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GROWTH AND BIOCHEMICAL RESPONSES OF MAIZE CULTIVATED IN SOILS CONTAMINATED WITH CADMIUM AND ZINC

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ABSTRACT – Contamination with trace elements is characterized by an abiotic stress that represents a limiting factor in agricultural production. Considering that cadmium (Cd) and Zinc (Zn) are physically and chemically similar, they can interact with the environment, causing antagonistic or synergistic effects. In this sense, physiological mechanisms to exclude, detoxify or compartmentalize the excess of those trace elements is crucial for the survival of the plants under conditions of high concentrations of these elements. In order to understand the responses of the species sensible to the presence of Cd and Zn, this study aimed to access the behavior of maize plants (*Zea mays* L.) cultivated in Cambisoils and Latosol with growing concentrations of Cd/Zn in a 21 days period. Growth and biochemical analyzes were performed such as antioxidant enzyme SOD, CAT and APX activity, hydrogen peroxide and lipid peroxidation. The data obtained evidenced the specific behavior of maize plants grown in Cambisoils compared to Latosol, showing a superior activity of all the enzymes analyzed, and also a lower content of hydrogen peroxide and lipid peroxidation. The interaction between both the elements resulted in a synergistic effect, negatively influencing all the analyzed parameters.
Keywords: trace elements, antioxidant enzymes, ROS, synergistic.

CRESCIMENTO E RESPOSTAS BIOQUÍMICAS DE PLANTAS DE MILHO CULTIVADAS EM SOLOS MULTICONTAMINADOS COM CÁDMIO E ZINCO

RESUMO - A contaminação por elementos traço caracteriza-se como um estresse abiótico que representa um fator limitante para a produção agrícola. Em função das semelhanças físicas e químicas entre cádmio (Cd) e zinco (Zn), esses elementos podem interagir no ambiente, podendo causar efeitos antagônicos ou sinérgicos. Nesse sentido, mecanismos fisiológicos para excluir, desintoxicar ou compartimentalizar o excesso de elementos traço são cruciais para sobrevivência dos vegetais quando expostos a elevadas concentrações desses elementos. A fim de compreender melhor as respostas de espécies sensíveis à presença de Cd e Zn, o presente estudo teve como objetivo avaliar o comportamento de plantas de milho (*Zea mays* L.) cultivadas em Cambissolo e Latossolo contendo concentrações crescentes de Cd/Zn. As plantas foram expostas a doses crescentes de Cd/Zn, por um período de 21 dias. Foram realizadas análises de crescimento e análises bioquímicas tais como, atividade das enzimas antioxidantes SOD, CAT, APX, assim como a quantificação do peróxido de hidrogênio e peroxidação lipídica. Os dados obtidos evidenciaram comportamento específico das plantas de milho cultivadas em Cambissolo, quando comparado ao Latossolo, apresentando uma atividade superior, em todas as enzimas analisadas, e um menor conteúdo de peróxido de hidrogênio e peroxidação lipídica. Observou-se que a interação entre ambos os elementos resultou em um efeito sinérgico, afetando negativamente todos os parâmetros analisados.
Palavras-chave: elementos-traço, enzimas antioxidantes, EROs, sinergismo.

Contamination by trace elements stands out among the factors that limit plant production. This abiotic stress affects the development of crops considered sensitive, with effects on productivity caused by physiological disturbances that harms the development and incomes (Srivastava et al., 2012). The biological effects of individual trace elements are well documented, and although the combinations between them are usual in nature, the mixed effects still need further investigations (Tkalec et al., 2014). The Cd often follows Zn minerals in the environment due to its chemical and physical resemblances (Gallego et al., 2012). It is usual to see antagonistic effects in studies that investigate the interactions between Cd/Zn and its accumulation and absorption, nevertheless synergistic effects are reported (Puga et al., 2015; Cherif et al., 2011). The Zn supplementation in lower concentrations can reduce the oxidative stress induced by Cd, while high levels of both minerals can induce accumulation of the oxidative stress (Cherif et al., 2011).

