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METAL ACCUMULATION, GROWTH AND NUTRITION OF *Vernonia polyanthes* EXPOSED TO LEAD NITRATE AND ARBUSCULAR MYCORRHIZAL FUNGI

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Abstract

The association between plants and arbuscular mycorrhizal fungi (AMF) can be used to bioremediate areas contaminated by metals. The objectives of this work were to evaluate the lead (Pb^{2+}) phytoaccumulation capacity, morpho-physiology and nutrition responses of *Vernonia polyanthes* exposed to a solution amended with concentrations of lead nitrate and arbuscular mycorrhizal fungi. The treatments consisted of increasing doses of Pb^{2+} as lead nitrate [$Pb(NO_3)_2$], two strains of AMF and an absolute control without lead and AMF. Lead negatively affected some morphophysiological variables, reduced 27.3, 25.63, 30.60, and 56.60% shoot length, root collar diameter, number of leaves and leaf area, respectively, besides reducing decreasing chlorophyll *a*. Lead accumulated in the shoot and roots, the latter at the highest concentrations. However, the translocation factor was above 1, indicating low efficiency. The bioaccumulation factor referring to the roots were above 1. The fungi colonization rate was low, 3.31% for *Gigaspora margarita* and 2.33% for *Acaulospora morrowiae*. However, the absorption of lead increased, reflecting in lower values of chlorophyll *a*, dry mass of root and diameter. Results indicated that the arboreal species *V. polyanthes* tolerate high concentrations of lead and can accumulate significant amounts in the roots. AMF increase the accumulation of lead in the shoot and can be used in projects aimed at the phytoextraction of metals.

Keywords: Bioaccumulation Factor. Phytoextraction. Phytostabilization. Potential Toxic Element. Translocation Factor.

1. Introduction

The lead is naturally found in rocks and the main lead mineral is galena (PbS), but anglesite ($PbSO_4$) and cerussite ($PbCO_3$) are also common minerals. However, it has become a serious environmental problem due to the contamination of soils and water worldwide because of anthropogenic activities, and it is currently considered one of the greatest pollutants of terrestrial and aquatic ecosystems (Kabata-Pendias 2011; Yongpisanphop et al. 2017). About 78% of the world production of Pb is used mainly to manufacture automotive batteries (Arias et al. 2015) and for other industrial processes, such as mining, foundry and galvanoplasty (Yang et al. 2015). The use of techniques that allow the removal of these contaminants, by environmental services at low cost and high capacity are acceptable to society and to the environmental authorities. Thus, bioremediation is underscored as an excellent option, since it refers to the use of plants

and rhizospheric microorganisms to minimize the toxic effects of potential contaminants in the environment. This technology is based on the association between plants and soil microbes to reduce concentrations or toxic effects of contaminants in the environments and known as economically feasible and very efficient (Kabata-Pendias 2011; Boechat et al. 2016a; Ma et al. 2019).

Many plants establish a symbiosis with the arbuscular mycorrhizal fungi (AMF) as an evolutionary mechanism to render their development feasible at sites that are severely polluted by heavy metals. These can help the phytoremediation of the contaminated areas, making the metals more available for absorption by the plants or reducing the metal toxicity in the host plants (Arias et al. 2015; Coninx et al. 2017; Ma et al. 2019).

The effect of AMF improving the absorption of nutrients that are essential for the plants is widely known (Smith and Smith 2012; Vergara et al. 2019). However, information is scarce regarding the absorption and accumulation of metals including Pb^{2+} in the species *Vernonia polyanthes* (Asteraceae), and the influence of this metal on biomass production and suitability in phytoremediation projects. *Vernonia polyanthes* (Asteraceae) is a native species of Brazil, known popularly as assa-peixe, adapted to adverse conditions, such as soils with low fertility, acid and with a high concentration of phytotoxic aluminum (Al^{3+}). It is frequently found at garbage dumps and close to highways. According to Lajayer et al. (2017) it is essential to identify native plant species adapted to the region for the phytoremediation of metal-contaminated soils.

Vernonia polyanthes plants that are grown in an environment where urban garbage is discarded accumulate chrome in the shoot even if the metal ions are at a low concentration in the soil (Pereira et al. 2013). It is believed that *V. polyanthes* inoculated with arbuscular mycorrhizal fungi can absorb and translocate a large amount of lead. However, research on plants with a potential for phytoremediation is scarce in tropical areas, particularly in the Brazilian Northeast Cerrado biomes.

Considering this scenario, we choose to assess whether the species *Vernonia polyanthes* can be used in lead phytoremediation and/or phytostabilization projects. Thus, the objective of this study is to evaluate the phytoextraction and phytoaccumulation potential of lead (Pb^{2+}) by the arboreal species in symbiosis with strains of arbuscular mycorrhizal fungi.

2. Material and Methods

Study site

The experiment was conducted in a greenhouse at 70% light with an impermeabilized floor, located at the geographic coordinates 09º 04' 28" S and 44º 21' 31" O, in the Northeast region of Brazil. The region climate is hot and humid and according to the Köppen classification it is of the Aw type, with mean precipitation between 900- and 1200-mm $year^{-1}$ distributed during the period of October to April. During the experimental period humidity reached a mean of 78% and the temperature ranged between the minimum of 21 ºC and the maximum of 34 ºC with a mean of 25.93 °C (INMET 2019).

