

**ORIGINAL RESEARCH PAPER**

# Toxic effect of metals (Al, Cd, Pb, Cr, and Fe) in soybean experiment (*Glycine max* L.) IPRO technology

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**Abstract:** Soil contamination by heavy metals is a serious environmental and agricultural problem. Among the toxic metals, aluminum, cadmium, lead, chromium, and iron represent production problems for agriculture around the world. This study aimed to evaluate the tolerance to Al, Cd, Pb, Cr, and Fe in the vegetative phase of the NEO 790 IPRO cultivar. Different concentrations of 0, 35, 85, and 125 mg L<sup>-1</sup> were prepared and added to the soil. The parameters evaluated were percentage of germination, plant length, root length, aerial and root fresh mass, and aerial and root dry mass. Al and Cd presented negative results for some vegetative parameters, being toxic at the concentrations evaluated. Positive effects were obtained for Pb and Cr on other morphological parameters, and for the Fe element, it was possible to observe double suitability as a toxic element in high doses (> 85 mg L<sup>-1</sup>) and in doses lower than 85 mg L<sup>-1</sup> effect stimulator. The NEO 790 IPRO cultivar proved to be resistant in most of the parameters evaluated at the applied doses.

**Keywords:** Heavy metals; *Glycine* genus; Aluminum; Cadmium; Lead; Iron; Toxicity.

## 1. Introduction

Several environmental problems are diagnosed every day. One of these environmental problems is the contamination of water, air, and especially soil by toxic elements that pose a danger to the health of different forms of human, animal, and plant life. Elements such as Aluminum (Al), Cadmium (Cd), Lead (Pb), Chromium (Cr), and Iron (Fe) in toxic concentrations can mainly contaminate large areas of agricultural soil. These elements can cause deleterious effects on several groups of terrestrial vegetables and aquatic species, including plant species of agricultural interest (Stefanello; Goergen, 2019).

Among vegetable groups, mechanisms of plant tolerance to high concentrations of heavy metals are described, such as restriction in transport from the root to the leaf; accumulation in trichomes; exudates that can complex metals; type of bond between the metal and the cell wall component; production of intracellular

compounds with chelating properties, and active pumping into the vacuoles (Oliveira et al., 2005). According to Steffens (1990) and Wang & Evangelou (1994), these mechanisms can occur alone or together, providing greater tolerance to stress caused by the presence of these toxic elements. Al is present in different levels in soils in a natural, available, or free form in the form of Al<sup>3+</sup>, which is in solution depending on the pH of the soil. In soil with a pH above 5.5, it generally occurs in low concentrations, but in a pH below 5, it is found in high concentrations. Studies aiming to elucidate the resistance capacity between the Al relationship and plants in tolerating different doses that can be toxic is still a great challenge for Science, as critical doses of this element can influence everything from the percentage of germination to grain productivity rates. However, some vegetables have a certain tolerance to Al, in studies, it was possible to verify beneficial effects, although at low concentrations (71.4 to 185 µmol L<sup>-1</sup>) in the soil and in

nutrient solution, where it positively influenced the development of corn, beet and in some legumes.

The nature of the beneficial effect of Al on the germination and development of plants is still not very clear, however, there is evidence that the effect of Al is direct, as studies discuss that this element can compete with other important elements, albeit in large quantities. toxic effects on the development of plants such as Copper (Cu), Zinc (Zn), and Phosphorus (P) (Stefanello; Goergen, 2019).

The Cd element presents accumulation and translocation in plant tissues, competing with other macro and micronutrients of interest to plants. Cd absorption occurs through the roots where it involves the concentration of the metal in the soil and the morphology of the root system, being absorbed by the cortical tissue and transported to the xylem through the apoplastic or symplastic route (Cataldo et al., 1983). This metal has deleterious effects on water absorption, affects enzymatic activities, transport, and use of macro and micronutrients such as Calcium (Ca), Phosphorus (P), and Potassium (K), and can also influence the inhibition of root elongation. In the aerial part, studies report that Cd affects leaf development, causes chlorosis and necrosis, and affects the photosynthetic process and respiration, which can induce plant death (Santos et al., 2013; Cogo et al., 2020).