All trace elements, essential and non-essential, when present in concentrations higher than optimum will affect different cellular components, interfering and disturbing the homeostasis of the plant cell straight through the accumulation of reactive oxygen species (ROS) (Tkalec et al., 2014). Toxic levels of ROS can generate oxidative stress, leading to inactivation and damage of the proteins and lipids on the cell membrane (Sharma et al., 2012). To avoid the harmful effects of the ROS, plants activate enzymatic antioxidants, including superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Sharma et al., 2012).

Among the species considered sensitive to contamination with trace elements, maize (*Zea mays*) is one of the crops with more physiological disturbances caused by this abiotic stress (OECD, 2006). This study aimed to evaluate the growth rates, antioxidant system enzyme activities and oxidative damages in maize plants cultivated in different classes of soil (Cambisoils and Latosol), in order to understand the physiological behavior of plants exposed to the interaction between Cd/Zn.

Material and Methods

This essay was performed in a greenhouse at the Departamento de Ciência do Solo from the Universidade Federal de Lavras/UFLA, in Lavras city, Minas Gerais state, Brazil (44º55'W; 21º05'S) using hybrid corn seeds Cultivar BM207, S-1, minimum purity 98%, germination 85%. Two classes of soil were detected and classified as dystrophic Cambisoils typic to moderate texture, and red yellow dystrophic Latosol, typical with medium to moderate texture, both in Tropical Forests. These soils are considered clear of Cd, with QRV < 0.4 mgCd. kg^{-1} dry weight (Fundação Estadual do Meio Ambiente, 2011). The soils were collected and sieved, and a sample was used to perform chemical and physical analysis (Tables 1 and 2).

Fertilization was accomplished using Malavolta's (1981) protocol, using 500g of soil in plastic pots and irrigation at 70% field capacity. After this, different doses (D) combining Cd and Zn were added, using $Cd(NO_3)_2.4(H_2O)$ and $ZnSO_4.7H_2O$ solutions as a source of these elements and a control: D0: 0.0 + 10.0 D1: 0.4 + 14.89; D2: 0.72+28.80; D3: 1.29+48; D4: 2.3 + 86.64; D5: 4.1+152.64; D6: 13.6+506.89; D7: 24.4+908.61 mg kg-1, respectively. Zn concentrations on D0 corresponded to the quantity of fertilization according to Malavolta (1981).

The doses were determined using the parameters established by the Conselho Nacional de

Table 1. Chemical analysis of the nutrients from Cambisoil and Latosol collected in Minas Gerais state, Brazil, used in the study to evaluate the toxicity of Cadmium and Zinc in maize (*Zea mays*).

Soils	pH	K	P	Ca	Mg	Al	CTCef	V	OM
		mg dm^{-3}		-------- cmol dm^{-3} --------				----- % -----	
Latosol	4.8	32	1.13	0.3	0.1	0.6	1.08	9.64	1.64
Cambisoil	5.3	34	2.60	1.6	0.4	0.5	2.59	34.05	2.87

1:25; P, K: Extractor Mehlich 1; S: Monocalcium phosphate extractor in acetic acid; Ca^{+2}, Mg^{+2} and Al^{+3}: Extrator KCl 1 mol L^{-1}, H+ Al: Calcium acetate extractor 0.5 mol L^{-1}, pH = 7.0; MO: oxidation Na_2CrO_7 4N + H_2SO_4 10N; SB:Base Sum; CTCef.: Effective cation exchange capacity; V: Base saturation index; OM:Organic matter.

Table 2. Physical analysis of the soils.

Soils	Texture (%)		
	Clay	Silt	Sand
Latosol	24	12	64
Cambisoil	31	22	47

Meio Ambiente (BRASIL, 2009), resolution 420. The values used in the experiment were multiples of 1.8, according to the normative suggestion of the OECD (2006). The doses were in a molar relation of 64/1 for Zn/Cd, approximately the same concentration found on a mining areas (Carvalho et al., 2013).