Preparation of seedlings and inoculation with AMFs

Vernonia polyanthes seedlings were produced from approximately 15 cm high stakes inserted into plastic tubes with a capacity for 230 cm^3, containing washed and autoclaved sand. The period of stake acclimation was approximately two months, and the plants were irrigated three times a day via an automated irrigation system.

After the acclimation period, the seedlings were transplanted to black plastic pots with a 3 L capacity, containing 1.5 kg of rough, washed and autoclaved sand, and filled up with 2.6 L of Hoagland and Arnon (1950) nutrient solution at 50% strength, without the heavy metals. Before transplanting the seedlings were standardized by the height of the shoot and then the roots were washed in tap water to remove the remainder of the sand that might potentially interfere in the experiment. The experiment followed a design in randomized blocks, with a factorial scheme of 4 x 3, with four concentrations of Pb (0, 72, 180 and 300 mg L^{-1}), two strains of arbuscular mycorrhizal fungi (*Acaulospora morrowiae* and *Gigaspora margarita*) and a control treatment (without inoculation), with three replications. The arbuscular mycorrhizal fungi (AMFs) strains were provided by the International Glomeromycota Culture Collection (CICG) of the Regional

University of Blumenau (FURB), Blumenau, Santa Catarina, Brazil. The inoculation was performed using a 3 g of inert vehicle (containing approximately 115 spores per tube).

The nutrient solution was renewed weekly and the inoculation was repeated. After 21 days of adaptation, the plants were submitted to treatment with increasing doses of lead, added in the form of lead nitrate [$Pb(NO_3)_2$].

The nutrient solution was oxygenated by a system consisting of an air compressor (Resun GF180, 300 L min^{-1} to 8 kPa of pressure), connected to a PVC pipe forming a main air distribution line. The secondary lines were constituted by silicone hoses, 16 mm in diameter, coupled to a porous stone at its extremity. Daily the solution volume was checked and, if necessary, its initial level was restored with deionized water; pH of the solution was maintained in the range of 5.5 and 6.5 and the electric conductivity of the solution at 3.4 mS cm^{-1} measured with the portable conductivitymeter (Kasvi®).

Collection and analysis of the plants

After 25 days of exposure to lead (Pb^{+2}), the plants were collected and separated into shoots and roots. The shoot was washed in deionized running water to remove particles that have adhered to the tissue and the roots were immersed for 5 minutes in a solution of HCl 0.1 mol L^{-1} to remove the metallic ions that have stuck to the surface and then washed with deionized water. The variables shoot length (SL), using a tape measure, diameter of the root collar (RC) obtained by digital pachymeter, number of leaves (NL) were measured using a graduated ruler. Then the plants were weighed to obtain the fresh mass of the shoot (FMS), fresh mass of root (FMR) and the volume of the roots measured using a graduated test tube.

The roots and shoot were placed in paper packages, submitted to drying in an oven with forced air circulation, at 65 ºC, until the plant material reached a constant weight (about 72 h) and the dry mass of the shoot (DMS) and of the roots (DMR) is obtained.

Once the dry masses had been looked at, the samples were ground in a Wiley type mill, digested in a nitro-perchloric acid solution, according to the methodology described by Tedesco et al. (1995) and the concentrations of the macronutrients phosphorus (P), potassium (K), sulphur (S), calcium (Ca) and magnesium (Mg) and of the metallic ion (Pb) were determined. The vandato-yellow colorimetric method was used to determine P. It forms a complex in a yellow color that absorbs light in the 420 nm region. K was determined by flame photometer. S was determined by turbidimetry and the concentration values of Ca, Mg and Pb were determined in an atomic absorption spectrometer (Varian® AA240FS).

Extraction and quantification of chlorophyll a, b and totals and carotenoids

The extraction of the photosynthetic pigments (chlorophyll *a*, *b*, totals and carotenoids) was performed using the methodology of Arnon (1949) and Hiscox and Tsraelstam (1979) with adaptations. Two hundred mg of plant material were weighed in a test tube wrapped in aluminum papers, adding to them 10 mL of dimethyl sulphoxide [$(CH_3)_2SO$] and incubated at 70 ºC for 30 min, in a water bath, followed by individual agitation, every 10 min. The equipment used for reading pigments was the Biomate® tm3 spectrophotometer, on the 470, 656 and 663 nm wavelengths, which were calibrated according to the transfer of an aliquot to a quartz cuvette with a 3 cm^3 volume. Based on the absorbances obtained the respective values of carotenoids (Cx+c), chlorophyll *a*, chlorophyll *b*, and total chlorophyll were calculated, expressed in µg mL^{-1} of extract, and were later converted into µg g^{-1} of dry mass.

Mycorrhizal colonization in the roots

In order to evaluate the mycorrhizal colonization, about 1 g of the fine and fresh roots were collected, clarified and stained with trypan blue (Koske and Gemma 1989) and evaluated using the method of intersections in reticulated plates (Giovannetti and Mosse 1980) obtaining the mycorrhizal colonization rate. For the percentage of mycorrhizal colonization, the roots were spread on a Petri plate with a 1.1x1.1 cm grid at the base, and both the horizontal and vertical lines of the grid were observed as well and the total number of intersections between roots and grid lines was written down, and also the number of intersections with mycorrhizal roots with some structure of the mycorrhizal fungi: vesicles, arbuscules, hyphae and spores.

