Meio Ambiente (Ag = 25 mg.Kg⁻¹, Pb = 180 mg.Kg⁻¹), dobro (Ag = 50 mg.Kg⁻¹, Pb = 360 mg.Kg⁻¹) e triplo (Ag = 75 mg.Kg⁻¹, Pb = 540 mg.Kg⁻¹) desta dosagem. Foram realizados testes de vigor e germinação das sementes e possíveis alterações anatômicas nas folhas e raízes das plantas de alface. A espécie apresentou alta capacidade de germinar sob estresse de Ag e Pb, e a germinação nunca foi completamente inibida; entretanto, a germinação diminuiu com o aumento das concentrações de Pb, mas não sob estresse de Ag. O uso de doses crescentes dos metais, reduziu o vigor das sementes e aumentou o teor de clorofila. Também foi observado aumento da biomassa nas plantas a partir dos tratamentos submetidos ao Pb. Os efeitos fitotóxicos dos metais foram mais acentuados aos 15 dias após a semeadura. Anatômica, *L. sativa* foi influenciada pelas concentrações de metais, e teve uma redução de até 79,9% na espessura da epiderme radicular na maior concentração de Pb, embora algumas estruturas não tenham sofrido alterações significativas. Os resultados sugerem que *L. sativa* apresenta tolerância a altas concentrações de metais pesados, mostrando possíveis mecanismos para superar o estresse causado por esses metais. Nesta pesquisa a alface possivelmente utilizou o mecanismo de exclusão de metais retendo Pb e Ag nas raízes preservando o aparato fotossintético na parte aérea das plantas. De forma geral o elemento químico Pb se mostrou mais tóxico que Ag, nestas condições experimentais.

Palavras-chave: alface, metais pesados, contaminação do solo, crescimento da planta, plasticidade do tecido.

*e-mail: jesse.marques@cesmac.edu.br

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1. Introduction

Lettuce (*Lactuca sativa* L.) is classified in the Asteraceae family, and it is one of the most consumed vegetables in Brazil and in the world. Due to its accumulating characteristic, it is subject to contamination by heavy metals dissolved in the soil solution (Silva et al., 2019; Eissa and Negim, 2018).

Contaminant metals in the soil can be classified as heavy metals or trace elements. Heavy metals are elements that normally give high toxicity to humans; among them, stand out the lead, cadmium, silver, mercury and chromium (Suryawanshi, et al., 2016; Baroni et al., 2020). Trace elements, on the other hand, are metals with lower toxicity and are often present in low concentrations, and some of them are considered biologically essential; however, some of these metals in specific situations may cause problems to ecosystems, causing pollution to water and soil (Ribeiro et al., 2019).

The fertilizers, metallurgical, mining, chemical, waste disposal, sewage sludge, tannery sludge, and fossil fuels industries are the main sources of soil contamination by heavy metals (Ćwieląg-Drabek, 2020; Eissa and Negim, 2018). The possibility that the metals enter human food raises many concerns since they can be in leafy tissues present in vegetables such as lettuce, bringing risks to human health (Eissa and Negim, 2018).

It has long been known that high concentrations of heavy metals such as silver, lead, mercury, among others, are responsible for causing numerous carcinogenic diseases, as well as other aggravating factors such as damage to the central nervous and the hepatic, renal, hematopoietic and skeletal systems (Kim et al., 2015; Shahbazi et al., 2017). For this reason, some countries have established boundaries and laws that govern the concentrations of heavy metals in agricultural soils.

The absorption, distribution and action mechanisms of heavy metals in plants depend on several factors, including species or varieties of the same species, plant development stage, chemical species of the element, metal concentration, time of exposure, soil pH, and organic matter content (Pendias, 2010; Baghaie and Fereydoni, 2019). When the heavy metals are absorbed by the roots, they accumulate mainly in the cell wall (Azevedo et al., 2019), and the exoderm and endoderm become a barrier to the movement of these ions (Tylová et al., 2017; Ismael et al., 2019).

When exposed to heavy metal contamination, lettuce undergoes significant interference in physiological processes. Therefore, this study aimed to evaluate the effect of different concentrations of lead nitrate ($Pb(NO_3)_2$) and silver nitrate ($AgNO_3$) on the emergence, initial growth and anatomical changes in *L. sativa*.

2. Material and Methods

2.1. Experiment Installation

The experiment was carried out in a greenhouse ($9^{\circ}42'03.9''S$ $36^{\circ}41'13.1''W$ and 324m altitude), using lettuce (*L. sativa*), cv 'babá de verão', purchased at a store

of agricultural products in the municipality of Arapiraca, (Alagoas State, Brazil). The average temperatures inside the greenhouse in the months of the experiment (June at September) were 6 am at 12pm (25.8 °C) and 12pm at 6pm (23.7 °C). And relative air humidity of 69%.