The experiment lasted twenty-one days, when the maize plants emerged. The essay used a completely randomized design (CRD) in an 8x2 factorial scheme, with eight treatments (seven doses that increased with Cd/Zn and one control), two soils (Cambisoil and Latosol), with six replications. The experimental units consisted of a pot with 500g of soil and five plants. The analysis of variance (ANOVA) was performed using the SISVAR 4.3 program (Ferreira, 1999). Mean values were compared using Scott-Knott test at 0.05% probability ($p \leq 0.05$).

Cadmium and Zinc on the plants

The concentrations of Cd and Zn were determined on the dry roots and the shoots in extracts obtained by acid digestion using a microwave, according to the method proposed by USEPA 3051A from the United States Environmental Protection Agency – USEPA, 1998. The data were obtained using a spectrophotometer with atomic absorption with atomization by flame of graffiti oven, according to the concentrations found on the extracts.

BCR-482–Institute for Reference Materials and Measurements (Geel, Belgium) was used as reference, with certified concentrations of Zn (100.6 mg kg^{-1}) and Cd (0.56 mg kg^{-1}). The recovered concentrations were 120% and 65% for Zn and Cd, respectively.

The limit for the detection (LD) was calculated using standard deviation and mean values of seven readings of the white sample, considering t value of Stuart for n=7 using the formula (American Public Health Association, 1998): $LDM = (x + t \times s) \times d$, where: $\times$ = mean concentration of the interest substance in seven white samples; t = Student value at 0.01 probability and n-1 degrees of freedom (for n = 7 and $\alpha = 0.01$, t = 3.14); s = standard deviation for the seven white samples; d = dilution factor used on the digestion of the sample. The limits for Zn and Cd detection were 1.22 mg kg^{-1} and 1.66 µg kg^{-1} for Zn and Cd, respectively.

Growth analysis and antioxidant metabolism

To obtain the mass of the roots and shoots, the maize plants were dried in an oven at 70ºC temperature until constant weight. To determine the activity of the antioxidant metabolism, leaves and roots were collected, maintained in liquid nitrogen and stored at -80ºC. Then 0.2g of each sampling were macerated in liquid nitrogen, with added buffer solution containing EDTA 0.01M; potassium phosphate 0.4M (pH 7.8); ascorbic acid 0.2M and distilled water. The homogenate was centrifuged at 1300g for 10 minutes at 4°C and the supernatant collected and stored at -20°C, for posterior analysis of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Giannopolitis & Ries, 1977; Havir & McHale, 1987; Nakano & Asada, 1981). To quantify the hydrogen peroxide (H_2O_2) and the lipid peroxidation, 0.2g of the leaves were macerated in liquid nitrogen, homogenized in 1500 mL of trichloroaceticacid (TCA) 0.1% (m/v) and centrifuged at 12,000g for 15 minutes at 4ºC, and the supernatant collected and stored at -20ºC. The lipid peroxidation was quantified in the species that reacted to the thiobarbituric acid (TBA) (Buege & Aust, 1978). Velikova et al. (2000) methodology was used to quantify the H_2O_2 content.

Results and Discussion

The soil analysis is shown in Tables 1 and 2. In a general way, the concentrations of zinc and cadmium increased in the roots and shoots of the maize plants with an increase of Cd/Zn doses. Higher values for zinc and cadmium were found on the roots and shoots of the plants cultivated in Latosol (Tables 3 and 4). It is important to emphasize that the plants cultivated in Latosols and dose seven perished.

The present study showed that Cd/Zn concentrations have a tendency to accumulate in the upper parts of the maize plants, despite the high mobility of the metal ions on plants (Clemens et al., 2013). On the other hand, higher concentrations of metals on the upper parts of the plants are similar to those found by Cherif et al. (2011) in tomato plants in the same conditions.

