Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v40i1.39392

CROP PRODUCTION

Metabolic alterations and X-ray chlorophyll fluorescence for the early detection of lead stress in castor bean (*Ricinus communis*) plants

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ABSTRACT. The remediation of lead-contaminated areas poses a serious challenge to soil chemists because Pb has low solubility in soil. Thus, Pb phytostabilization is considered to be an attractive remediation technique. Castor bean (*Ricinus communis*) is an oilseed crop known for its tolerance to heavy metals, and our aim was to assess the early detection of Pb toxicity and the effects of Pb on the biomass, photosynthetic pigments, antioxidative enzyme activities, and total soluble proteins of this plant. Specimens were grown in a nutrient solution spiked with Pb concentrations of 25, 50, 100, 150, or 200 μmol L^{-1}. A control without Pb was also grown. The results show that X-ray chlorophyll fluorescence is an efficient technique for the early detection of photosystem II alterations driven by Pb toxicity. Castor bean was tolerant to the Pb doses tested; plants presented no changes in photosynthetic pigments, defense enzyme activities, or total soluble proteins in leaves. Given its ability to tolerate and accumulate Pb in its roots, castor bean is a viable alternative for phytostabilization and phytoattenuation of lead-contaminated areas. It is also economically attractive for industrial and biofuel oil production while being used for remediation.

Keywords: tolerance; oilseed crop; photosystem II; Fr/FFr ratios.

Alterações metabólicas e fluorescência de clorofila para detecção precoce da toxicidade de chumbo em mamona (*Ricinus communis*)

RESUMO. A remediação de áreas contaminadas com Pb é uma prática relevante e difícil, pois este é um elemento praticamente imóvel no solo. A fitoestabilização é uma prática considerada ambientalmente atraente para manejo de áreas contaminadas por metais pesados. A mamona é uma espécie produtora de óleo não comestível e apresenta relativa tolerância a metais pesados. O presente trabalho avaliou a toxicidade por Pb utilizando a técnica da fluorescência de clorofila e as alterações provocadas pelo metal na produção de biomassa, produção de pigmentos fotossintéticos, na atividade de enzimas antioxidantes e concentração de proteínas solúveis total. Os resultados demonstraram que a fluorescência de clorofila é um indicador eficiente para detectar precocemente as alterações no fotossistema II causadas pela toxicidade por Pb. As doses de Pb não provocaram alterações nos pigmentos fotossintéticos, na atividade das enzimas antioxidantes e nas proteínas solúveis total nas folhas. A mamona, por sua tolerância e capacidade de acumular Pb nas raízes, pode ser uma alternativa ambiental e economicamente atraente para fitoestabilização e fitoatenuação de áreas contaminadas por Pb. A mamona apresenta adicional vantagem econômica decorrente da utilização do óleo para produção de bioenergia e fins industriais durante o processo de remediação.

Palavras-chave: tolerância; oleaginosa; fotossistema II; razão Fr/FFr.

Introduction

Environmental contamination by lead (Pb) could originate from anthropogenic sources such as sewage sludge, mining, metallurgy, or waste and pollutant emissions from various industrial activities. Once Pb enters the soil system, it can be transferred to various trophic levels, compromising environmental quality (Ren, Wang, & Zhang, 2006; Gamiño-Gutiérrez, González-Pérez, Gonsebatt, & Monroy-Fernández, 2013; Li, Lin, Cheng, Duan, & Lei, 2015; Santos, Nascimento, Matschullat, & Olinda, 2016).

In areas contaminated with Pb, its removal is an onerous and difficult task because it is a practically immobile element in soil, and it presents low translocation in most plants. Phytoremediation practices can be used to remediate impacted areas, and among these practices, phytostabilization can be

an environmentally attractive alternative. Some researchers have demonstrated that castor bean is highly tolerant of heavy metals and metalloids (Costa et al., 2012; Silva, Silva, Araújo, & Nascimento, 2017). Because it is a non-food crop, it has great potential for remediation of contaminated areas, and it has the additional advantage of economic exploitation during the recovery period because it can be used for biofuel production (Berman, Nizri, & Wiesman, 2011) with no restrictions on metal accumulation in the oil (González-Chávez, Ruız Olivares, Carrillo-González, Leal, 2015).

