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Cadmium toxicity on seed germination and seedling growth of wheat *Triticum aestivum*

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ABSTRACT. Cadmium toxicity in seed germination and seedling growth of wheat (*Triticum aestivum*, var. IAC-370) is assessed. The ecotoxicological effects of six experimental concentrations of cadmium (0.03; 0.06; 0.12; 0.6; 1.2; 2.4; 4.8 mM), and control were evaluated. All assays were performed in quadruplicates with 25 seeds per replication in Petri dishes. Responses for toxic effect comprised the variables germination percentage, index of velocity of germination (IVG), length of aerial section and root of the seedlings, green and dry mass of the seedlings. Results showed that *T. aestivum* seeds exposed to cadmium decreased their normal germination percentage as from 0.03 mM concentration, with a 31% reduction of germination percentage and a 20% decrease in IVG. Cadmium´s inhibition effect on initial growth of seedlings influenced growth of roots and aerial part as from concentration 0.12 mM and reduced the production of green and dry mass of seedlings as from 0.06 mM. The presence and accumulation of cadmium in the soil cultivated with *T. aestivum* may trigger liabilities in productivity and/or viability caused by its toxicity as from 0.03mM concentrations absorbed by the plant roots.

Keywords: ecotoxicology, metal, contamination.

Toxicidade do cádmio na germinação de sementes e no crescimento de *Triticum aestivum*

RESUMO. Esta pesquisa avaliou os efeitos tóxicos do cádmio na germinação e crescimento inicial do trigo (*Triticum aestivum*, var. IAC-370). Foram avaliados os efeitos ecotoxicológicos de seis concentrações de cádmio (0,03; 0,06; 0,12; 0,6; 1,2; 2,4; 4.8 mM). Os experimentos foram em quadruplicatas, com 25 sementes por replicata, em placas de Petri. As variáveis respostas foram o percentual de germinação, o índice de velocidade de germinação (IVG), o comprimento da parte aérea e da raiz das plântulas, a massa fresca e seca das plântulas. Os resultados reportaram que as sementes de *T. aestivum* expostas ao cádmio apresentaram decréscimo do seu percentual germinativo de plântulas normais a partir a concentração de 0,03 mM, com redução de 31% do percentual germinativo e decréscimo de 20% no IVG. O efeito inibitório do cádmio no crescimento inicial das plântulas afetou o crescimento das raízes e da parte aérea a partir da concentração 0,12 mM, causando a redução da produção de massa fresca e seca das plântulas a partir de 0,06 mM. A presença e acumulação do cádmio, no solo de cultivo da *T. aestivum*, poderá causar perdas de produtividades e/ou viabilidade provocadas pela sua toxicidade a partir de concentrações de 0,03 mM que estejam absorvidos pela raiz da planta.

Palavras-chave: ecotoxicologia, metal, contaminação.

Introduction

Wheat (*T. aestivum* L.) is a species of the Poaceae family with high economic relevance and great importance for human consumption. In fact, it is a world-wide consumed cereal with high grain adaptation and productivity rates (MARINI et al., 2011).

Seed germination comprises various processes of the early development of the plant (SILVA et al., 2013) that culminates in metabolic transformations that trigger the development of the embryonic axis and, consequently, the emission of the radicle (OLIVEIRA et al., 2013). Favorable environmental conditions with important roles in the regulation of seedling growth and development are required so that the process occurs (OLIVEIRA et al., 2015).

Studies on seed germination deepen physiological and morphological knowledge of the embryo and seedlings, and discuss the influences of environmental factors on seed quality (GORDIN et al., 2012).

On the other hand, heavy metals are abiotic factors that may interfere in the development of seed germination. The contamination of the environment by heavy metals and other pollutants hails from the 18^{th} century (ANDRADE et al, 2014), among which cadmium (Cd) is acknowledged to be one of the most toxic metals for live organisms.

The permanence of cadmium in the soil contaminates water and impedes the absorption of plant nutrients causing morphological, physiological, biochemical and structural changes in plants (AUGUSTO et al., 2014). The immobilization of cadmium outside the plant or in the root may be one of the first barriers of the plant against toxicity to the heavy metal since cadmium levels are highest in roots rather than in leaves, with low transport level from the root to the aerial segment of the plant (CHAVES; SOUZA, 2014).

Exposure of plants to toxic amount of cadmium affects germination and compromises the growth and development of the plants (GUIMARÃES et al., 2008), featuring intervention in several physiological processes and causing reduction in agricultural productivity (GONÇALVES et al., 2009).