The experimental unit consisted of a container filled with 500g of soil (Medium texture), in which the solutions of lead (Pb), in the form of lead nitrate ($Pb(NO_3)_2$) and silver (Ag) in the form of silver nitrate ($AgNO_3$), were applied in 3 increasing concentrations constituting the respective treatments. The watering shift that was used these solutions occurred in the morning, between 7am and 8am. The standard concentration of each metal was based on the maximum value allowed by the Resolution 460/2013 (CONAMA, 2013 – National Environment Council) for agricultural soils ($Ag= 25 \text{ mg.Kg}^{-1}$, $Pb= 180 \text{ mg.Kg}^{-1}$), and this value was doubled ($Ag= 50 \text{ mg.Kg}^{-1}$, $Pb= 360 \text{ mg.Kg}^{-1}$) and tripled ($Ag= 75 \text{ mg.Kg}^{-1}$, $Pb= 540 \text{ mg.Kg}^{-1}$), in addition to the control groups for the two metals. The lettuce chemical fertilization was carried out using NPK 4:14:8, applied at the time of planting.

Each plot consisted of four containers, in which sixteen seeds were sown per plot. At 15 DAS (days after sowing), thinning was performed, leaving only four plants per plot. The evaluations were carried out at 15, 21 and 35 DAS.

2.2. Germination and evaluation of seed vigor

The Emergence Speed Index, Emergence Time and Emergence Percentage were determined using the following formulas, respectively:

$$ESI = (G1 / N1) + (G2 / N2) + \dots + (Gn / Nn);$$

where: ESI= Emergence Speed Index, G= number of normal seedlings computed in the counts, and N= number of days from sowing to the first, second... umpteenth time of evaluation) (Maguire, 1962);

$$tE = (S_{ni} \cdot ti) / S_{ni};$$

where: tE= the mean time of emergence, ni= the number of seedlings emerged at the i-th day, and ti= time (days) (Labouriau, 1983);

$$E = (Ne / Ae) \cdot 100;$$

where: E= emergence percentage, Ne= number of emerged seedlings, and Ae= total number of seeds placed to emerge (Brasil, 2009).

The analyses were performed from the first to the eleventh day after sowing.

2.3 Growth analysis and chlorophyll and dry biomass determination

Plant height, leaf area, and dry matter were obtained to analyze the growth of the crop. Leaf area was calculated by the non-destructive method, using leaf number, length, width and correction factor of 0.68, using the equation:

$$AF = [(C \times L) / 2 \times CF \times NF];$$

Where: C= leaf length in cm, L= leaf width in cm, CF= correction factor, and NF= number of leaves per plant. The SPAD index readings were performed in the morning, on fully expanded terminal leaves by using the indirect chlorophyll meter SPAD-502 (Soil Plant Analysis Development), Konica Minolta Camera Co. Ltd. Japan. Three leaves were randomly analyzed in each plot with ten measurements on each leaf.

At the end of the experimental period, all plants were collected, divided into root and aerial part (shoots), conditioned in Kraft paper bags, dehydrated in a forced air ventilation oven at 70°C for 48 hours, and then weighed on an analytical balance (Mars AD500) to obtain the dry biomass of the respective parts.

2.4. Anatomical analyses

For anatomy analysis, five leaves and five roots of each treatment were randomly collected at 35 DAS. The samples were fixed in F.A.A. 70% (Johansen, 1940) for 48 hours, and later they were stored in 70% ethyl alcohol. Cross sections were obtained manually, and root cuts were clarified in 1% sodium hypochlorite solution, washed three times for 5 minutes in distilled water, stained with 0.05% toluidine blue (Sakai, 1973) and mounted on 50% aqueous glycerol (Kraus; Arduin, 1997).

The slides were photographed with a camera (Canon Powershot A630) coupled to the optical microscope (Nova 107) and analyzed in the Image Tool software version 3.0. The anatomical tissues evaluated in the leaves were: epidermis thickness of the abaxial face (EAB), epidermis thickness of the adaxial face (EAD), chlorophyll parenchyma thickness (CP), vascular bundles thickness, and the distance

between vessels. In the root, epidermis thickness, cortical parenchyma thickness, and vascular bundle thickness were observed.

2.5. Statistical analyses

From the raw data (data set) the normality test was applied by Shapiro-Wilk (Shapiro and Wilk, 1965). The deviances were estimated according to data distribution for each factor and interaction, as well as for null and saturated models, whence residual deviance derives. Inferences of the deviance analysis for binomial distribution were based on Scott Knott test, because the dispersion parameter is known. All the analysis were performed with software R version 3.4.1 (R Core Team, 2018), considering the significance level of $\alpha=0.01$ for all applied tests. A completely randomized design with 50 replicates, in a factorial scheme 2x4 (metals x concentrations), was used.