Lead accumulation and the mechanisms involved in its tolerance and toxicity can lead to distinct responses in various plant species. Tolerant plants can sequester and accumulate Pb in the cell wall and/or vacuole, thus restricting its toxicity (Kopittke et al., 2008; Meyers, Auchterlonie, Webb, Wood, 2008; Chandra & Kumar, 2017). In addition, antioxidant enzyme activity in plants cultivated under Pb stress is reportedly a relevant defense mechanism against this element (Kumar, Prasad, & Sytar, 2012; Hamdouche, Aoumeur, Djediai, Slimani, & Aoues, 2012). On the other hand, plants susceptible to Pb toxicity exhibit visual symptoms such as reduced dry matter production (Karimi, Khanahmadi, & Moradi, 2012), chlorosis followed by necrosis and decreased assimilation of nitrogen (Hamdouche et al., 2012; Alkhatib et al., 2011), nutritional imbalance (Sinha, Dube, Srivastava, & Chatterjee, 2006), slower photosynthetic rate, and lower CO_2 concentration in leaves.

Aside from investigations of the toxic effects of Pb on plant tissues (visual symptoms; nutritional imbalance; and morphological, metabolic, and physiological disorders), techniques allowing the identification of toxicity or tolerance in early-stage plants are of great importance for monitoring environmental contamination. Chlorophyll fluorescence uses information about the photochemical activity of plants, allowing the early detection of environmental stress (Corcoll, Bonet, Leira, & Guasch 2011, Marques, Nascimento, Silva, Gouveia-Neto, & Silva, 2017; Silva, Nascimento, & Gouveia-Neto, 2017). This is possible because the chlorophyll molecule is fluorescent, and through photon dissipation, changes in electron transfer at the level of chloroplast membranes can be detected (Lin, Liu, Lin, Pan, & Peng, 2007). Another significant advantage of this technique is that it is sensitive to photosynthetic cell membrane disorders but does not destroy plant tissue (Cherif et al., 2010; Silva, Nascimento, Gouveia-Neto, & Silva-Jr., 2015; Marques & Nascimento, 2013).

In this study, we evaluated the toxicity of Pb using the non-destructive chlorophyll fluorescence technique and evaluated changes in the production of biomass and photosynthetic pigments, the activity of antioxidant enzymes, and the concentration of total soluble proteins. We aimed to use this species in phytostabilization or phytoattenuation remediation programs.

Material and method

Castor bean seeds (*Ricinus communis* cv. BRS Energia) were germinated in trays containing vermiculite moistened with a 0.67 mmol L^{-1} Ca solution ($Ca(NO_3)_2.4H_2O$) (Vilela & Anghinoni, 1984). Twenty-eight days after sowing, the seedlings were transferred to plastic pots with 6 L of nutrient solution, which was replaced weekly (Hoagland & Arnon, 1950), containing: 105.05 mg L^{-1} N, 15.5 mg L^{-1} P, 117.3 mg L^{-1} K, 100.2 mg L^{-1} Ca, 24.3 mg L^{-1} Mg, 32.1 mg L^{-1} S, 0.65 mg L^{-1} Cl, 0.5 mg L^{-1} Mn, 0.05 mg L^{-1} Zn, 0.02 mg L^{-1} Cu, 0.5 mg L^{-1} B, 0.01 mg L^{-1} Mo, and 7.35 mg L^{-1} Fe. Deionized water was added daily to replace the water lost by evapotranspiration. The pH was adjusted with H_2SO_4 or NaOH 1 mmol L^{-1} to values close to 5.6 (+/- 0.2) whenever necessary. Doses of 25, 50, 100, 150, and 200 μmol L^{-1} Pb [$(CH_3COO)_2Pb_3H_2O$] were added to the solution after 14 days of culture. The control did not contain added Pb.