Phytoextraction efficiency

Based on the concentrations of the metals in the plant tissues and in the solutions, the following were determined: Total concentration of metals (TCM) obtained by the sum of the concentrations of metals in the root and in the shoot of plants, according to the equation: TCM = $[Metal]_{root}$ + $[Metal]_{shoot}$. The efficiency of phytoextraction can be quantified calculating the bioconcentration factors (BCF), the translocation factor (TF) and the phytoextraction coefficient (PEC). The BCF indicates the biota efficiency to accumulate metal from the surrounding medium (solution) in its tissues (roots) (Mackay 1982), according to the equation BCF = $[Metal]_{root}$ / $[Metal]_{solution}$. The TF indicate the plant capacity to translocate the metallic ion of the roots to the shoot (stem and leaves) according to the equation: TF = $[Metal]_{shoot}$ / $[Metal]_{root}$ and PEC measures the plant efficiency in extracting the metal from the nutrient solution, according to equation: PEC $[Metal]_{shoot + root}$ / $[Metal]_{solution}$.

Statistical analysis

The data obtained were submitted to analysis of variance applying the F test (ANOVA), considering the significance at the traditional levels of 5 and 1% probability. Since a significant difference was found between the doses, they were submitted to regression analysis and between the fungal strains submitted to Tukey´s test at 5% of probability. All analyses were performed in the Sisvar statistical program (Ferreira 2011).

3. Results and Discussion

In the response variables, dry mass of root (DMR), chlorophyll *a*, lead concentration in the shoot (sPb) and calcium in the root (rCa) have a significant effect on interaction between increased lead concentrations and arbuscular mycorrhizal fungi (AMFs) inocula. To AMFs isolated has significant effect on root collar diameter (RC), fresh mass of root (FMR), mycorrhizal colonization (MC), phosphorus (sP) and potassium (sK) in the shoot and, magnesium in the root (rMg). A significant effect was observed in the increasing lead concentration in shoot length (SL), number of leaves (NL), leaf area (LA), fresh mass of shoot (FMS) and root (FMR), dry mass of shoot (DMS), root volume (RV), and lead concentration in the root (rPb). As observed by Alaboudi et al. (2018) when the concentration of heavy metals in the substrate increased, the fresh and dry weights of the growing plants gradually decreased.

The plant weights and lengths were smaller in all plants grown in a metal rich nutrient solution (300 mg L^{-1}) compared with the same plants grown in control and at low to moderate (0 and 70 mg L^{-1}, respectively) metal contaminated nutrient solution (Figure 1).

V. *polyanthes* plants exposed to increasing lead concentrations in the nutrient solution had a significant decrease of approximately 27.3, 25.8, 30.6, 59.6, 49.1, 40.5, 57.1, and 29.1% in the morphological variables SL, SD, NL, LA, FMS, FMR, DMS and RV, respectively, with negative linear behavior (Figure 1A to 1H). However, in the variables related to the root system of *V*. *polyanthes* plants, fresh mass of root (FMR) and root volume (RV), the behavioral adjustment was a quadratic polynomial with reduction in concentrations above 250 and 270 mg L^{-1}, respectively (Figures 1F and 1H). A possible response is an increase in the synthesis of polysaccharides of the cell wall and cell wall thickness (Rossato et al. 2012). Furthermore, according to Baligar et al. (1998), concentrations of lead below the toxic level stimulate the growth of the plant roots, which could explain the increase of the average values observed in the present study (Figures 1F and 1H).