3. Results

The data, when submitted to variance normality and homogeneity tests presented normal distribution and it was not necessary to transform them. After obtaining ANOVA only for the variable average time of germination, there was interaction, the other variables the behavior was due only to the functions of the elements Pb and Ag.

3.1. Emergence and growth

Seed emergence and vigor test showed an interaction between the metals for both the emergence speed index (ESI) and emergence percentage (E). Both Pb and Ag

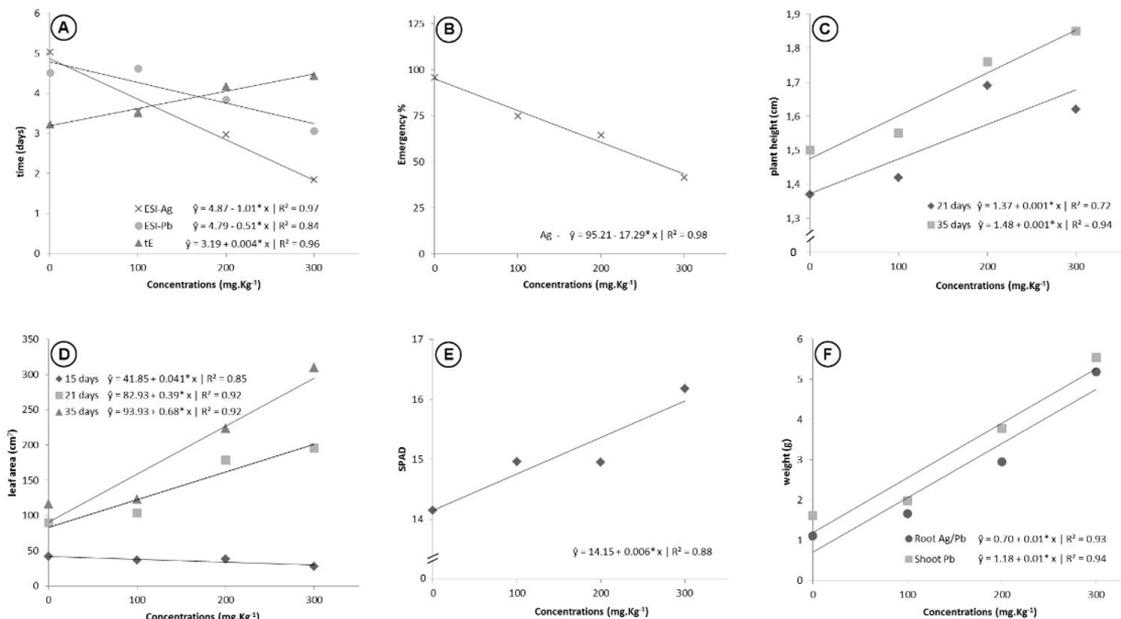


Figure 1. Germination and growth characteristics of lettuce plants subjected to increasing lead and Ag concentrations. A) Emergence speed index and emergence mean time; B) Emergence percentage; C) Plant height as a function of lead and silver concentrations; D) Behavior of leaf area in plants subjected to lead; E) SPAD as a function of lead and silver concentrations; F) Weight of dry matter. Each point on the graphs represents an average of 50 repetitions. The standards variations vary between ± 2.5 and ± 6.8 around the average from the graphs at the different points.

reduced the ESI; however, as their concentrations were increased, there was a higher decrease when Ag was used (Figure 1A). There was no difference or interaction, being only significant the metals concentration variable (Pb and Ag), being used, in regression, for the construction of Figure 1A, representing the pattern both metals. The tE was affected by both metals, increasing as the concentrations of these metals were increased. The percentage of Pb emergence has not been explained, so the graph refers only to the percentage of Ag. In this case, the emergence percentage was not affected by the different concentrations of Pb, whereas the Ag concentrations significantly reduced this factor, increasing it as the concentrations were increased (Figure 1B).

The different concentrations of heavy metals did not significantly affect plant height in the first 15 DAS, whereas the analyses performed at 21 and 35 DAS (Figure 1C) showed a linear behavior, expressing an elongation in the stem as the Pb and Ag concentrations were increased.

No significant differences were observed in leaf area in plants subjected to Ag concentrations, and there was no interaction between the analyzed factors either. However, at 15 DAS, leaf area was inversely proportional to Pb concentrations, and at 21 and 35 DAS, leaf area was directly proportional to these concentrations, increasing the leaf as the highest concentrations of heavy metals were applied (Figure 1D).

As there was no significant difference between Pb and Ag, but there were different options, the graph was then constructed from the linear regression of SPAD and customs, representing the pattern for both metals. (Figure 1E).