Current research evaluates the effects of toxicity by cadmium on seed germination and on the initial growth of wheat (*T. aestivum*).

Material and methods

Seeds of *T. aestivum* (var. IAC-370) were donated by the Agronomic Institute (IAC), São Paulo State, Brazil. The germination test to verify seed viability was performed by disinfecting seeds in a solution of sodium hypochlorite (2.5%) during 10 minutes and then washed in distilled water. Two hundred seeds, with four replications with 50 seeds each, were seeded in 150 mm petri plates with two sheets of germitest paper moistened with 6 mL of distilled water.

The seeds were sterilized in deionized water (H_2O_2 30%) to evaluate the eco-toxicological effects of cadmium. Prior to seeding, the seeds were immersed during 30 minutes in 15 mL of different concentrations of cadmium used to moisten the plate. Seed were sown in 150 mm diameter petri plates to germinate on double layer germination paper (germitest, sterilized in an autoclave at 120°C for 20 min.). The paper was moistened in a solution with cadmium at concentrations 0.03; 0.06; 0.12; 0.6; 1.2; 2.4; 4.8 mM, in quadruplicates with 25 seeds per plate. Cadmium solutions were prepared from its salt $CdSO_4$. $8H_2O$ and pH 6.6 was maintained for the cadmium source solution. A Petri plate with only deionized water for paper moistening was used for the variance control.

The variables responses to evaluate cadmium´s toxic effect comprised germination percentage, index of velocity of germination (MAGUIRE, 1962), changes in the seedlings´ morphological parameters (length of root and aerial part, percentage and abnormal types), amount of green and dry matter produced.

Germinated seeds were counted daily by radicle protrusion till the number of germinated seedlings remained constant. Growth of root and aerial part of 20 seedlings (totaling 80 seedlings per treatment) were measured (mm) at 24, 48, 72, 96 and 120h after seeding, by digital caliper. Total dry mass of the seedlings, types of abnormalities in the plants, and classification of non-germinated seeds as hard or dead, were determined, following RAS (BRASIL, 2009).

Laboratory studies were performed under controlled conditions in which all plates with seeds were maintained at 30°C ± 2, in a 16-8h light-dark photoperiod.

Results and discussion

Triticum aestivum seeds exposed to cadmium decreased in normal seedling germination percentage as from 0.03 mM concentration and had a 31% reduction in germination percentage when compared to control experiment (Figure 1).

Most non-germinated seeds (92%) behaved as hard seeds and failed to reveal any change or tumescence. However, other seeds which did not germinate (8%) but showed some type of tumescence and/or darkening were classified as dead seeds. In both cases, none of these germinating seeds recovered development, even after five further days in the substrate.

Linear regression analysis confirmed a high co-relationship between percentage decrease and increase in cadmium concentration to which the seeds were exposed, with a highly significant R^2 rate (0.95). In fact, in *T. aestivum*, cadmium reached half (EC50) of its maximum toxic effect at concentration 0.12 mM and thus inhibited the germination of 50% of seeds. Its toxic effect was almost total (99%) at concentration 1.20 mM. The concentration of 1.20 mM showed no germination of normal seedlings, developing only two abnormal seedlings. The inhibition of germination was complete at a concentration of 4.8 mM.

Increase in cadmium concentrations also had a high linear co-relationship (r^2 = 0.95) with a reduction of the index of velocity of germination (IVG) of the seeds (Figure 2). Cadmium interfered on IVG as from concentration 0.06 mM, with a significant statistical variation with regard to control, at a decrease of approximately 20% of IVG.

The seedlings´ morphological structure revealed that cadmium provided an increase in the number of abnormal seedlings and thus revealed its toxic capacity at cell level (Figure 3). Abnormalities and their percentage comprised atrophy of primary root (51.4%), absence of primary root (37.1%), thin and weak primary root (6.2%), twisted primary root (3.3%), absent aerial part (1.4%) and twisted aerial part (0.5%).