Pb is another heavy and toxic element not only for plants but also for animals and humans, as it has no benefit. Pb in its cationic form ( $Pb^{2+}$ ) is absorbed by plants due to its similarity with essential transition metals, differing in translocation due to its ease of forming complexes with high steric hindrance (Silva et al., 2007a/b). According to Baligar et al. (1998) and Bertoli et al. (2011), high concentrations of this element interfere with cell division and inhibit the extension of the root system and concentrations below the level considered toxic can stimulate root growth. In aerial systems, Pb slows down the respiratory process and causes reduced plant growth. Another deleterious effect of Pb is the interruption of Ca metabolism and enzymatic inactivation (Bergmann, 1992).

This element moves very easily with other elements such as K, Barium (Ba), Sulfur (S), and Ca in minerals and adsorption sites, this is due to its similarity with alkaline earth metals, which also gives this metal the characteristics being one of the least mobile metals that accumulate in the surface horizons of the soil (Kabata-Pendias; Pendias, 2001).

Cr metal is the twenty-first most found metal in the Earth's crust (Sousa; Santos, 2018). As for plant nutrition, it has no potential function for plants. However, studies describe positive results for

vegetable growth with the application of low levels of soluble Cr (Kabata-Pendias; Pendias, 2001). This metal occurs in nature as a result of weathering on different types of soil and also through the deposition of waste of industrial origin such as tanneries and steelmaking (Sousa; Santos, 2018).

Iron is considered an essential micronutrient for the development of plants where it is involved in DNA synthesis, respiration, and photosynthesis and as a constituent of several enzymes (Routh; Sahoo, 2015). However, at higher concentrations, Fe becomes a toxic element for several groups of plants. In flooded soil environments, microbial activity favors the reduction of insoluble  $Fe^{3+}$  into soluble  $Fe^{2+}$ , however, tolerance rates are in the range of  $0.1 \text{ mg L}^{-1}$ . The microorganisms in these areas, favoring this conversion, drastically increase this element, reaching up to  $600 \text{ mg L}^{-1}$ , being mainly toxic to rice plants (Dobermann; Fairhurst, 2000). Furthermore, Fe toxicity in plants triggers the formation of reactive oxygen species (ROs) that cause several cellular anomalies and consequently the death of the plant (Sahrawat et al., 2004; Silveira et al., 2007).

Soybean (*Glycine max* L.) is one of the most produced grains in the world and is considered an example of a vegetable for analyzing tolerance and resistance in studies with toxic metals, this is possible due to the large number of cultivars that adapt to different types of soils, climate and metal concentrations (Poudel et al., 2023). However, it is important to highlight that even if a plant can tolerate soils with a high level of toxic metals, studies must be carried out aiming at the final deposition of this metal in the plant (Zhi et al., 2020; Ikhajiagbe et al., 2021).

Considering that high concentrations of Al, Cd, Pb, Cr, and Fe negatively affect the development of the vegetative phase of soybeans, this study aimed to evaluate different doses of these toxic metals in the cultivation of IPRO technology soybeans at 45 days after germination and evaluate the possible effects of these metals on morphological results during full development.

## 2. Material and Methods

### 2.1. Study location

The study was carried out at the Goiano Federal Institute, Rio Verde, Goiás State, Brazil, between November 2023 and February 2024. The experiment had two phases. First phase: carried out in vitro at the Technological Chemistry Laboratory in the Department of Agrochemistry. Second phase: carried out in a

greenhouse at the Hydraulic and Irrigation Laboratory.