Ag concentrations provided no significant increases or decreases in shoot dry biomass production. Pb increased the biomass as the highest concentrations were applied

(Figure 1F). This behavior was also observed for root dry mass for both metals (Figure 1F).

3.2. Anatomy

Analyzing the micrographs of the anatomical slides, it is noticeable that the structural aspect of the tissues has not been visually altered (Figure 2), but the differences between the metals used and their respective concentrations can be visualized on a micrometric scale (Table 1).

An interesting fact that occurred in this work, it is possible to observe when comparing Figures 2A and 2G, the tissue changes are clear when the lettuce plants come into contact with the different concentrations of lead and silver nitrate. Where, from Figure 2 B, ontogenesis of accessory vessels occurs, as well as greater distance between the vascular bundles.

Visually, with the increase in the concentrations of metals, those conduction vessels (xylem and phloem), present discreet robustness and this structure are perceived, mainly from Figure 2B. Vessels xylem in the details. In Figure 3C, the diameters of the vessels are quite pronounced, when compared with the vessel details of Figures 3 A-F. In Figure 3G, the vessels have higher density when compared to Figure 3C.

For the epidermis thickness of the adaxial face (EAD) of *L. sativa* leaves, more compared to their controls, pronounced differences were found at the highest concentration of Ag, in this case, was observed an increase of 15.7%. Observed again an increase of 24.9% in the EAD in plants subjected to the concentration of 180 mg.Kg⁻¹ of Pb, and a decrease of 51.8% in the EAD in those grown at the concentration of 360 mg.Kg⁻¹. There was no statistical difference regarding the EAD between plants

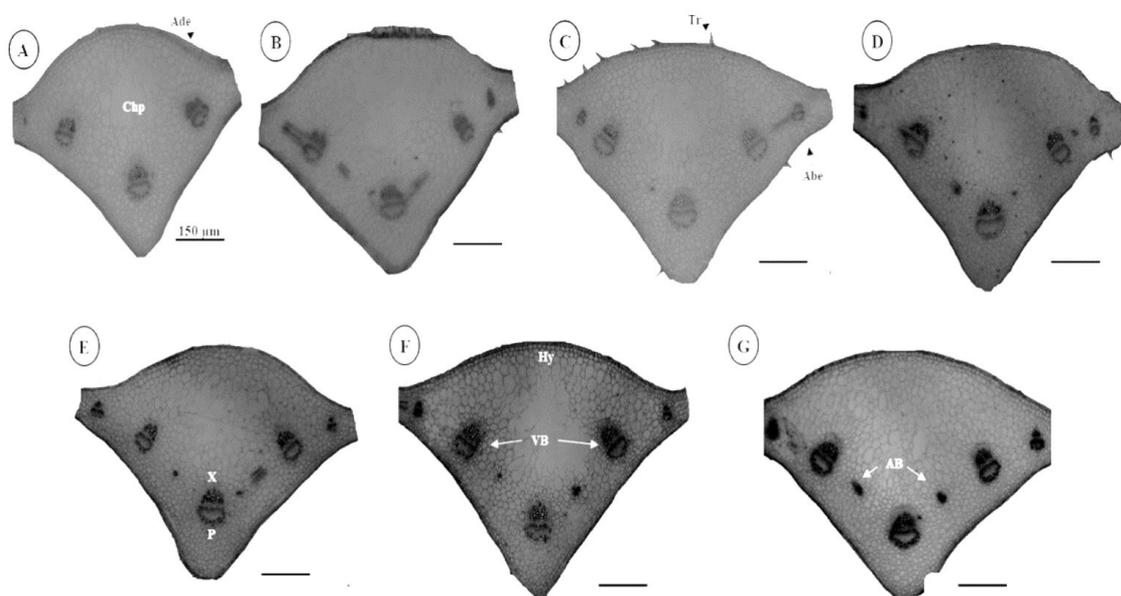


Figure 2. Leaves of lettuce under lead and silver nitrate concentrations. A: 0 mg.Kg⁻¹ Pb and Ag; B: 12.5 mg.Kg⁻¹ Ag; C: 25 mg.Kg⁻¹ Ag; D: 37 mg.Kg⁻¹ Ag; E: 90 mg.Kg⁻¹ Pb; F: 180 mg.Kg⁻¹ Pb and G: 270 mg.Kg⁻¹ Pb. Ade: adaxial epidermis; Chp: Chlorophyll parenchyma; Tr: trichome; X: xylem; Abe: abaxial epidermis; P: phloem; Hy: Hypodermis; VB: vascular bundles; AB: Accessory bundles. Bars 50 μm.

Table 1. Means from the anatomy of stem and root tissues in *L. sativa* under concentrations Pb and Ag.