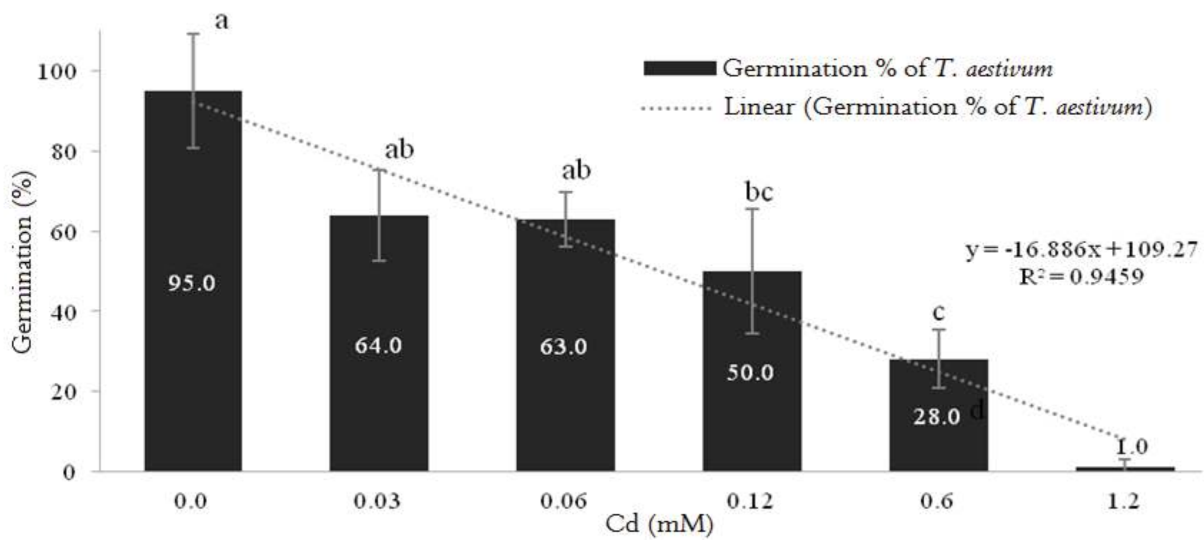

Figure 1. Percentage of normal seedling germination of *T. aestivum* seeds with several cadmium concentrations. Different letters between column show statistically different rates by ANOVA test, followed by Tukey´s test ($p < 0.01$).

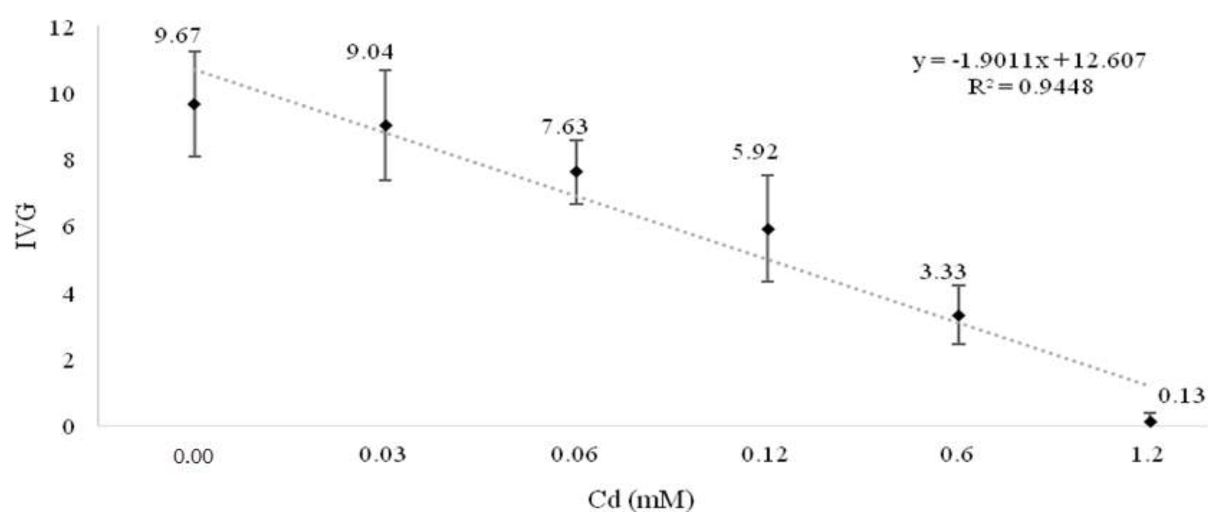

Figure 2. Index of velocity of germination (IVG) of *T. aestivum* seeds at different cadmium concentrations.