## 2.2. Reagents and equipment

Aluminum Chloride ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ) Dinâmica, Brazil, Cadmium Chloride ( $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ ), Chromium Nitrate ( $\text{Cr}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ ) Êxodo Científica, Brazil, Lead Nitrate ( $\text{Pb}(\text{NO}_3)_2$ ) Dinâmica, Brazil, and Iron Chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) Synth, Brazil. Analytical balance (Shimadzu, Mod. AY220, Japan), electric oven with air circulation (SolidSteel, Mod. SSDic 1600, Brazil), measuring tape (Megha Zine, Mod. T2858), Brazil, and digital caliper (MTX, Mod. 316119, China).

## 2.3. Soybean cultivar

Soybean seeds cultivar Neo 790 IPRO was used, with medium size, indeterminate growth habit, medium branching, and moderate resistance to lodging. It has a white flower, green hypocotyl, gray pubescence, medium to high fertility (4), flowering 28 days, average height 87 cm, maturation group 7.9. Stem canker resistance, moderately susceptible frogeye leaf spot, and resistant bacterial pustule; susceptible root-knot nematodes (*Meloidogyne* spp.).

## 2.4. Soil analysis

Soil parameters were determined in a layer between 0-20 cm deep with the following results: Ca = 2.13, Mg = 1.43, K = 0.30, P = 3.0, S = 9.0, Na = 1.0, organic matter (OM) = 61.1 and pH = 4.9. Clay = 30.3, Silt = 25.2 and sand = 44.4.

## 2.5. Experimental design

The experiment was carried out in a randomized block design for (toxic metals) where the type of design was  $5 \times 3 \times 7 + 1$ : five metals, three concentrations, seven replications, and one control.

## 2.6. Germination test

The germination test was carried out with five replications of 50 seeds, distributed in germination boxes (gerbox) on three sheets of germination paper moistened with distilled water or metal solution, in the proportion of 2.5 times the weight of the paper. After sowing, the germination boxes were kept in B.D.O, at a constant temperature of 25 °C with a photoperiod of 10 h of light and 14 h without light. The count was carried out daily. Germinated seeds with a root protrusion of at least 2 mm were considered using a digital caliper, the results were expressed as a percentage of germination (%) *in vitro*.

## 2.7. Toxicological effect

To evaluate the toxic effect of the metals Al, Cd, Cr, Fe, and Pb, aqueous solutions with concentrations (35, 85, and 125 mg L<sup>-1</sup>) were prepared. The solutions containing the metals were added to the soil 7 days before sowing. 100 mL of metal solution was added to each experimental plot. During this period, the soil was watered 4 times with

150 mL of distilled water at a field capacity of 80% RH (relative humidity) (Silva et al., 2024).

## 2.8. Planting and cultural treatments

For planting, plastic cups (700 mL) made of colorless polystyrene were used, where two holes were made to drain excess water. 550 g of high-productivity soil collected in a soybean and corn planting area more than 5 years ago was used. For each replication, two seeds were sown in a hole 0.5 cm deep. Then, each repetition was watered with 100 mL of distilled water. After germination, watering was carried out every two days. The experiment was kept in a greenhouse with controlled temperature humidity and air circulation for 24 h. There were no fungicide or insecticide sprays applied during the experiment period.

## 2.9. Seedling analyzes

Soybean plants were analyzed 45 days after sowing. The phytotechnical quality indicators evaluated were aerial length (AL) and root length (RL) expressed in centimeters (cm), aerial fresh mass (AFM) and root fresh mass (RFM) expressed in grams (g) and aerial dry mass (ADM) and root dry mass (RDM) after gravimetric drying at 65 °C for 24 h with results expressed in g.

## 2.10. Statistical analysis

The experimental design was in randomized blocks, where treatments consisted of different concentrations of metals in an aqueous solution. The data were subjected to analysis of variance using the *F* test and, when a significant effect was found, regression analysis was performed using the Sisvar software (Ferreira, 2019).

# 3. Results

## 3.1 Germination result

The germination test corresponded to 97.70% ± 0.41 of germinated seeds.

## 3.2. Effect of Aluminum doses

The analysis of variance indicated significant differences depending on the treatments only for the plant length variable (Table 1).