Analyzed part	Tissue (μm)		Heavy metal concentrations mg.Kg^{-1} *				
			0	100	200	300	
Root	Epidermis	Ag	189.97aA**	129.57bBC	172.39aAB	114.55aC	
		Pb	175.36aA	201.59aA	163.42aA	97.50aB	
	Cortical parenchyma	Ag	334.84aAB	430.87aA	252.21aB	357.48aA	
		Pb	381.98aA	367.92aAB	298.95aAB	276.45bB	
	Thickness of vessels	Ag	325.85aB	382.76bB	355.15bB	515.00aA	
		Pb	358.66aB	501.89aA	451.65aA	516.34aA	
	Adaxial epidermis	Ag	28.66aB	24.23bC	24.55aC	33.15aA	
		Pb	27.17aB	33.93aA	22.35aC	25.14bBC	
	Abaxial epidermis	Ag	22.78aAB	17.87bB	28.26aA	23.55aAB	
		Pb	22.04aA	24.08aA	17.70bA	22.54aA	
	Leaf	Distance between vessels	Ag	629.53aC	750.99aB	817.12aAB	881.53aA
			Pb	629.56aB	648.22bB	569.87bB	861.70aA
Thickness of vessels		Ag	252.56aB	275.42aB	284.94aB	353.44aA	
		Pb	278.06aA	262.28aAB	226.11bB	271.58bA	
Chlorophyll parenchyma		Ag	1827.25aC	1983.65aB	2067.62aB	2243.27aA	
		Pb	1859.88aB	1723.77bB	1556.41bC	2148.26aA	

*0% concentration is equivalent to the absence of the metal for both metals; 100% at 25 mg.kg^{-1} and 180 mg.kg^{-1} ; 200% at 50 mg.kg^{-1} and 360 mg.kg^{-1} ; and 300% at 75 mg.Kg^{-1} and 540 mg.Kg^{-1} for Ag and Pb, respectively. **Means followed by the same lowercase letter, in the columns, and upper case, in the lines, do not differ by Scott Knott test at the 5% significance level.

subjected to the concentrations of 50 mg.Kg^{-1} of Ag and those grown at the concentration of 360 mg.Kg^{-1} of Pb. In plants grown at the concentration of 180 mg.kg^{-1} of Pb, the EAD was increased by 40%, when compared to those subjected to 25 mg.Kg^{-1} of Ag. At the maximum applied concentration, Ag caused an increase in EAD than when was Pb applied (Table 1).

Regarding the epidermis thickness of the abaxial face (EAB), there was no difference up to 75 mg.Kg^{-1} between the plants subjected to Ag and those from the control group, except at the concentration of 50 mg.Kg^{-1} , in which there was an increase of 58.1% in the EAB in comparison with the concentration of 25 mg.kg^{-1} . Regarding the effect of both metals, Pb concentrations had no significant effect on the EAB. Pb provided an increase of 34.7% in the EAB in plants grown at the concentration of 180 mg.Kg^{-1} and a reduction of 37.4% at the concentration of 360 mg.Kg^{-1} , in comparison to the concentrations of 25 mg.kg^{-1} and 50 mg.kg^{-1} of Ag, respectively (Table 1).

Comparing the distances between vessels, the higher the concentration of Ag, the larger the distance between them. However, the distance between vessels in plants subjected to the concentration of 50 mg.Kg^{-1} of Ag did not differ from the distance found in plants subjected to 25 and 75 mg.Kg^{-1} of Ag, (Table 1). Regarding the Pb, the largest distance was observed at the highest concentration. Comparing the effect of both metals, plants grown at concentrations

of 25 and 50 mg.Kg^{-1} of Ag showed an increase of 15.9% and 43.4% in the distance between vessels, respectively, compared to those subjected to the concentrations of 180 and 360 mg.Kg^{-1} of Pb (Table 1).

The thickness of vessel bundles in plants subjected to the Ag treatment showed no difference up to the concentration of 50 mg.Kg^{-1} ; however, in plants at the concentration of 75 mg.kg^{-1} , it was 39.9% higher than the thickness of vessels in plants from the control treatment. At Pb concentrations, there was no difference regarding this variable in plants subjected up to the concentration of 540 mg.Kg^{-1} , except for those under 360 mg.Kg^{-1} , which presented lower mean and did not differ from the plants of the 180 mg.Kg^{-1} treatment. In contrast, the metals did not influence the thickness of vessels in plants at the concentration of 100% (25 mg.Kg^{-1} of Ag and 180 mg.Kg^{-1} of Pb), whereas at concentrations of 200% and 300% (50 mg.Kg^{-1} to 75 mg.Kg^{-1} of Ag; 360 mg.Kg^{-1} to 540 mg.Kg^{-1} of Pb), the thickness of vessels in plants from the Ag treatment was 26% and 30.1% higher, respectively (Table 1).