There are physical and chemical differences on the soils. The edaphic characteristics provide conditions to the Cambisoil adsorbing higher concentrations of Cd, disponibilizing lower amounts of this element for the plants (Gallego et al., 2012). With lower concentrations of Cd available, the maize plants cultivated in Cambisoil presented lower values of Cd in its tissues than those cultivated in Latosol, for most of the doses.

Growth analysis is the most sensitive parameter to an increase on the concentrations of Cd/Zn, since *Zea mays* is a sensitive species for non-phytoremediation of trace element (Carvalho et al., 2013). The exposition of maize plants to increasing doses of Cd/Zn resulted in a decrease on the growth parameters evaluated (Table 5).

As a response to the oxidative stress induced by trace elements, the corn plants use the antioxidant defense system to eliminate the ROS and prevent destructive oxidative reactions (Gallego et al., 2012). Lower concentrations of Cd/Zn found on corn plants cultivated in Cambisoil allowed that plants had a minor impact of the trace elements on its metabolism, and thus presented lower oxidative damage than those found in plants cultivated in Latosol. A decrease on the activity of the enzymes SOD, CAT and APX was observed as the doses increased (Figure 1), and this

Table 3. Zinc concentrations on the roots and shoots of the maize plants cultivated in Cambisoil and Latosol with increasing doses of Cd/Zn.

Treatments	Zinc (mg kg^{-1})			
	Upper parts		Roots	
	Cambisoil	Latosol	Cambisoil	Latosol
D0	132.06 Bh	181.16 Ag	100.53 Ag	109.76 Ah
D1	222.90 Bg	325.13 Af	97.66 Bg	225.30 Ag
D2	472.53 Bf	554.26 Ae	223.60 Bf	444.30 Af
D3	672.46 Be	845.60 Ad	303.86 Be	643.86 Ae
D4	801.93 Bc	834.40 Ad	359.19 Bd	808.56 Ad
D5	723.60 Bd	1061.26 Ac	594.86 Bc	3003.63 Ac
D6	1138.80 Bb	1629.73 Ab	4147.90 Ba	4434.60 Ab
D7	1185.36 Ba	0000.0	3559.96 Bb	0000.0

The capital letters compare soils while the lower-case letters compare the doses according to Scott-Knott ($p < 0.05$).

Table 4. Concentrations of cadmium in the roots and shoots of the maize plants cultivated in Cambisoil and Latosol with increasing doses of Cd/Zn.

Treatments	CADMIUM (µg kg^{-1})			
	Upper part		Root	
	Cambisoil	Latosol	Cambisoil	Latosol
D0	<LDM	<LDM	<LDM	<LDM
D1	10.00 Bd	15.63 Af	5.33 Ac	2.43 Ac
D2	12.80 Ad	13.76 Af	6.00 Bc	11.66 Ab
D3	17.73 Ac	18.23 Ae	1.36 Ad	2.06 Ac
D4	26.06 Ab	21.60 Bd	1.23 Ad	3.46 Ac
D5	32.90 Aa	33.06 Ac	9.63 Bb	23.70 Aa
D6	32.96 Ba	54.43 Ab	13.60 Bb	21.03 Aa
D7	36.03 Ba	00.00	38.40 Aa	00.00

LDM (Cd) = 1.66 µg kg^{-1}

The capital letters compare soils while the lower-case letters compare the doses according to Scott-Knott ($p < 0.05$).

Table 5. Analysis of the growth parameters on the upper parts and roots of maize plants cultivated in Cambisoil and Latosol at different concentrations of Cd/Zn.