Table 1. Analysis of variance for the variables plant length (PL), root length (CR), aerial fresh mass (MFA), root fresh mass (RFM), aerial dry mass (MSA), and root fresh mass (MSF) in doses of Aluminum.

Al	PL	RL	AFM	RFM	ADM	RDM
	*	ns	ns	ns	ns	ns
CV%	4.62	10.42	21.39	20.36	33.28	30.70

Note: \* Significant. ns = not significant according to test

F. Source: Authors, 2024.

Only for plant length was a significant difference ( $p < 0.05$ ) observed in treatments with values that varied between 17.3 (Control) and 17.0 for the highest concentration of 125 mg L<sup>-1</sup> (Figure 2).

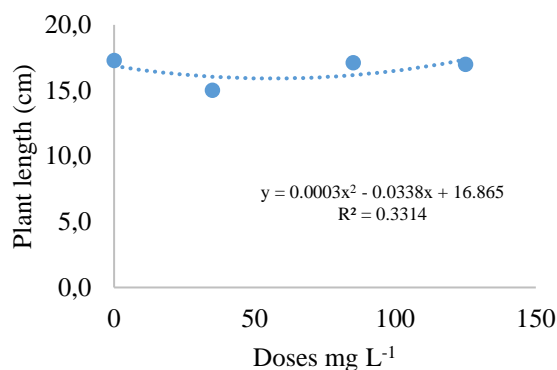


Figure 2. Effect of different doses of Aluminum metal on soybean plant length. Source: Authors, 2024.

### 3.3. Effect of Cadmium doses

The analysis of variance indicated significant differences depending on the treatments only for the PL and RL variables (Table 2).

Table 2. Analysis of variance for the variables plant length (PL), root length (CR), aerial fresh mass (MFA), root fresh mass (RFM), aerial dry mass (MSA), and root fresh mass (MSF) in doses of Cadmium.

Cd	PL	RL	AFM	RFM	ADM	RDM
	*	*	ns	ns	ns	ns
CV%	6.13	10.89	24.29	22.66	28.87	32.69

Note: \* Significant. ns = not significant according to test F. Source: Authors, 2024.

Cadmium showed a positive and significant effect ( $p < 0.05$ ) only for the PL and RL parameters. The other morphological parameters were not affected by the concentrations applied via soil ( $p > 0.05$ ) (Figures 3 and 4). For aerial length, there was a significant difference with values between 17.3 cm (Control) and a reduction of 14.25 cm (125 mg L<sup>-1</sup>) at the highest concentration (Figure 3).

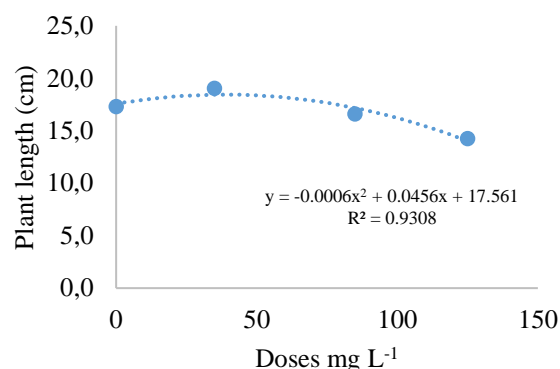


Figure 3. Effect of different doses of Cadmium metal on soybean plant length. Source: Authors, 2024.

A significant reduction caused by the high concentration of Cd was observed in root length (Figure 4) with values between 18.7 cm (Control) and 18.5 cm at the maximum concentration of 125 mg L<sup>-1</sup>.

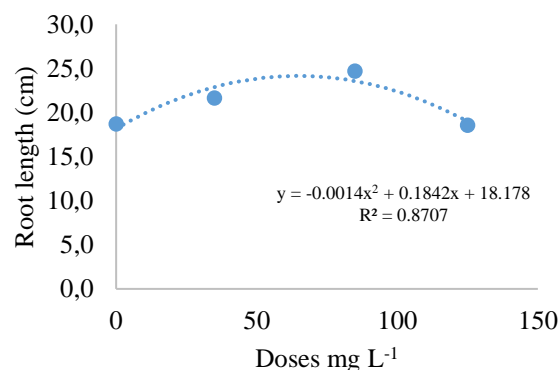


Figure 4. Effect of different doses of Cadmium metal on soybean root length. Source: Authors, 2024.