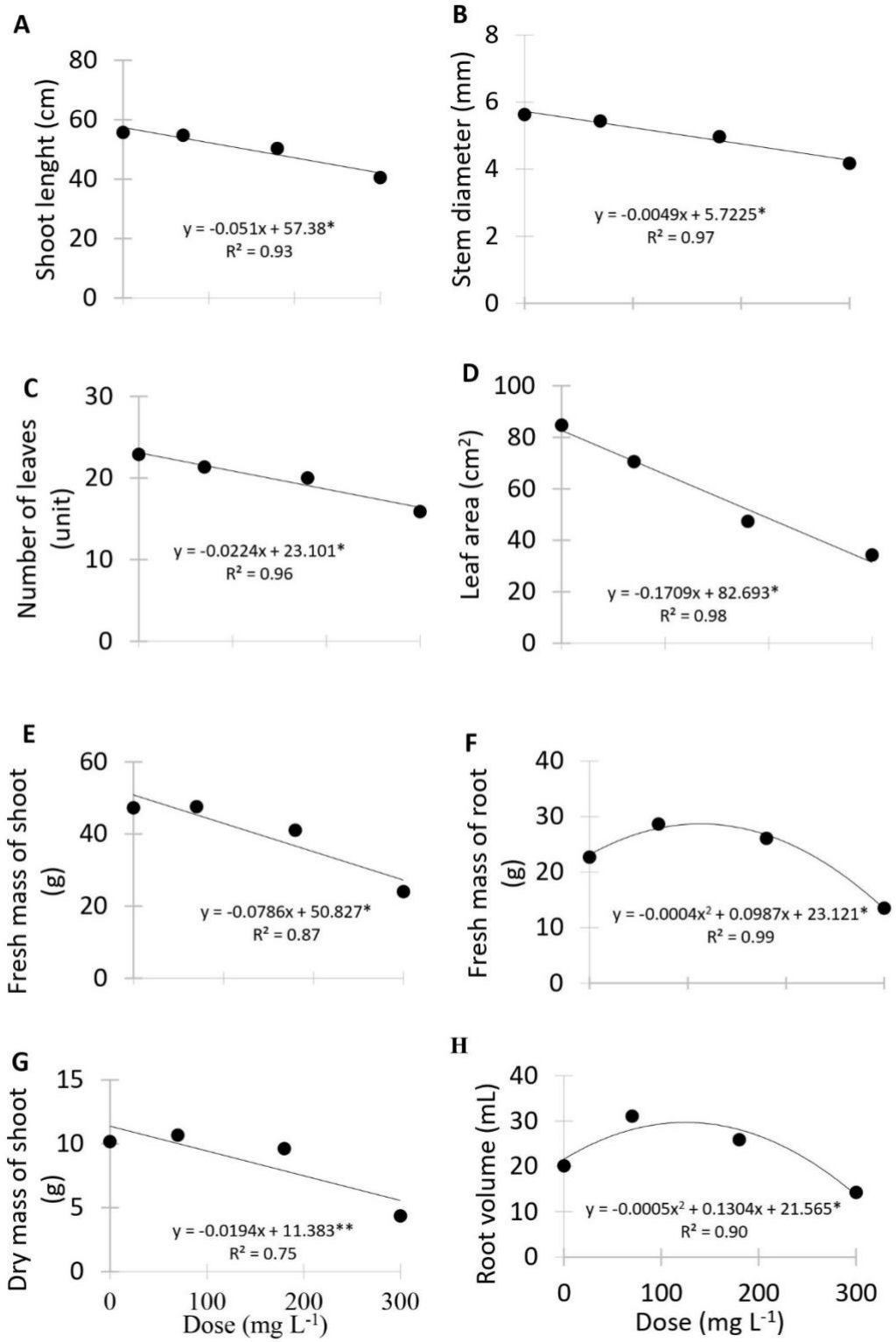

Figure 1. Morphological responses of *Vernonia polyanthes* plants grown in nutrient solution with increasing lead concentration. A – shoot length; B – stem diameter; C – number of leaves; D – leaf area; E – fresh mass of shoot; F – fresh mass of root; G – dry mass of shoot; H – root volume. * e ** significant at 1 and 5%, respectively.

In the study by Alves et al. (2008), vetiver [*Vetiveria zizanioides* (L.) Nash], jureminha [*Desmanthus virgatus* (L.) Willd] and algaroba [*Prosopis juliflora* (SW) DC] plants grown in a nutrient solution containing lead presented a reduction of DMS of 22.9, 32.7, and 24.3%, respectively, at the dose of 200 mg L^{-1}. Decreases in the leaf number and leaf area reflect Pb toxicity. Quebra-pedra (*Phyllanthus niruri* L.) plants also showed a reduction in leaf numbers when exposed to Pb concentrations (100, 200, 400, 800 and 1600 mg kg^{-1} of soil), showing sensitivity to Pb toxicity (Chandrasekhar and Ray 2019). The excessive amount of lead in soil may cause stress-induced changes in plants including growth reduction, decreased biomass,

leaf chlorosis, many other physiological and biochemical changes (Yongsheng et al. 2011). In a study by Zhang et al. (2018), sunflower plants had organelles (thylakoids) damaged, inhibiting their growth in soil contaminated with multi-metals, including Pb. Decline in growth and biomass are common indications of heavy metal-induced toxicity in plants (Rehman et al. 2017). The results of this study were similar to those reported by Chandrasekhar and Ray (2019), where plants of the species *Eclipta prostrata* L. *known as* quitoco, arnica, erva-botão, belonging to the same *V. polyanthes* family (Asteraceae) did not decrease the shoot length, fresh and dry weight and number of leaves, when grown in 100, 200, 400, 800 and 1600 mg kg^{-1} of soil, and the authors considered the results an indication of the potential of that species to survive in soils contaminated with Pb.

In the variable dry mass of root (DMR) there was a negative linear reduction with the increase of the lead concentration and inoculation with *Gigaspora margarita* fungus (GM). However, without inoculation and with the use of *Acaulospora morrowiae* fungus (AM), the adjustment was a quadratic polynomial negative with an increase in DMR and decrease from the approximate concentration of 125 mg Pb L^{-1} (Figure 2A). It´s mean that the plants decreased the grown of the roots only after the toxic level, and below the toxic level the growth of the plant roots were stimulate (Baligar et al. 1998). Furthermore, phytotoxicity of Pb depends on the species, plant tissue, exposure period and concentration (Moraes et al. 2014).

Rossato et al. (2012) observed similar behavior in *Pluchea sagittalis* (Asteraceae) *popularly known as* quitoco or as arnica, where the plants decreased the grown of the roots only after the toxic level. The authors reported an increase of fresh and dry weight of the roots with the application of 200 µM Pb L^{-1} of nutrient solution, decreasing the means with increasing of the Pb levels. Lead causes phytotoxicity by altering cell membrane permeability, reacting with active groups of different enzymes involved in plant metabolism, inhibition of ATP production, lipid peroxidation and DNA damage by excessive production of reactive oxygen species (ROS) (Kumar et al. 2017).