Plants subjected to the highest concentrations of Ag and Pb had chlorophyll parenchyma thickness 33.7 and 15.57% higher, respectively than those subjected to the control treatment. Plants grown at concentrations of 25 and 50 mg.Kg^{-1} of Ag showed chlorophyll parenchyma thickness 15.1% and 32.8% higher than those subjected

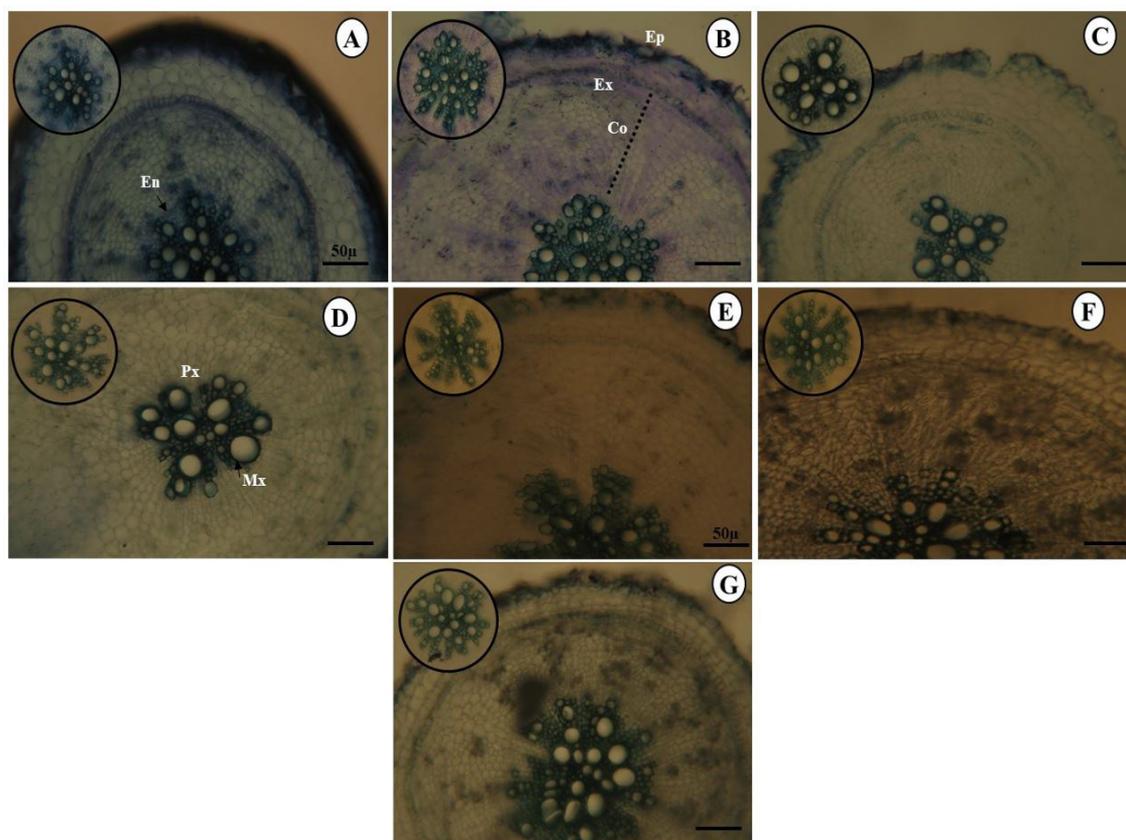


Figure 3. Roots of lettuce under lead and silver nitrate concentrations. A: 0 mg.Kg⁻¹ Pb and Ag; B: 12,5 mg.Kg⁻¹ Ag; C: 25 mg.Kg⁻¹ Ag; D: 37 mg.Kg⁻¹ Ag; E: 90 mg.Kg⁻¹ Pb; F: 180 mg.Kg⁻¹ Pb and G: 270 mg.Kg⁻¹ Pb. En: endoderm; Ep: epidermis; Ex: exoderm; Co: cortex; Px: protoxylem; Mx: metaxylem. Bars: 50μm.

to concentrations of 180 and 360 mg.Kg⁻¹ of Pb, respectively (Table 1).

The results of the anatomy of the root tissues for both analyzed factors (metal and Concentration) showed an interaction. The epidermis thickness had a reduction of 65.3% and 79.9% in plants subjected to the highest concentrations of Ag and Pb, respectively, in comparison to the control. Plants subjected to the concentration of 180 mg.Kg⁻¹ of Pb had the thickness of their epidermal cells increased by 55.6% in comparison to those from the Ag treatment. Regarding the other concentrations, there was no significant difference (Table 1).