### 3.4. Effect of Lead doses

The analysis of variance indicated significant differences depending on the treatments only for the root length variable (Table 3).

Table 3. Analysis of variance for the variables plant length (PL), root length (CR), aerial fresh mass (MFA), root fresh mass (RFM), aerial dry mass (MSA), and root fresh mass (MSF) in doses of Lead.

Cd	PL	RL	AFM	RFM	ADM	RDM
	ns	*	ns	ns	ns	ns
CV%	9.68	14.58	24.57	23.51	35.22	31.20

Note: \* Significant. ns = not significant according to test

F. Source: Authors, 2024.

Pb showed a significant difference between the control with 18.9 cm and the highest dose with 125 mg L<sup>-1</sup> with 26.7 cm ( $p < 0.05$ ).

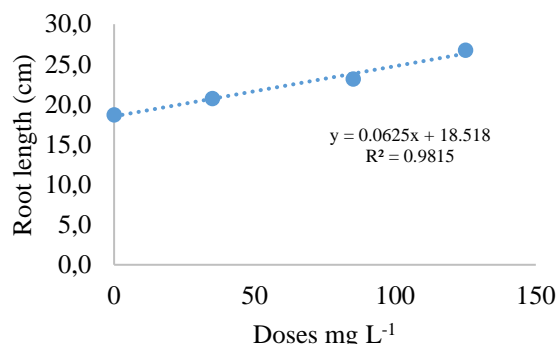


Figure 5. Effect of different doses of Lead metal on soybean root length. Source: Authors, 2024.

### 3.5. Effect of Chrome doses

The analysis of variance indicated significant differences depending on the treatments only for the aerial and root fresh mass variables (Table 4).

Table 4. Analysis of variance for the variables plant length (PL), root length (CR), aerial fresh mass (MFA), root fresh mass (RFM), aerial dry mass (MSA), and root fresh mass (MSF) in doses of Chrome.

Cr	PL	RL	AFM	RFM	ADM	RDM
	ns	ns	*	ns	*	ns
CV%	16.70	21.73	21.09	26.19	18.81	23.73

Note: \* Significant. ns = not significant according to test F. Source: Authors, 2024.

Cr showed a positive and significant effect ( $p < 0.05$ ) only for the AFM and ADM parameters. The other morphological parameters were not affected by the concentrations applied via soil ( $p > 0.05$ ) (Figures 6 and 7). For AFM there was a significant difference with values between 2.7 g (Control) and 4.2 g (125 mg L<sup>-1</sup>) at the highest concentration, showing a linear result (Figure 6).

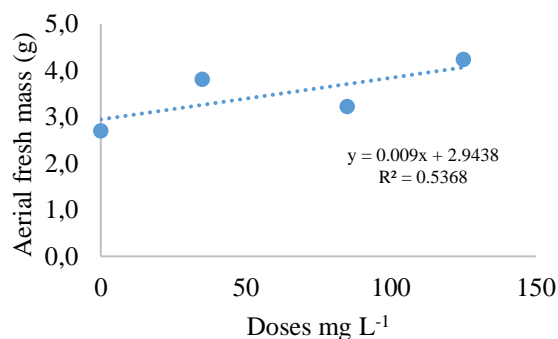


Figure 6. Effect of different doses of Chrome metal on soybean aerial fresh mass. Source: Authors, 2024.

For ADM there was a significant difference with values between 0.6 g (Control) and 0.9 g (125 mg L<sup>-1</sup>) at the highest concentration, showing a linear result (Figure 7).