The plants were kept in a greenhouse for 28 days once Pb was added. The plants were collected at the end of the growth period. Leaves, stems and roots were separated, dried and weighed to obtain their respective biomasses. Digestion of powdered plant material was carried out in a microwave oven. In the digestion extract, Pb concentrations were determined by atomic absorption spectrophotometry (Perkin Elmer, AAnalyst 800).

Chlorophyll fluorescence measurements were carried out with a UV LED light, with red (685 ηm) and far-red (735 ηm) peaks obtained by the appliance's software (Ocean Optics SpectraSuite). Four evaluations were performed throughout the experiment. The first was carried out before metal addition, and the last was carried out the day before plant collection. These evaluations were performed at night, after leaving the plants in the dark for 20 min. in order to ensure the inactivation of electron transport in

photosynthesis. Analyses were carried out using the second pair of leaves below the apical meristem, with four readings per plant submitted to light emission for 10 seconds.

The spectra were fitted to two Gaussian curves corresponding to 685 ηm and 735 ηm. The peak height of the fluorescence intensity F685/F735 ratio (Fr/FFr) was calculated from the fitted curve for Pb concentration and used to infer the effect of the element on the biosynthesis of chlorophyll and PSII via Origin 6.0 software.

One sample per plant was collected from the same pair of leaves used in the evaluation of chlorophyll fluorescence for the analyses of photosynthetic pigments. The determination of chlorophylls *a* and *b* as well as carotenoids was carried out using extraction with 80% acetone (Arnon, 1949). The equation suggested by Lichtenthaler (1987) was used for carotenoid determination.

The crude extract used in the determination of enzyme activity and protein content was obtained by mixing 200 mg of plant material in a mortar with liquid N_2 and 2.0 mL of potassium phosphate buffer (100 mmol L^{-1}, pH 7.0). The homogenate was centrifuged at 14,000 g for 25 min. at 4°C. The supernatant was collected and stored in a freezer at 80°C. Ascorbate peroxidase (Nakano & Asada, 1981), catalase (Havir & McHale, 1987), polyphenoloxidase (Kar & Mishra, 1976), and total soluble protein content (Bradford, 1976) activities were determined by spectrophotometry.

The experiments were conducted in a randomized block design with three replicates. Data were analyzed using ANOVA and regression analysis.

Result and discussion

Biomass production and phytotoxicity symptoms

During the growing period, the dry matter yield from the leaves, stems, and roots did not change with the dose of Pb in the nutrient solution (Table 1). Some researchers have shown that Pb toxicity causes a reduced transpiration rate and inhibition of the photosynthetic rate, resulting in visual changes such as slowed growth, foliar chlorosis, leaf wilt, and fruit deformation (Zhao, Ye, & Zheng, 2011; Lou, Luo, Hu, Li, & Fu, 2012). However, our castor bean plants did not exhibit any of these symptoms, suggesting they are tolerant to the metal. Differences between cultivars could result in lesser or greater susceptibility to heavy metal stress (Romeiro et al., 2006; Niu, Sun, & Sun, 2009; Costa et al., 2012).

Table 1. Biomass of castor bean plants grown under different doses of Pb in the nutrient solution. Values between parentheses refer to standard deviation of the mean.

Pb doses	Dry matter (g pot^{-1})							
(μmol L^{-1})	Leaves ^{ns}		Stem ^{ns}		Roots ^{ns}		Total ^{ns}	
0	28.13	(11.27)	17.06	(7.18)	17.73	(3.34)	62.91	(21.52)
25	23.44	(5.90)	13.61	(3.32)	15.32	(4.36)	52.37	(12.04)
50	29.77	(5.90)	18.53	(6.12)	17.68	(1.44)	65.98	(12.14)
100	23.04	(5.58)	21.45	(7.22)	19.97	(1.60)	64.46	(3.75)
150	27.68	(5.07)	16.74	(4.36)	19.22	(0.93)	63.65	(9.74)
200	28.53	(9.74)	15.88	(7.05)	18.45	(4.64)	62.85	(21.24)

^{ns} Not significant.