	Dry mass from the upper part ($g.plant^{-1}$)		Root dry mass ($g.plant^{-1}$)	
Doses	Cambisoil	Latosol	Cambisoil	Latosol
D0	0.85Aa	0.72Ba	0.28 Ab	0.28 Aa
D1	0.81Aa	0.67Ba	0.38 Aa	0.22 Bb
D2	0.61Ab	0.42Bb	0.28 Ab	0.16 Bc
D3	0.52Ac	0.43 Bb	0.25 Ab	0.16 Bc
D4	0.47Ac	0.38 Bb	0.20 Ac	0.16 Bc
D5	0.38Ad	0.31 Bc	0.17 Ac	0.15 Ac
D6	0.29Ae	0.29Ac	0.12 Ad	0.11 Ad
D7	0.22Af	-	0.10 Ad	-

Capital letters compare soils while lower-case letters compare doses, using Scott-Knott ($p< 0.05$).

can be attributed to the inactivation of the enzymes by the H_2O_2 (Figure 2), that is formed in different compartments of the cell (Gill & Tuteja, 2010).

Analyzing the activity of the SOD enzyme in plants cultivated in Cambisoil (Figure 1, A and B), it is possible to notice that, in a general way, an increase is followed by a decrease on the activity, and the effects are similar in the roots and shoots of maize plants. Plants cultivated in Latosol showed higher activity of the SOD enzyme on the upper parts. Comparing both soils, plants cultivated in Cambisoil presented a higher activity of the enzyme SOD that was similar in all the doses analyzed. This enzyme is the first defense on the metabolism of the plants, which highlights that the plants were actually in a distress situation (Cherif et al., 2011).

The activity of the enzyme CAT was affected by the treatments (Figure 1, C and D), demonstrating higher activities of the enzyme on the roots compared to the leaves of the plants cultivated in Cambisoil. The upper parts of the maize plants cultivated in D7 didn't show any expressive activity of the enzyme. On the other hand, analyzing the results from Latosol, the activity of this enzyme in leaves was higher when compared to roots.

The activity of APX showed a different behavior in different doses (Figure 1, E and F). The results obtained for the roots and upper parts were similar in the maize plants cultivated in Latosol.

The levels of hydrogen peroxide were higher on plants cultivated in Latosol, and this increment was directly proportional to an increase in Cd/Zn concentrations (Figure 2, A and B). The levels of lipid peroxidation were expressed in malondialdehyde (MDA) content. Data obtained were divergent between the soils, showing a significant increase on the MDA levels (Figure 2, C and D).

Treatments containing the doses with the interaction of Cd/Zn resulted in an increment of the MDA content in all the tissues when compared to the controls. It is important to highlight that the presence of Cd on plant tissues induces the oxidative stress, which can damage the membrane and, consequently, increase MDA concentrations (Solti et al., 2016). Due to its chemical properties, Cd can't interact directly with oxygen, but can cause oxidative stress indirectly by the inhibition of the metabolic reactions (Cherif et al., 2011). The results showed a synergistic effect between both trace elements, as Zn did not show any