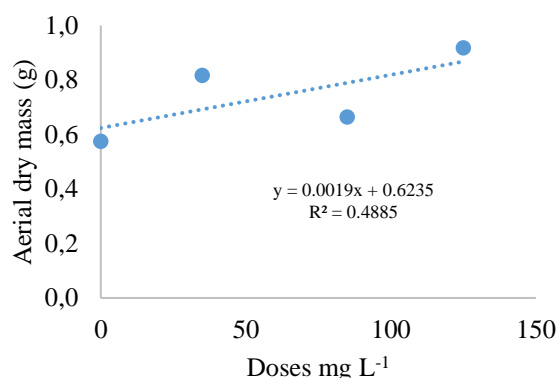


Figure 7. Effect of different doses of Chrome metal on soybean aerial dry mass. Source: Authors, 2024.

### 3.6. Effect of Iron doses

The analysis of variance indicated significant differences depending on the treatments only for the RL and RDM variables (Table 5).

Table 5. Analysis of variance for the variables plant length (PL), root length (CR), aerial fresh mass (MFA), root fresh mass (RFM), aerial dry mass (MSA), and root fresh mass (MSF) in doses of Iron.

Cd	PL	RL	AFM	RFM	ADM	RDM
	ns	*	ns	ns	ns	*
CV%	9.51	7.55	23.47	20.14	26.76	19.62

Note: \* Significant. ns = not significant according to test F. Source: Authors, 2024.

Fe showed a positive and significant effect ( $p < 0.05$ ) only for the RL and RDM parameters. The other morphological parameters were not affected by the concentrations applied via soil ( $p > 0.05$ ) (Figures 8 and 9). For RL there was a significant difference with values between 18.7 cm (Control) and 28.5 cm ( $85 \text{ mg L}^{-1}$ ) presenting a polynomial result (Figure 8).

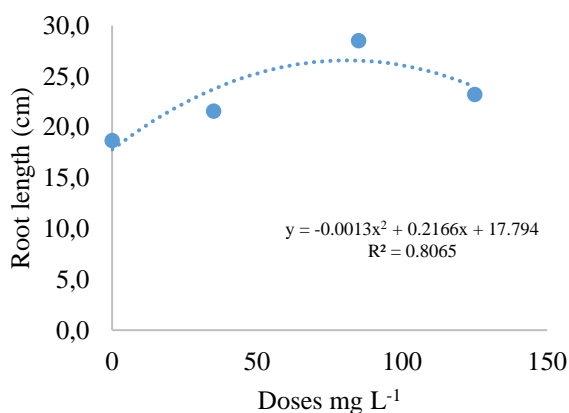


Figure 8. Effect of different doses of Iron metal on soybean root length. Source: Authors, 2024.

For RDM there was a significant difference with values between 0.3 cm (Control) and 0.5 cm ( $85 \text{ mg L}^{-1}$ ) presenting a polynomial result (Figure 9).

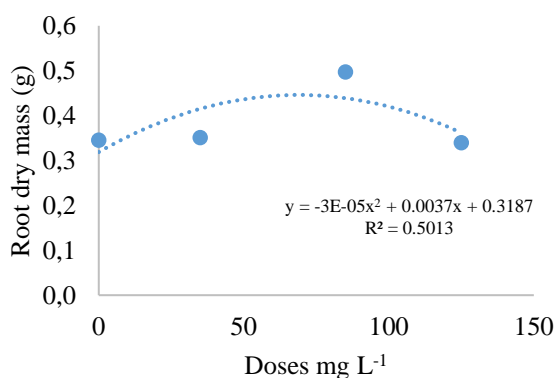


Figure 9. Effect of different doses of Iron metal on soybean aerial dry mass. Source: Authors, 2024.

#### 4. Discussion

Possible toxic effects of Al on plants are easily observed in the roots where they present thickening and yellowing at the tips, tortuous, with several secondary branches, dark in part due to the oxidation of phenolic

compounds and without absorbent hairs in the cap. Other negative factors of Al toxicity are observed in the reduction in root cap size and disarray of the meristematic tissue, in addition to the formation of protoxylem and endoderm in regions close to the root apex with high lignin levels. In the aerial system, an accumulation of soluble phenols is observed, especially in plants that are less tolerant to Al ions. This accumulation is due to the link between Al and Boron (B) (Codognotto et al., 2002; Peixoto et al., 2007; Miguel et al., 2010).