Pb distribution in plants

Greater Pb doses were accompanied by increased Pb concentrations in the leaves and roots (Figure 1). From the control to the highest Pb dose (200 μmol L^{-1}), the increase was 52% in the leaves and 48572% in the roots, similar to what was found by Romeiro et al. (2006). In that case, accumulation was approximately 500 mg kg^{-1} and 24,000 mg kg^{-1} in the leaves and roots, respectively, at a Pb dose of 200 μmol L^{-1}. Costa et al. (2012) verified that the distribution of metal is 1.4% in the leaves and 98.6% in the roots in castor bean plants grown with a Pb dose of 96 mg L^{-1}.

We found that when dosing with Pb at 200 μmol L^{-1}, the roots contained 382 times the amount in the leaves. Metal sequestration in the vacuoles of the root cells is a defense mechanism of plants that prevents the absorbed metal from being translocated to the aerial areas (Kumar et al., 2012). The ABC transporter gene is one responsible for detoxifying Pb in the roots of plants (Pal, Banerjee, & Kundu, 2013). The highest accumulation (1,312 mg kg^{-1} of Pb) in the roots of plants grown with the 200 μmol L^{-1} dose of Pb demonstrates the defense strategy in which the roots contribute to the plant's tolerance when high doses of this metal are in the soil. This is also an outstanding, advantageous feature for the phytostabilization or phytoattenuation of lead-contaminated areas. The benefit of vegetal coverage is accompanied by the advantage of a natural barrier, preventing the metal from being transported by erosion, leaching, or runoff (Melo, Costa, Guilherme, Faquin, & Nascimento, 2009; Andreazza, Bortolon, Pieniz, & Camargo, 2013; Pandey, 2013).

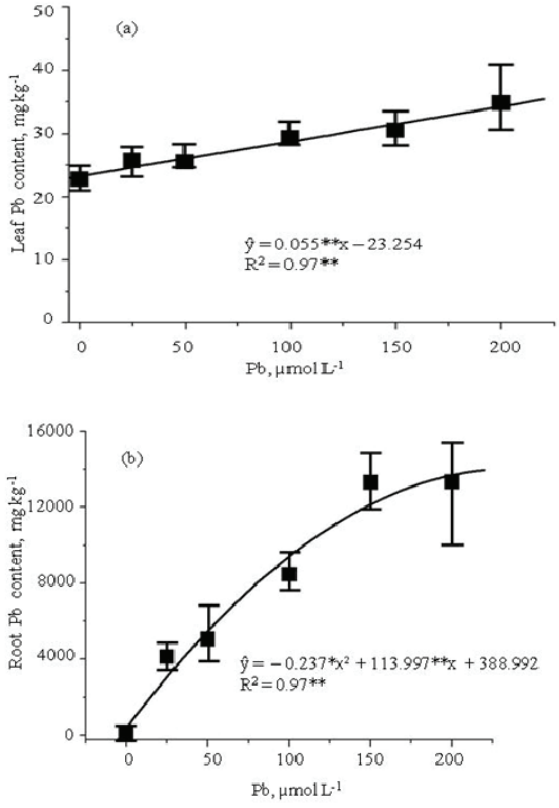

Figure 1. Pb levels in leaves (a) and roots (b) of castor bean plants grown under different Pb doses in the nutrient solution. * and **: significant at the 5% and 1% probability levels, respectively.