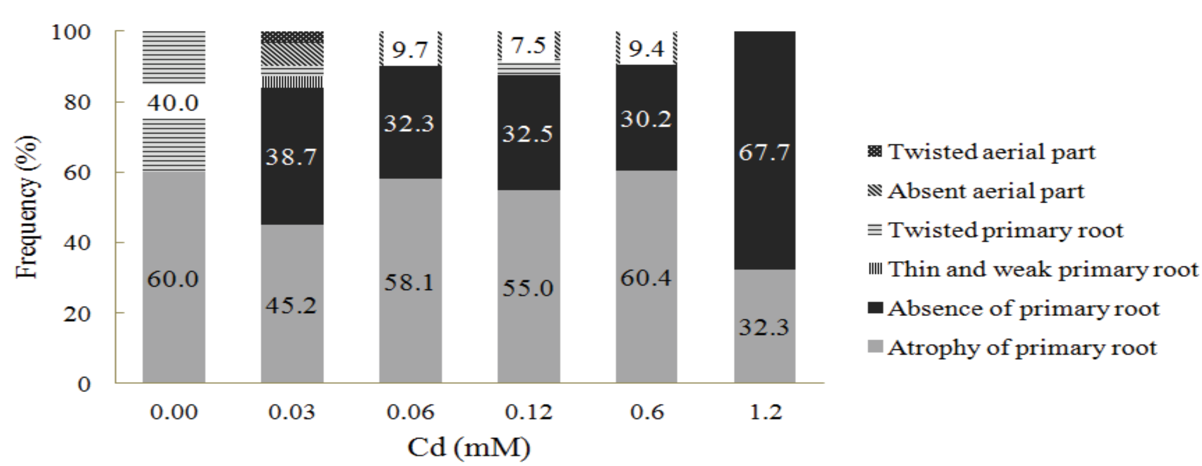

Figure 3. Frequency (%) of the types of abnormalities observed in *T. aestivum* for each experimental concentration of cadmium.

Cadmium inhibited the length of aerial part and root of *T. aestivum* seedlings since the heavy metal reduced their growth in 18.5 and 15.6%, respectively, as from concentration 0.06 mM. Since growth inhibition of the seedlings´ vegetal segments by cadmium almost reached 50% at 0.60 mM concentration (Table 1), its high toxic capacity for *T. aestivum* at such low concentrations was demonstrated.

Table 1. Mean length, standard deviation (SD) and percentage of coefficient of variation (CV) of the seedlings´ aerial segment and root of wheat exposed to different concentrations of cadmium for 120h.

Concentration of cadmium (mM)	Shoot		Root	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)
Control	21.06 ± 2.02^{a}	9.6	17.44 ± 1.44^{a}	8.3
0.03	19.31 ± 1.04^{a}	5.4	16.88 ± 1.62^{a}	9.6
0.06	17.17 ± 1.19^{b}	6.9	14.71 ± 1.46^{b}	9.9
0.12	12.20 ± 1.69^{c}	13.9	11.24 ± 1.41^{c}	12.5
0.60	11.93 ± 1.93^{c}	16.2	8.58 ± 1.63^{d}	19.1
1.20	5.04 ± 1.72^{d}	34.1	5.06 ± 1.29^{e}	25.6
2.40	5.31 ± 2.45^{d}	46.2	2.75 ± 0.42^{e}	15.4

Different letters in the same column show statistically different rates by Kruskal-Wallis test, followed by Mann-Whitney test ($p < 0.05$).

Figure 4 shows the growth of the aerial segment and roots after 120h of seeding. The 0.03 mM concentration did not demonstrate any significantly statistical variation with regard to the growth of seedlings of control. Contrastingly, concentrations as from 0.12 mM of cadmium revealed high liability in root growth, which restricted the establishment and development of the seedlings in normal soil conditions.

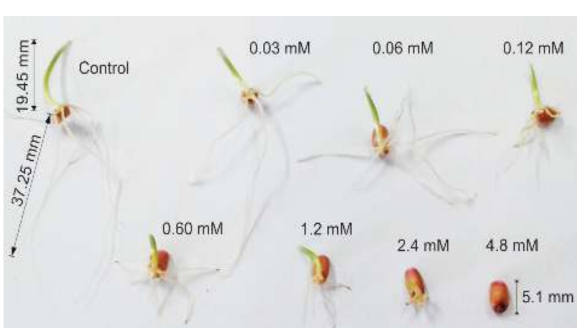

Figure 4. Development of *T. aestivum* after 120h exposed to different concentrations of cadmium.

Total green and dry mass of the seedlings also had a significant decrease in mean rates (Table 2), respectively ranging between 0.174 and 0.322 mg and between 0.041 and 0.061 mg. The inhibitory effect of cadmium started from 0.06 mM concentration for green mass and from 0.12 mM for dry mass.