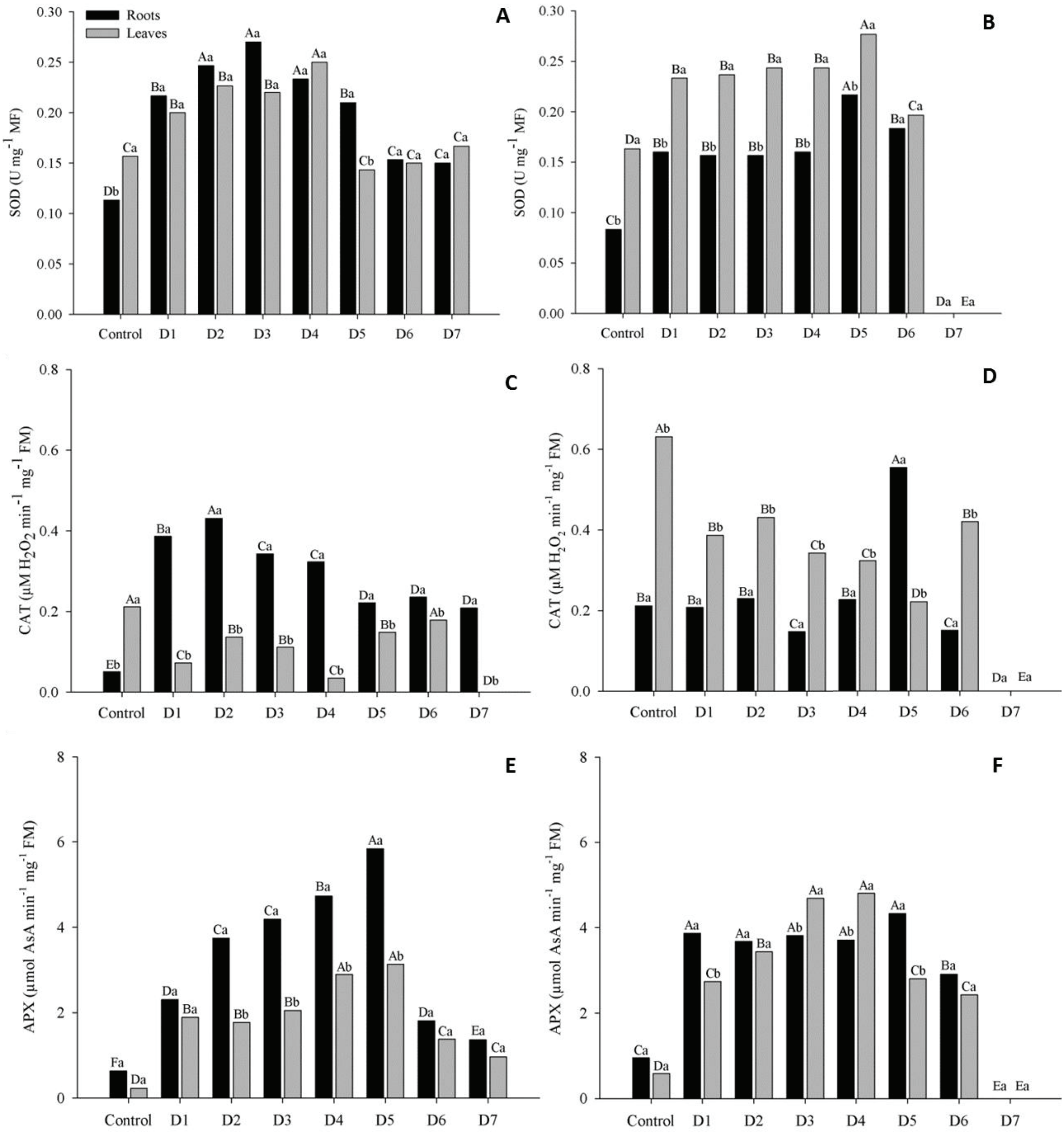

Figure 1. Activity of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) in leaves and roots of maize plants submitted to different doses of Cd/Zn and cultivated in Cambisoil (A,C and E) and Latosol (B, D and F). Capital letters compare soils while lower-case letters compare doses, according to Scott-Knott test ($p < 0.05$).