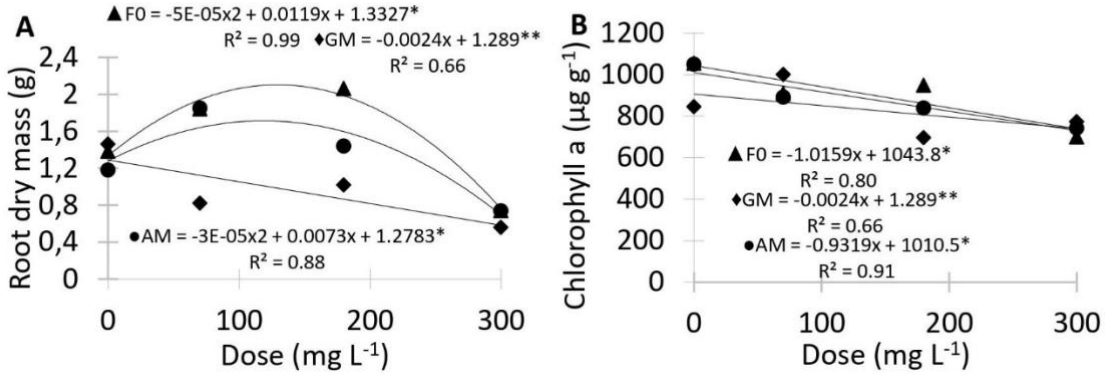

Figure 2. Dry mass of root and chorophyll a of *Vernonia polyanthes* plants grown in a nutrient solution with increasing lead concentration and arbuscular mycorrhizal fungi inocula. A – root dry mass; B – chlorophyll a. * and ** significant at 1 and 5%, respectively. F0 – treatment without fungi inoculation; GM – *Gigaspora margarita*; AM – *Acaulospora morrowiae*.

Figure 2B shows reduction in chlorophyll *a* content of 29.19, 33.71 and 8.57% at the high concentration of lead (300 mg L^{-1}) with AM, F0 and GM, respectively, compared to the control (0 mg Pb L^{-1}). This result is explained because Pb considerably damages the antioxidant system, inducing oxidative stress due to the improved synthesis of reactive oxygen species (ROS) including superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (•OH) (Wu et al. 2018). This process prevents the maintenance of chlorophyll *a* and conversion to chlorophyll *b* through an enzyme called chlorophyll *a* oxygenase, which catalyzes the oxidation of the methyl group to the aldehyde group of these molecules (Xu et al. 2001). In addition, high concentrations of heavy metals damage the thylakoid in the chloroplast stroma negatively affecting photosynthesis (Yang et al. 2015).

Morphological variables have different responses to AM fungi (Table 1). Compared with the non-inoculated control treatment, the stem diameter (SD) of the single inoculation of *Acaulospora morrowiae* (AM) decreased by 30%, and the fresh mass of root (FMR) with *Gigaspora margarita* (GM) width by 30%

(Table 1). Similar results were observed for dry mass of roots (Figure 2A). These results were probably related to the high concentrations of lead in nutrient solutions and to plant exposition.

Table 1. Stem diameter (SD), mycorrhizal colonization rate (MCR), fresh mass of root (FMR) and root Mg content (rMg) of *V. polyanthes* plants exposed to lead in a nutrient solution.

Treatment	SD	MCR	RFM	rMg
AM	4.68[b]	2.34[a]	22.41[ab]	2.82[b]
GM	4.88[ab]	3.32[a]	18.90[b]	2.98[b]
F0	5.61[a]	0.00[b]	27.00[a]	3.91[a]
C.V.	14.64	118.96	26.52	17.98

*AM – *Acaulospora morrowiae*; GM – *Gigaspora margarita*; F0 – Treatment without fungi inoculation. Means followed the same letter do not differ by Tukey´s test at 5%.

No root infection was found in control plants (F0), and the mean proportion of root length colonized in inoculated plants ranging from 2.3 to 3.3% (Table 1). Some publications reported the great efficacy of AM fungi and high sensitivity of isolates from unpolluted soils not adapted to the stress of contaminated soils (Hua et al. 2009). In addition, one of the effects of heavy metals on AMFs is the inhibition of spore germination and hyphae development, rendering mycorrhizae formation difficult, inhibition of colonization and decreased nutrition provided to the AMF (Chen et al. 2015; Yang et al. 2016).

The macronutrient concentrations (P, K and Mg) in the shoot and root of *V. polyanthes* decreased with increasing lead concentration in the nutrient solution (Tables 1 and 3; Figures 3A, 3B, and 3C). The P-concentrations in the shoot of *V. polyanthes* decreased by 26.9, 44.8 and 38.1% according to increasing lead concentrations (0, 70, 180 and 300 mg L^{-1}, respectively) in the nutrient solution of (Figure 3A). A favorable P regime in growth media is known to reduce the effects of Pb toxicity in plants since the ability of Pb to form insoluble phosphates in plant tissues is the answer to decreasing P absorption by plant root and translocation to the shoot (Kabata-Pendias 2011).