Some researchers have shown that Pb accumulation in the cells of roots can be observed in several cellular components, inducing tolerance or indirectly causing disturbances in the photosynthetic, nutritional, and metabolic apparatuses of plants. Piechalak, Tomaszewska, Baralkiewicz, and Małecka (2002), evaluating the accumulation and distribution of Pb in fava (*Vicia faba*), pea (*Pisum sativum*), and bean (*Phaseolus vulgaris*) plants, found that after a 96-h exposure to a dose of 0.001 mol L^{-1}, only 5% to 10% of the accumulated metal was translocated to the shoots, and the greatest Pb content, found in the roots, was located in cell walls and nuclei. These authors also noted that if 1% of the Pb accumulated in the cytoplasm of the root cells, then it was sufficient to activate the plant's defense mechanism and raise the production of phytochelatins. Samardakiewicz and Woźny (2000), evaluating the accumulation of Pb in the root cells of an aquatic plant (*Lemna minor* L.), verified that after 1h of exposure to the metal (at 15 µmol L^{-1}), greater accumulation could be found in the cell walls, vesicles, and small vacuoles. Kopittke et al. (2008), evaluating Pb accumulation in the root cells of Brachiaria (*Brachiaria decumbens* Stapf) plants and Rhodes grass (*Chloris gayana* Knuth), found the initial presence of the metal in the cytoplasm and cortical cells when the dose was no more than 20 µmol L^{-1} and 5.5 µmol L^{-1}, respectively. A greater part was sequestered by the vacuole in the form of pyromorphite [Pb_5 (PO_4) 3Cl]. These authors suggested that the presence of pyromorphite in the Golgi complex is an additional defense mechanism against Pb accumulation in the cell wall of the roots of Brachiaria plants, a behavior not observed for Rhodes grass, a plant that is sensitive to Pb. Meyers et al. (2008), evaluating the distribution of Pb in the root system of Indian mustard (*Brassica juncea*), verified the deposition of this metal in extracellular compartments, suggesting that this complexation occurred as a result of Pb binding to anionic sites. Małecka, Piechalak, Morkunas, and Tomaszewska (2008), studying the defense mechanism of pea plants (*Pisum sativum*) under conditions of Pb toxicity, verified changes in root mitochondria treated with Pb at a dose of 0.5 or 1.0 mmol L^{-1}. They found reductions in mitochondrial crests, increases in mitochondrial volume, changes in mitochondrial shapes, and the presence of granules within peroxisomes and mitochondria when Pb was present. The authors state that Pb toxicity is relevant in these non-photosynthetic organelles because they are responsible for ATP generation and the storage of antioxidant enzymes, which is performed in the peroxisomes (Mhamdi, Noctor, & Baker, 2012).

Chlorophyll fluorescence

Differences in the absorption peaks in chlorophyll fluorescence spectra were observed (Figure 2a). The lowest fluorescence reabsorption was observed in plants grown at the greatest Pb dose (Figure 2b), demonstrating that chlorophyll fluorescence is sensitive enough to detect changes in photosystem II (PSII) caused by Pb toxicity in plants.

The Fr/FFr ratio shows that Pb doses promoted temporal changes in photosynthetic biosynthesis, and these were detected after as few as 10 days of cultivation and became more intense 18 days after the addition of the metal (Figure 3a). It is interesting to note that the increase in chlorophyll fluorescence ratios corroborated the Pb levels in the leaves (Figure 3b), indicating that the plants presented stress in the photosynthetic apparatus even without displaying visual Pb toxicity symptoms. The stress resulting from heavy metals causes poorer efficiency in the PSII reaction, triggering inhibition in the

phosphorylation reaction (Romanowska, Wasilewska, Fristedt, Vener, & Zienkiewicz, 2012) at the association of thylakoids with polyamine molecules, in turn inducing a process of re-adaptation in the photosynthetic apparatus at the molecular level (Abreu, Coscione, Pires, & Paz-Ferreiro, 2012).