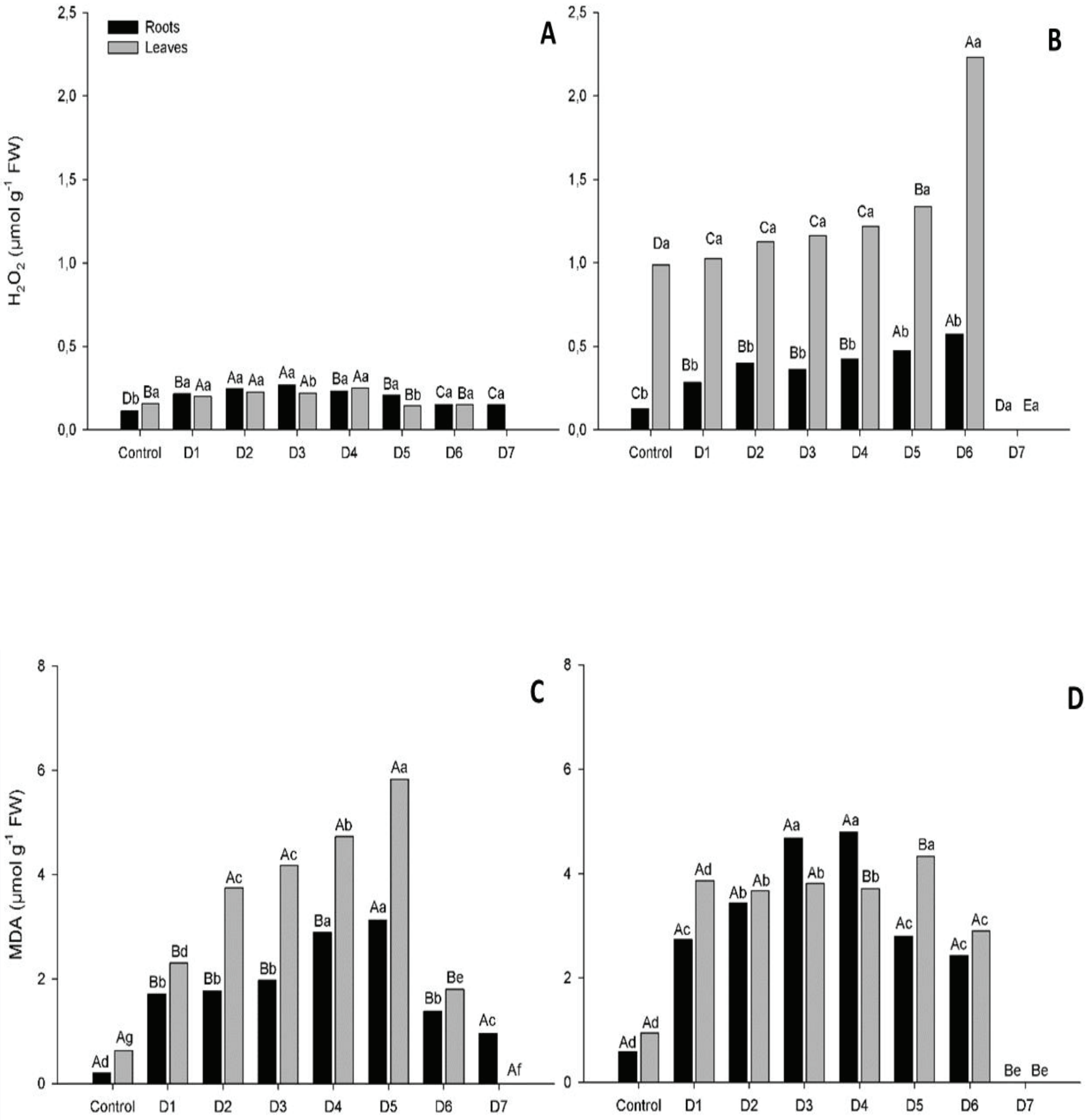

Figure 2. Concentration of hydrogen peroxide and lipid peroxidation levels expressed in malondialdehyde (MDA) content on the roots and upper parts of maize plants submitted to different doses of Cd/Zn and cultivated in Cambisoil (A and C) and Latosol (B and D). Capital letters compare soils while lower-case letters compare doses, according to Scott-Knott test ($p < 0.05$).

antagonistic effect when reducing Cd concentrations on leaves.

Higher concentrations of Zn can induce the oxidative stress, as described in many plants (Tkalec et al., 2014). The formation of the ROS can't be directly induced by Zn, which means that the reactive forms of oxygen that cause lipid oxidation are formed as a consequence of the interaction between Cd and Zn, producing alterations that are able to activate the NADPH-oxidases on the plasma membrane, leading to oxidative stress (Hussain et al., 2013).

Conclusions

The interaction between Cd and Zn presented a synergistic effect, regardless of the dose where the maize plant was grown, resulting in different damage concerning growth parameters and oxidation stress.

Physical and chemical characteristics of both soils affected directly the development and growth of the plants, justifying that different mechanisms are involved on its protection against the oxidative stress caused by trace elements. The maize plants cultivated in Cambisoils showed a specific behavior.

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