Several researchers have reported the toxic effect of cadmium demonstrated in the decrease of germination and growth rates of seedlings of different crops. Research on other cultivars of *T. aestivum* also revealed low tolerance rates with cadmium, with toxic effects as from 5 mg L^{-1} (AHMAD et al., 2012) and 7 mg L^{-1} (YADAV; SINGH, 2013) concentrations, respectively with 18.4 and 10.0 – 16.6% decrease. Results in current assay showed toxic effect as from concentration 0.03 mM = 6.21 mg L^{-1}, with significant reduction in germination percentage.

Table 2. Green and dry mass (mg) produced by seedlings at different concentrations of cadmium (mM).

Concentration of cadmium (mM)	Green mass	Standard deviation	CV (%)	Dry mass	Standard deviation	CV (%)
Control	0.322^{a}	0.022	6.75	0.061^{a}	0.008	12.29
0.03	0.293^{a}	0.008	2.79	0.056^{a}	0.007	11.76
0.06	0.287^{ab}	0.018	6.24	0.058^{a}	0.004	7.36
0.12	0.249^{b}	0.024	9.48	0.044^{b}	0.005	11.74
0.60	0.174^{c}	0.024	13.60	0.041^{b}	0.003	6.68

Seeds at concentrations higher than 0.60 mM failed to produce any seedlings with sufficient mass for analysis. Different letters in the same column show statistically different rates by ANOVA test, followed by Tukey´s test ($p < 0.01$).

Lack of tolerance has been reported in other crops such as spinach (*Spinacia oleracea*) with 19.0 and 87.5% decrease in germination when exposed to 5.2 mg L^{-1} and 15 mg L^{-1} of cadmium, respectively (HOSSEINI et al., 2012; BAUTISTA et al., 2013); soybean (*Glycine max*) with 5 mg L^{-1} cadmium reducing 8% germination (LI et al., 2013); chard (*Beta vulgaris*) and lettuce (*Lactuca sativa*), with 18.0 and 19.0% reduction in germination when exposed respectively to 5.2 mg L^{-1} cadmium (BAUTISTA et al., 2013) and seablite (*Suaeda salsa*), with a 18.0% decrease when exposed to 6.0 mg L^{-1} cadmium (LIU et al., 2012).

Since its inhibitory effect on the growth of *T. aestivum* seedlings was higher in the root than in the aerial segment, the dry weight of the seedlings decreased significantly with increase in cadmium concentration. High sensitiveness of roots for cadmium may be due to the fact that the root is the first organ to contact the heavy metal and be involved in the absorption process (MAGNA et al., 2013).

The literature has registered similar trends in growth inhibition of seedlings of the species *Silybum marianum* (KHATAMIPOUR et al., 2011), *Cucurbita máxima* (SUBIN; STEFFY, 2013) and *Cicer arietinum* (FAIZAN et al., 2011). Further, researches by Khatamipour et al. (2011) and Subin and Steffy (2013) also demonstrated a greater trend of the inhibiting effect on roots than on the aerial segments.

Cadmium may actually cause negative effects on absorption. transport and the employment of the macro-elements calcium, phosphorus, potassium, sulfur and water, with the onset of several symptoms, such as leaf chlorosis, reduction of photosynthetic activity, enzymatic activity, respiration (GUIMARÃES et al., 2008) and inhibition of root and aerial segment growth (SOUZA et al., 2009).

Cadmium in the soil may also cause its accumulation in the plant (ROJAS-CIFUENTES et al., 2012) and thus *T. aestivum* cultivars may affect crop productivity due to the metal in the soil.

Heavy metals accumulate in plant tissues by a complex toxicity process (SILVA et al., 2015). After mobilization of the soil for root cells, they tend to accumulate in the vacuole cells or they are transported to the aerial parts through the xylem (CLEMENS et al., 2002).

When the plant accumulate metals, such as cadmium, at phytotoxic levels, biochemical and molecular disturbances may occur that cause oxidative stress in cells and, consequently, such effects as cell membrane changes, DNA damage, genetic mutation, rust protein, lipid peroxidation and growth inhibition (HOSSAIN et al., 2012).

Although the plants possess antioxidant mechanisms to develop in oxidative stress conditions (WANG et al., 2011), they may not maintain homeostasis of the toxic heavy metal when exposed to high concentrations of the contaminant.

Conclusion

Triticum aestivum is a non-tolerant plant to cadmium during its germination and initial growth. The toxic effects of cadmium may be observed in low concentrations (0.03 mM) since they significantly reduce germination and IVG percentage. However, its toxicity during the seedling´s initial growth occurs in concentrations as from 0.06 mM and 0.12 mM when growth of root and aerial parts are respectively inhibited, coupled to decrease in the green and dry mass of the seedlings.

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