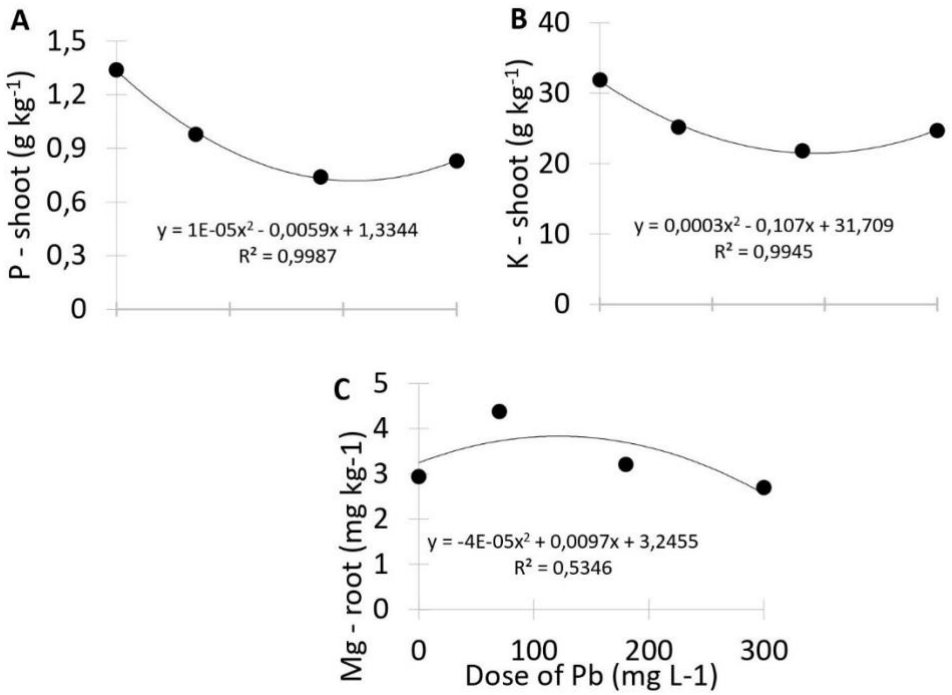

Figure 3. Macronutrient contents of *V. polyanthes* plants grown in nutrient solution with increasing lead concentration. A – P in the shoot; B – K in the shoot; C – Mg in the root.
* e ** significant at a 1 e 5%, respectively.

No effect on phosphorus absorption was observed with inoculation of arbuscular mycorrhizal fungi. However, Meyer et al. (2017) observed increases in phosphorus concentration in the shoot and roots of *vetiver* grown on a heavy metal contaminated substrate.

The same results were observed for K concentration in the shoot of *V. polyanthes* (Figure 3B). According to Sharma and Dubey (2005), the strong interaction of K ions with lead could result from their similar radii (Pb = 1.29 Å and K = 1.33Å). These two ions may compete for entry into the plant through the same potassium channels. Similarly, lead effects on K -ATPase and -SH groups of cell membrane proteins cause an efflux of K from the roots, which explains the decrease in K concentration with the increase of lead in the nutrient solution.

In Figure 3 it was observed that magnesium concentration (rMg) in the root of *V. polyanthes* decreases after exposure to 150 mg L^{-1} lead concentration and it is known that lead exposure decreases the concentration of divalent cations (Zn, Mn, Mg, Ca, and Fe) in leaves and roots (Zhang et al. 2018). Furthermore, Pb inhibits chlorophyll synthesis by causing impaired uptake of essential elements such as Mg and Fe by plants. It damages the photosynthetic apparatus due to its affinity for protein N- and S- ligands (Burzynski 1987; Ahmed and Tajmir-Riahi 1993; Sharma and Dubey 2005; Moraes et al. 2014).

The calcium concentration in the root of *V. polyanthes* decreased linearly with *Gigaspora margarita* (GM) inoculation. However, the opposite response was observed with *Acaulospora morrowiae* (AM) inoculation and in the non-inoculated plants these increases were observed after a dose of 180 mg L^{-1} (Figure 4).

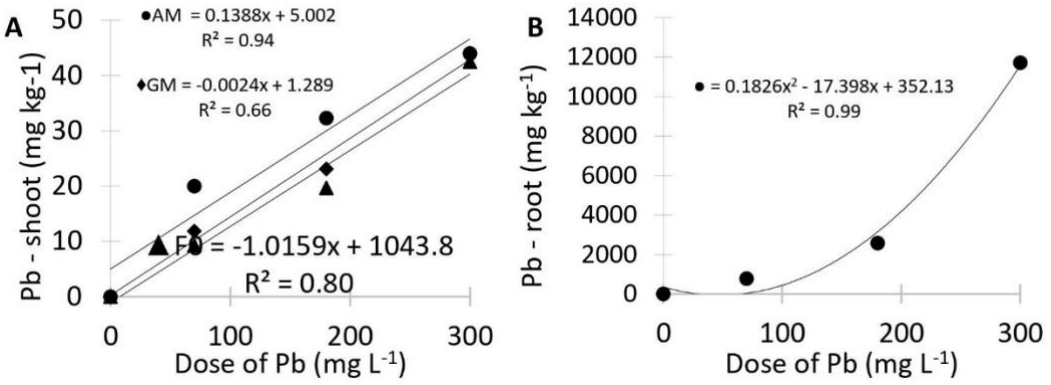

Figure 4. Lead contents in the shoot and root of *V. polyanthes* plants grown in nutrient solution with increasing lead concentration. A – Pb in the shoot; B – Pb in the root. * e ** significant at a 1 and 5%, respectively. F0 – Treatment without fungi inoculation; GM – *Gigaspora margarita*; AM – *Acaulospora morrowiae*.