In the leaf blade treated with doses of Al, the consequence is yellowing due to Al interference in the biosynthesis of chlorophyll a/b and total, purple color in the sheaths and margins of the blade and may present atrophy due to P deficiency. In our results, the concentrations of Al were toxic mainly at the two highest concentrations 85 and  $125 \text{ mg L}^{-1}$  where the PL was lower compared to the control. In this parameter, the investigated soybean cultivar proved to be intolerant, for the other parameters there was no significant difference. The results of this study corroborate Stefanello & Goergen (2019) who studied the toxic action of Al at different concentrations of  $0\text{-}120 \text{ mg L}^{-1}$  where they found a reduction in the growth of *Cynara scolymus* L. plants between 8.65 cm (control) and 6.05 cm ( $120 \text{ mg L}^{-1}$ ). Machado et al. (2015) found significant reductions in the total length of jatropha plants (*Jatropha curcas*) as toxic Al concentrations increased up to  $80 \text{ mg L}^{-1}$ .

In the literature, uncontaminated soil is considered to have a value of  $0.5 \text{ mg kg}^{-1}$  (reference value) for Cd (Júnior et al., 2000). As observed in this study, the Cd element presented significant toxic effects for the PL and RL variables. This toxic element is absorbed through the roots, due to competition between macro and micronutrients essential for plant development. According to Chang et al. (1987) and McBride, 1995) the content of heavy metals in plant tissues depends exclusively on the pH of the soil, the nature of the metal, the OM content, and the soil's capacity to retain cations.

Furthermore, the most common forms of contaminants in agriculture are the use of industrial chemical fertilizers or sewage sludge, which can increase the concentration of toxic metals in the soil-plant system, and which are used in agriculture as an alternative way of supplying plants with essential nutrients (Kabata-Pendias; Pendias, 2001). Our results demonstrate that the soybean cultivar NEO 790 IPRO is intolerant to Cd dosages applied to the soil, as it interfered with the full development of roots and shoots. Willinghoefer et al. (2020) found that Cd in different early and mid-cycle soybean cultivars presents marked toxicity at a dose of  $2.5 \text{ } \mu\text{g L}^{-1}$  with 18% seed death. In late-cycle soybeans, these researchers found that the dose of  $5 \text{ } \mu\text{g L}^{-1}$  of Cd presented the highest toxicity rate with 25% death and 10% seed abnormalities. It then suggests that the Cd element negatively interferes with the germination process at concentrations greater than  $2.5 \text{ } \mu\text{g}$

L<sup>-1</sup>, this is supported by several studies. However, some authors disagree with this thought, Benavides et al. (2005), for example, suggest that during the germination process, Cd has low toxicity, acting mainly in the vegetative phase of the plant. This is easily discussed because during the initial stage of development after germination, seedlings are sensitive to water stress, and the most pronounced effect on this parameter is likely the interference with the internal water balance since Cd has a deleterious effect on permeability of the membrane.

Willinghoefer et al. (2020) describe that the toxic effects of Cd are visible in morphological parameters in the vegetative phase such as PL and RL in 11 cultivars evaluated with dose variation between 0-5.0 µg L<sup>-1</sup>. Regarding root length, in this study, it was observed that among the 11 soybean cultivars, only three cultivars CM7739, M7198, and NS7901 presented the lowest root length indices, which were significantly different from the others, and the control ( $p < 0.05$ ). According to Lux et al. (2010), the Cd element has an inhibitory effect on root growth and is also attributed to the reduction of mitosis, causes damage to the Golgi apparatus, in addition to reducing the synthesis of cell wall components, and promotes negative changes in polysaccharide metabolism. The action of toxic elements is still very confusing, as these elements act in different ways without respecting the same method of harmful action, this is discussed by Pal et al. (2006) who observed in an experiment with corn that the presence of Cd did not interfere with cell division but inhibited cell elongation.