The thickness of the cortical parenchyma cells in plants grown at Ag concentrations up to 75 mg.Kg⁻¹ did not differ from that in plants subjected to the control group, except for those grown at the concentration of 50 mg.Kg⁻¹, which, compared to the control, had a decrease of 32.8% in the thickness of the cortical parenchyma cells. For plants grown at Pb concentrations, only those from the 540 mg.Kg⁻¹ treatment had a reduction (38.1%) in the thickness of the cortical parenchyma cells in comparison to the control group. At the highest concentrations of the two metals, this variable in plants subjected to Pb had a reduction of 29.3% compared to those from the Ag treatment (Table 1).

For the thickness of vessels, up to the concentration of 50 mg.Kg⁻¹ of Ag, there was no significant difference in comparison to the control group, but at the concentration of 75 mg.Kg⁻¹, the vessels were 58.0% thicker than those in plants grown with no metal. In plants subjected to 180 mg.Kg⁻¹ of Pb, there was a vessel thickening of 39.9%, differing from the control group only. Plants grown at 180 and 360 mg.Kg⁻¹ of Pb had thickness of vessels 31.1% and 27.2%, respectively, higher than those grown at the same concentrations of Ag (Table 1).

4. Discussion

When analyzing the data from the germination physiology experiment in *L. sativa* seed, it was evident that the ability of the metal to interfere with seed germination and emergence can often be related to seed permeability to heavy metals and internal structure, which, once in contact with the embryo, causes damage to the tissues. The effects on germination may be associated with the fragile seed tegument of *L. sativa*, increasing the contact of the embryo to the metal and negatively interfering with the speed, time and percentage of germination and emergence.

Analyzing the phytotoxicity of lead on germination and initial growth of lettuce under laboratory conditions, Pires-Lira et al. (2020) found that seed germinations was affected at higher concentrations of this metal, although they recognized that seedling growth is more sensitive to the effects of heavy metals than seed germination is.

In this research, lettuce seeds germinated in 5 days when in contact with the metals Pb and Ag and in 3 days in the absence of the metals. Apparently, this difference may be associated with metals, however, according to the manufacturer, the sativa seeds can germinate in up to 7 days.

Under laboratory conditions, Jelea et al. (2015) verified a reduction in germination with the increase in the concentration of lead nitrate. The decrease in the percentage of germination may be related to the interference of the metal in the activity of amylases and proteases (Nas and Ali, 2018; Boi et al., 2020).

Several studies indicate that silver nitrate is toxic for seeds of species such as barley, wheat, and cruciferous plants, showing that in sensitive species, toxicity is already observed from a silver nitrate concentration of 7.5 mg.L⁻¹ (Vishwakarma et al., 2017). Considering that there was an emergence of the plants, although the maximum concentration (75 mg.Kg⁻¹) used in this study was ten times higher than those observed in other studies with more sensitive plants, it can be assumed that for emergence, lettuce presents good tolerance to silver nitrate. Probably, the studied species has some enzymatic mechanism that can help it to overcome the stress caused by Ag.

Excess metals in plants cause a decrease in their growth due to the inhibition of photosynthesis, hormonal changes and interference in the regulation of mineral nutrition (Rucińska-Sobkowiak, 2016). Due to these factors, even with the chemical fertilization performed in the initial stage of growth, the plants presented characteristics of nutrient deficiency, among which, chlorosis was the most apparent one, showing clear signs of nitrogen deficiency.

Subsequently, the plants responded by increasing the chlorophyll content. The tolerance mechanism of plant species subjected to excess heavy metals is related to their intrinsic, physiological and biochemical response, as well as to the chemical element and its ability of translocation through the plant (Tiwari and Lata, 2018). Changes in the expression of some genes may contribute to the acquisition of higher tolerance (Chen et al., 2019); however, genotypic differences among species and even cultivars allow different physiological responses to chemical agents.

The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms such as: (i) uptake and accumulation of metals through binding of extracellular exudates and cell wall constituents; (ii) efflux of heavy metals from cytoplasm to extra nuclear compartments including vacuoles; (iii) complexation of heavy metal ions inside the cell of various substances, for example, organic acids, amino acids, phytochelatins, and metallothioneins; (iv) accumulation of osmolytes and osmoproteins and induction of antioxidative enzymes; and (v) activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures (Cho et al., 2003).

Despite numerous studies, the specific mechanisms involved in heavy metal accumulation and detoxification are yet to be well described. Characterization of these mechanisms could be important in improving the potential of Salicaceae plants for phytoremediation (Cho et al., 2003).

In spite of observing negative effects on the plants subjected to Pb, some defense mechanisms against the phytotoxic agents may have been activated since in longer periods of exposure to the metal, there was a significant increase in plant leaf area and height, even at the maximum used dosage. Thus, the phytotoxic effect of the metals did not negatively affect the plant in longer periods. The seemingly positive responses can be explained by the fact of lettuce plants reduce or neutralize the toxic effects of some metals, which can be reduced by activating plant antioxidant defense systems, reduction in metal transport over long distances, and storage of metals in the vacuoles (Emamverdian et al., 2015).