In treatments F0 and AM the concentrations of Ca in the root, in general, do not decrease or increase as observed in AM (Figure 4). This occurs, because the interference of Pb with Ca is metabolically important since Pb can mimic the physiological behavior of Ca and thus can inhibit some enzymes (Kabata-Pendias 2011). On the other hand, exposure to lead may decrease the concentration of divalent cations, including Ca^{2+} (Kumar et al. 2017). The reduction of Ca concentration with the increase of Pb concentration was observed in bean plants cultivated in a nutrient solution (Cannata et al. 2013). These results suggesting that the enhancements of P, K, Mg, and Ca in mycorrhizal and non- mycorrhizal plants grown on substrate containing metals probably depend on plant species, AMF species and soil conditions (Clark and Zeto 2000).

The doses of 70, 180, and 300 mg L^{-1} presented the lowest averages for Pb concentration in the shoot of plants without the application of AMF (9.2; 19.7; 42.5 mg kg^{-1} dry mass, respectively). The highest averages (20.0, 32.3 and 44.0 mg kg^{-1}) were observed with the application of the AM fungus at all doses applied in the present study (Figure 4A). Probably, Pb may have increased in the shoots of inoculated plants due to the reduction of biomass as a consequence of the higher metal absorption (Figures 1 and 2; Table 1).

Regarding Pb concentration in the roots of *V. polyanthes* plants, there was no significant effect for factor interaction (Pb dose and fungus) and for the fungus factor. However, the dose factor had a significant effect (Table 1; Figure 4B). Zhang et al. (2018) observed that inoculation of AMF (*Funneliformis mosseae* and

Funneliformis caledonium) did not significantly affect the concentration of Cd, Cu, Pb, Cr, Zn and Ni in sunflower roots grown in heavy metal contaminated soil (Cd 33.7; Cu 3,444; Pb 2,883; Cr 386; Zn 14,437 and Ni 373 mg kg^{-1}), also no differences were found in shoot Pb concentrations of legumes (*Robinia pseudoacacia*, *Trifolium pretense* and *Medicago sativa*) and grass (*Lolium perenne*) between non-mycorrhizal and mycorrhizal plants at 500 mg kg^{-1} Pb stress level (Yang et al. 2016).

Gigaspora margarita fungi positively aided the growth of *vetiver* (*Chrysopogon zizanioides*) even with a low colonization rate (6.4%) (Meyer et al. 2017). As previously mentioned, AMF inoculation resulted in higher Pb translocation, resulting in a smaller diameter in *V*. *polyanthes* plants (Table 2). Mnasri et al. (2017), when evaluating the effect of AMFs on heavy metal absorption, in *Medicago sativa* L. observed that AMFs increased the concentration of heavy metals in both roots and shoots. According to Ferrol et al. (2016) AMFs increase the transfer of essential micronutrients, but also nonessential elements to plant tissues.

The lead content in dry mass of root ranged from 780.0 (70 mg L^{-1}) to 11,724.4 mg kg^{-1} Pb (300 mg L^{-1}). Exposure of the *V*. *polyanthes* plant at 180 and 300 mg L^{-1} increased 230 and 1403% of the root metal content compared to 70 mg L^{-1} (Figure 4B). The increase in Pb accumulation in plant roots due to the application of increasing doses of Pb was reported by Roongtanakiat and Sanoh (2011) and Alves et al. (2016). The latter authors found in the roots a maximum Pb content of 24,762 mg kg^{-1} (dose of 303 mg L^{-1} Pb) in vetiver; 12,897 mg kg^{-1} (dose of 400 mg L^{-1}) in castor bean; 9,517 mg kg^{-1} (dose of 400 mg L^{-1}) in sunflower. Pb accumulation was greater in roots than that in shoots of all plants. Lead accumulates mainly in root cells due to blockage by caspary striae within the endoderm, sequestration in rhizodermal and cortical cell vacuoles, immobilization by negatively charged pectins within the cell wall, and accumulation in plasma membranes (Kopittke et al. 2007; Jiang and Liu 2010).

Phytoextraction coefficient (FC), bioaccumulation factor (BF), Translocation factor (TF) and, total concentration of metals (TCM) values ranged from 0.0 to 41.4; 0.0 to 41.39; 0.0 to 0.03 and; 0.0 to 12,460.67, respectively (Table 2). Plants can be classified as accumulators, excluders and indicators according to the bioaccumulation factor, taking into consideration the capacity to accumulate heavy metals (Ma et al. 2001). Phytoremediation efficiency is evaluated by bioaccumulation factors of metals in different plant tissues and is used in several phytoremediation studies in hydroponic systems such as Batista et al. (2017) in the phytoremediation of Pb or in the environmental impact assessment in areas contaminated by multiple metals, as in the research conducted by Boechat et al. (2016a; 2016b).

In general, for all factors increasing lead concentration increased values, except to TF (Table 2). BCF and TF can be used as indicators of the phytoremediation potential exhibited by plant species. For a plant to be considered efficient in translocating metal from roots to the shoot, TF must be equal to/or greater than 1. Plants with BCF greater than 1 and translocation factor less than 1 (BCF > 1 and FT < 1) have a potential for phytostabilization. Phytostabilization is a technology that involves altering soil and metal-tolerant plants to establish ground cover by reducing metal migration to air, surface and groundwater and reducing soil toxicity (Kodre et al. 2017).