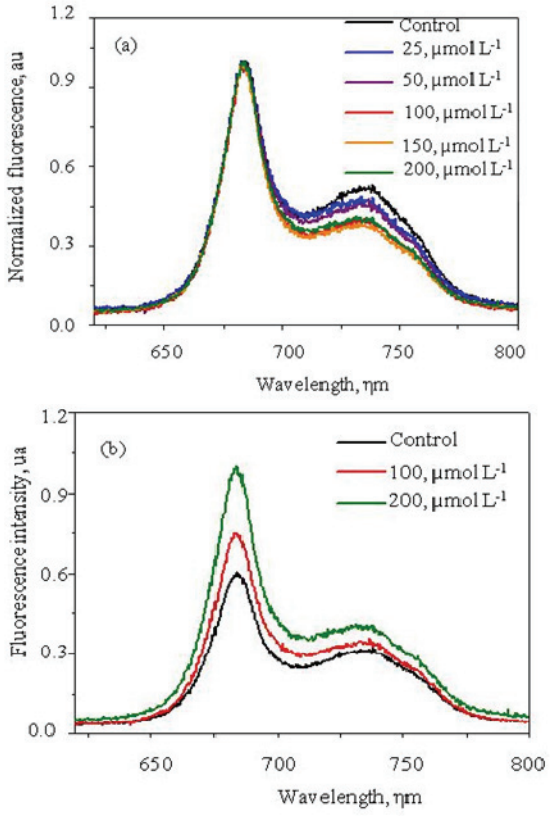

Figure 2. Gaussian curve-fitted chlorophyll fluorescence spectra of the control and Pb treated castor bean plants leaves (a) and Maximum intensity of the chlorophyll fluorescence for the highest doses of Pb in the nutrient solution (b).

Measuring chlorophyll fluorescence is non-destructive, and it can evaluate changes in chloroplast membranes and elucidate damage in the photosynthetic apparatuses of plants (Krause & Weis, 1991). The values for the Fv/Fm ratio (maximum fluorescence / maximum fluorescence emission variation) and Fr/FFr ratio (maximum peak in the red / peak region in the red-distal region), obtained by measuring the chlorophyll fluorescence bands, can be used to detect stress in PSII. A decline in chlorophyll concentration indicates an abnormal condition in the photon metabolism and, consequently, a reduction in the Fv/Fm ratio. However, the Fr/FFr ratio increases when PSII is disturbed. The Fv/Fm and Fr/FFr ratios demonstrate opposite behaviors under normal photosynthetic conditions (Marques & Nascimento, 2013; Silva et al., 2015).

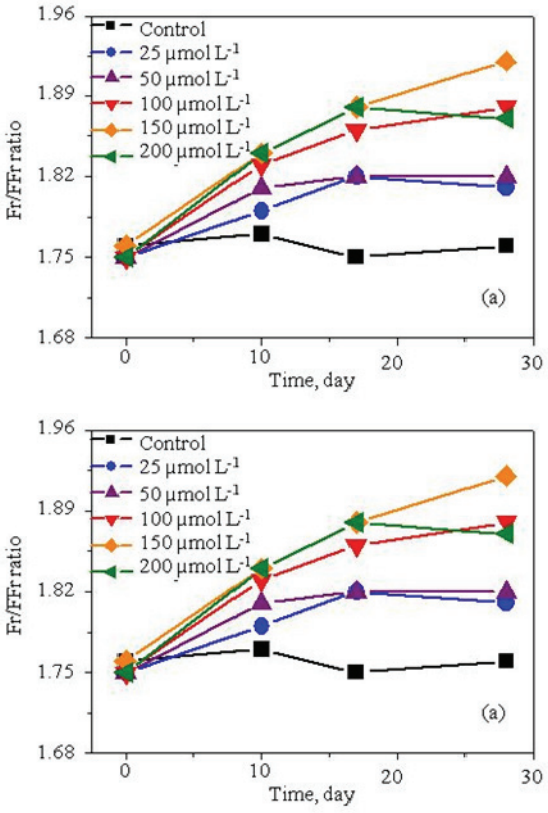

Figure 3. Ratio of chlorophyll fluorescence spectra as a function of the cultivation time: red = 680–700 nm and far-red = 730–740 nm (Fr/FFr) (a). Ratio of chlorophyll fluorescence spectra at 28 days in castor bean plants grown under different Pb doses in the nutrient solution, referring to the red and farred (Fr/FFr) readings and Pb content in the leaves (b).