Different concentrations of metals, among them the Pb, in different species and parts of plants are directly related to the concentrations of metals in the soil. The concentrations of metals may be higher in the cell walls of the root system and lower in the aerial part. In some cases, the metals act positively on the increase of biomass, both in root and aerial part, depending on the species (Eissa and Negim, 2018). These results corroborate the ones obtained in this study, in which either the biomass increase was verified, or no difference was observed between the controls. The metal concentrations increased the root system and, consequently, increased the capacity of water and nutrient absorption, which directly reflected the increase in the aerial part. In sequence, given the increases in biomass in both root and shoot systems, it can be considered that the applied concentrations had no toxic effects on lettuce in longer periods of exposure to the applied metals.

The Pb interferes with several physiological processes in lettuce, such as the increase of chlorophyll content and leaf and root area; in response, the plant has bigger structures for the accumulation of biomass, considering they can positively interfere with the increase in photosynthesis and water and nutrients absorption, acting with a set of factors that allows this increase.

In a study with lettuce (*L. sativa*), radish (*Raphanus sativus*), tomato (*Solanum lycopersicum*) and ryegrass (*Secale cereale*), it was verified the maximum accumulation of Pb in the roots (Rai et al., 2019), suggesting that immobilization may be linked to the amino acid histidine, consisting of a plant strategy to avoid toxicity in parts above ground (Dawuda et al., 2020). Pb detoxification mechanisms also include storage in the vacuole, synthesis of phytochelatins, and binding to glutathione (Pires-Lira et al., 2020).

Even at low concentrations of cadmium, there was a significant increase in *Tetraena qataranse* biomass production, in both root and shoot (Usman et al., 2019). A similar result was verified in the present study with the increase of lead and silver concentrations, characterizing a stimulating response of adaptation to stress caused by the presence of metal. Usman et al. (2019) point out that in *Tetraena qataranse*, the metals improved the gas exchange by stimulating the stomatal opening; this condition may

have stimulated the increase of biomass in the plants analyzed in our study.

Physiological stresses caused by metals to different organisms can be observed to a greater or lesser extent, depending on the response given by the organism. *L. sativa* proves to be a species tolerant to metals such as Cd and As (Wang et al., 2017; Musa et al., 2017) as well as to Ag and Pb, as shown in our study, which proves that the changes in seedlings subjected to concentrations of Ag and Pb vary, occurring in the tissue structure of roots and leaves. The reduction in the root epidermal cell thickness in plants grown at higher concentrations of metals possibly presented oxidative effects by the action of these metals (Ismael et al., 2019; Hamim et al., 2018).

As a survival strategy, some plants increase the thickness of the vascular bundles when subjected to metal concentration (Hamim et al., 2018) to maintain the hydraulic safety by increasing the thickness of the vascular tissue cells, increasing the flow of photo assimilates and nutrients in the vegetable (Pires-Lira et al., 2020). The increase in vessel thickness can be directly related to the increase in shoot mass, in which a proportional increase was observed (Figure 1F), also observed in the increase of vessels (Table 1 and Figure 3 in special G).

In the leaves, the increase in the distance between vascular bundles may have occurred due to the increase in the shoot and leaf area (Figure 1D and Figure 2 in special G) since as the concentrations have increased the distance between vascular bundles followed the same behavior, similar to that observed in leaf area and distance between vessels.

Changes in epidermal cell thickness, both abaxial and adaxial, and in chlorophyll parenchyma can be related to the plant ability to get around the stress caused by the metal (Hamim et al., 2018). It is suggested that the adaptations in the thickness of epidermis and parenchyma may have increased the rate of transpiration and photosynthetic capacity of the plant, which was compensated by the increase in root biomass and consequently increase in water and nutrients absorption, resulting in the increase of biomass in the aerial part.

In summary, lettuce plants subjected to silver nitrate and lead nitrate metals presented phytotoxic effects in the first days up to 15 DAS. However, silver nitrate promoted negative effects in the emergence percentage, reducing the vigor of *L. sativa* seeds with a marked fall in the number of emerged seeds. Despite the initial interference of the metals in the lettuce plants, the final biomass production had no negative interference compared to the control groups (with no metals), proving to be tolerant to high concentrations of Ag and Pb. The 'babá de verão' cultivar has some tissues modified in response to the stress caused by the metals Ag and Pb. Even with no deleterious effects on the biomass production of lettuce plants, because it is an accumulating species, the growth of this vegetable in contaminated soils can lead to serious damage to human and animal health.

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