Table 2. Bioaccumulation factors in *V. polyanthes* plants cultivated in a hydroponic system with increasing lead concentration.

Treatment	PEC	BCF	TF	TCM
	Treatment without fungi			
0	0.00	0.00	0.000	0.00
70	6.15	6.02	0.012	430.45
180	14.63	14.52	0.005	2633.70
300	37.69	37.55	0.004	11306.85
	Gigaspora margarita			
0	0.00	0.00	0.000	0.00
70	18.02	17.85	0.022	1261.23
180	15.71	15.58	0.008	2828.13
300	38.45	38.31	0.004	11536.42
	Acaulospora morrowiae			
0	0.00	0.00	0.000	0.00
70	9.87	9.58	0.030	690.68
180	13.00	12.82	0.014	2339.67
300	41.54	41.39	0.004	12460.67

PEC – phytoextraction coefficient; BCF – bioconcentration factor; TF – translocation factor; TCM – total concentration of metals.

TF values ranged from 0.004 to 0.03 (Table 2). These results indicate that the *V. polyanthes* plant was not efficient in translocating large amounts of Pb from roots to shoots and was not considered a plant suitable for phytoextraction in contaminated areas. In these cases, the biomass production of the shoot of the plants and the amount of metal removed should be considered, as in these cases it would be justified to use it due to growth tolerance in extremely contaminated environments. Therefore, the *V. polyanthes* plant is a strong candidate for phytostabilization programs considering that it has such characteristics, since the lowest dose (70 mg L^{-1}) studied. According to Garg and Chandel (2010) in some cases, mycorrhizal plants show the characteristic of phytostabilization, reducing the translocation of heavy metals to the shoot. However, in the present study, arbuscular mycorrhizal fungi increased Pb translocation from roots to shoots (Figure 4A).

Correlation analyses were performed between the concentration of Pb and macronutrients in the shoots of the *V. polyanthes* plant grown at increasing doses of Pb. Significant negative correlations were found between Pb levels with Ca and P (Table 3).

Table 3. Pearson correlation coefficients between lead concentration and macronutrient concentration in the shoot and root of *V. polyanthes* plants.

Ion	Ca^{2+}	Mg^{2+}	PO_4^{3-}	K^+	SO_4^{2-}
			Shoot		
Pb^{2+}	-0.593^{*}	0.242^{ns}	-0.738^{**}	-0.553^{ns}	-0.333^{ns}
Ca^{2+}	1	0.166^{ns}	0.626^{*}	0.455^{ns}	0.601^{*}
Mg^{2+}	na	1	-0.115^{ns}	-0.0179^{ns}	0.439^{ns}
PO_4^{3-}	na	na	1	0.795^{**}	0.603^{*}
K^+	na	na	na	1	0.364^{ns}
SO_4^{2-}	na	na	na	na	1
Ion	Ca^{2+}	Mg^{2+}	P	K^+	S
			Root		
Pb^{2+}	0.127^{ns}	-0.525^{ns}	0.210^{ns}	-0.625^{*}	0.206^{ns}
Ca^{2+}	1	0.166^{ns}	0.868^{**}	0.532^{ns}	0.749^{**}
Mg^{2+}	na	1	-0.0104^{ns}	0.484^{ns}	0.211^{ns}
PO_4^{3-}	na	na	1	0.417^{ns}	0.551^{ns}
K^+	na	na	na	1	0.481^{ns}
SO_4^{2-}	na	na	na	na	1

** significant at 1%; * significant at 5%; ns - not significant at 5%; na – not apply.

The decrease of P with increasing Pb concentration was also evidenced in Figure 3A and explained by antagonism between these elements. According to Kabata-Pendias (2011) P absorption is reduced with the increase of Pb concentration. To Ca^{2+}, its reduction with the increase of Pb concentration can be explained by the competition between them. Marschner (1995) states that divalent cations such as Pb^{2+} compete with other cations such as Ca^{2+}. Regarding the roots of cultivated *V. polyanthes* plants in increasing doses of Pb2+, it was noted that there were significant negative correlations for Pb^{2+} and K^+ contents only (Table 3). Increased Pb^{2+} absorption decreae K+ absorption. High Pb concentrations decrease K^+ absorption, similarly to Mg^{2+} and Ca^{2+} which at high concentrations decrease K^+ absorption by plant roots (Cannata et al. 2013).

4. Conclusions

The present study indicates that the *Vernonia polyanthes* plant is tolerant to high concentrations of lead and capable of accumulating significant amounts of Pb in the roots. It is classified as a bioaccumulator of Pb in the root system and can be used in phytostabilization projects. Mycorrhizal fungi increase the accumulation of Pb in the shoot and can be used in projects aimed at heavy metal phytoextraction. Lead at high concentrations decreases the quality of morpho-physiological variables and the absorption of magnesium, phosphorus and potassium in *V. polyanthes* plants. The results suggest that the increase of the major elements in mycorrhizal and non-mycorrhizal plants grown on substrate containing lead nitrate depend on the plant species, AMF species and conditions of the culture medium.

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