Use of the Fr/FFr ratio allowed detection of changes in PSII when plants showed no visual symptoms of toxicity. Therefore, chlorophyll fluorescence is a useful tool for monitoring Pb toxicity in castor bean plants, corroborating results found in studies of Cd toxicity (Silva, Nascimento, Gouveia-Neto, & Silva-Jr., 2012; Marques & Nascimento, 2013; Silva, et al., 2017), As toxicity (Stoeva, Berova, & Zlatev, 2004; (Silva et al., 2015), Ni toxicity (Gopal, Mishra, Zeeshan, Prasad, & Joshi, 2002; Mishra & Gopal, 2008), Cu and Hg toxicities (Ventrella, Catucci, Piletska, Piletsky, & Agostiano, 2009), Zn (Cherif et al., 2010; Marques & Nascimento, 2014; Marques et al., 2017) and Pb toxicity (Marques et al., 2017).

Pigments, enzyme activities, and total soluble protein

Pigment concentrations were not influenced by Pb (Table 2). Interestingly, these results corroborate the non-visualization of any chlorosis symptoms in

the leaves as well as the absence of nutritional imbalance (data not shown). In addition, although Pb did not cause damage to chlorophyll biosynthesis, a remarkable change in chlorophyll fluorescence was observed, indicating that this technique is efficient for early detection of Pb toxicity at the membrane level of chloroplasts in castor bean plants. According to Buschmann (2007), the Fr/FFr ratio depends primarily on chlorophyll content and, to a lesser extent, the photosynthetic activity, optical characteristics and cellular arrangements of the leaf tissue. According to Siedlecka and Krupa (2004), Rubisco (ribulose-1.5-bisphosphate carboxylase/oxygenase) is an abundant and very important enzyme in the Calvin cycle because it participates in the catalysis of carboxylation and oxygenation reactions. Under stress by heavy metals, these can substitute the Mg in the active center or subunits of the Rubisco and, consequently, hinder its normal activity, causing changes in the Calvin cycle function. This inhibits electron transport in the photosynthetic apparatus and damage to PSII.

No significant response to the presence of Pb was observed in the activities of the enzymes ascorbate peroxidase, catalase, and polyphenoloxidase or in total soluble protein concentration (Table 3). Heavy metal toxicity could induce production of reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, and hydrogen peroxide, all of which interact with cellular components, causing oxidative damage and subsequent cellular deterioration (Gadjev, Stone, & Gechev, 2008). In plant species tolerant to heavy metals, ROS content can be controlled by an efficient mechanism of antioxidant enzymes (Jamil, Abhilash, Singh, & Sharma, 2009; Lin & Aarts, 2012; Juknys, Vitkauskaite, Račaite, & Vencloviene, 2012). This is an important defense mechanism in the homeostatic balance that reduces heavy metal toxicity in plants (Sun, Zhou, Sun, & Jin, 2007; Yadav et al., 2009).

Nautiyal and Sinha (2012) did not observe changes in chlorophyll *a* concentration in pigeon pea (*Cajanus cajan*) leaves with Pb doses up to 0.2 mmol L^{-1}, but the production of carotenoids was stimulated at a dose of 0.05 mmol L^{-1}. These authors also observed that Pb doses up to 1 mmol L^{-1} caused proline accumulation and induced elevation in the activities of the enzymes ascorbate peroxidase and superoxide dismutase in the leaves as well as an increase in non-protein substances with thiol groups in the roots. Alkhatib et al. (2011), evaluating the toxicity of Pb in tobacco (*Nicotiana tabacum*), verified that the metal did not affect pigment content. The authors did not observe anomalies in the thylakoid membranes when Pb was less than 10 μmol L^{-1}; however, chloroplasts treated with 500 μmol L^{-1} exhibited alterations in their compositions and fewer thylakoids. On the other hand, our results were contrary to those found by Kiran and Prasad (2017), who verified a reduction in chlorophyll *a* and *b* content in the leaves of *R. communis* by approximately 50% and 30% at Pb doses of 200 and 400 μM, respectively. Pal et al. (2013) found a 23% reduction in chlorophyll *a* and *b* content in *R. communis* when the soil was dosed with 800 mg kg^{-1} Pb.

Table 2. Pigment contents in castor bean plants grown under different doses of Pb in the nutrient solution. Values between parentheses refer to standard deviation of the mean.

Pb doses (μmol L-1)	Chlophyll *a* [ns]	Chlophyll *b* [ns]	Chlophyll Total [ns]	Carotenoids [ns]
	(mg g^{-1}) of dry matter			
0	0.92(0.07)	0.39(0.05)	1.31(0.11)	0.33(0.05)
25	0.73(0.12)	0.34(0.05)	1.07(0.17)	0.29(0.11)
50	0.96(0.17)	0.41(0.07)	1.36(0.25)	0.34(0.27)
100	0.95(0.06)	0.39(0.02)	1.33(0.08)	0.32(0.03)
150	1.08(0.16)	0.45(0.07)	1.53(0.23)	0.37(0.23)
200	1.01(0.19)	0.43(0.05)	1.43(0.24)	0.33(0.26)

[ns] Not significant.

Table 3. Ascorbate peroxidade (APX), catalase (CAT), polifenoloxidase (PPO), and total soluble protein (TSP) of castor bean plants grown under different doses of Pb in the nutrient solution. Values between parentheses refer to standard deviation of the mean.

Pb doses (μmol L-1)	APX [ns]	CAT [ns]	PPO [ns]	TSP [ns]
	(μmol H_2O_2 g^{-1} $min.^{-1}$ FM)		(μmol piragalol g^{-1} $min.^{-1}$ FM)	(μg g^{-1} FM)
0	58570.24 (1579.81)	1347.89 (228.20)	39705.02 (5617.19)	34.88 (5.53)
25	66974.65 (15066.66)	1702.598 (511.47)	30284.00 (9322.16)	33.08 (7.03)
50	42934.14 (22361.32)	1165.469 (275.87)	37862.42 (5885.33)	33.08 (6.91)
100	39481.17 (3728.97)	1510.042 (310.55)	31888.84 (5764.55)	40.11 (7.69)
150	51012.79 (6957.08)	1135.065 (243.86)	30343.43 (9740.82)	36.55 (9.48)
200	48471.93 (2254.05)	1520.177 (30.40)	29808.49 (669.18)	45.29 (2.85)

[ns] Not significant.

R. communis cv. BRS Energia showed integrity in chlorophyll *a* and *b* content, the activities of antioxidant enzymes, and the concentration of total soluble protein under Pb stress. An important defense mechanism used by the plants was metal accumulation in the roots and survival without greater damage from the metal.

Conclusion

The use of chlorophyll fluorescence is efficient for detecting changes in photosystem II that result from Pb toxicity. Lead doses do not cause alterations in photosynthetic pigments, antioxidant enzyme activities, and total soluble proteins in the leaves, showing metal tolerance. The castor bean, because of its tolerance of Pb and ability to accumulate Pb in the roots, can be an environmentally and economically attractive alternative for phytostabilization and phytoattenuation of lead-contaminated areas. It has the additional economic advantage of providing oil for industrial purposes and bioenergy production while it is used during remediation.

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Received on September 9, 2017.
Accepted on December 15, 2017.