Aerial and root fresh mass values analyzed by Willinghoefer et al. (2020) did not differ between the 11 soybean cultivars evaluated with doses between 2.5-5 µg L<sup>-1</sup> of Cd. Diverging results from our study were observed for corn in the experiment by Lagriffoul (1998) where he evaluated nutrient solutions with Cd, where the effect of this metal negatively influenced the length of the shoot and reduced the biomass content.

Nava et al. (2011) found in soybean plants that the Cd levels evaluated did not interfere with the morphological parameters evaluated, which were below the critical level for plants similar to some of our morphological results for the same crop. In the study by Júnior et al. (2000) researchers disagree with our results, as it was observed that the morphological parameters in soybean crops are negatively influenced by Cd at different levels available in plant tissue grown in pots. The researchers also add that, in experiments carried out in pots or cups there is a low rate of losses through percolation, where the toxic element remains longer in the soil solution, thus being, in the beginning, more available to plants. This Discussion is also supported by Miller (1976), although our concentrations did not demonstrate such facts.

Davis et al. (1978) discuss that Cd concentrations above 15 mg kg<sup>-1</sup> initiate toxic effects in vegetables, however, this dose is still debatable, as the different

groups of vegetables, including bioremediation, present a drastic variation in the dose of this toxic element. In this idea, Kabata-Pendias & Pendias (2001) consider that a critical range of Cd contamination in plant biomass is between 5-30 mg kg<sup>-1</sup>. Reaching common sense on the minimum dose at which it is possible to detect toxic effects in vegetables will still take a long way with new studies on different plant groups.

Among other heavy elements, Pb is considered not essential for the development of plants in any of their phases (germination, vegetative or reproductive). This element is highly toxic and can accumulate in organisms such as animals and humans. The phytotoxicity exerted by Pb follows the same pattern as other non-essential or essential toxic elements at levels higher than those allowed for crops. Vegetables that grow in an environment contaminated by Pb present physiological, biochemical, and structural effects, such as leaf chlorosis, changes in enzymatic activities, inhibition or reduction of germination and photosynthesis, structural malformation, darkening of the root system, causing changes in water and hormonal balance, presents high production of stomata and membrane permeability problems (Ruley et al., 2006; Romeiro et al., 2007; Pereira et al., 2013; Wójcik; Tukiendorf, 2014).

Our results for the Pb element demonstrated a significant difference ( $p < 0.05$ ) only for the root length parameter. The other parameters of the vegetative phase evaluated were not influenced by the doses applied ( $p > 0.05$ ), possibly the doses were low for detecting these other parameters. However, other studies have shown that Pb has phytotoxicity, and this was studied by Nava et al. (2011) where they found a decrease in leaf content in soybean plants fertilized with different sources of NPK+Zn caused by the high concentration of Pb. Similar results were also observed by Rangel et al. (2006) for corn plants grown in a dystroferic Red Oxisol with a clayey texture where sewage sludge with Pb was applied. There are several studies on the toxic effect of Pb on vegetables, Júnior et al. (2000) also found significant levels of Pb in plant tissue in in vivo experiments with soybeans.

The authors add that in sandy soils Pb will be more available for absorption by plants. For soils with high OM content and high clay content, there will be a greater Pb adsorption effect, therefore, this element will be less available for plants, which explains the adsorption capacity for the availability of metals in the soil, this idea is also supported by Hassett (1974). This can be observed in the OM content in the sandy Red Dystroferic soil used in our experiment. Although our results demonstrated that the graph is linear, we did not arrive at a dosage that negatively influenced root length. At the highest concentration of 125 mg L<sup>-1</sup>, the soybean cultivar was still tolerant.

Still, in other cultures, Alves et al. (2008) studied the toxic effects of Pb in *Vetiveria zizanioides* (vetiver),

*Desmanthus virgatus* (jureminha) and *Prosopis juliflora* (mesquite) where they described that the dry mass of these vegetables showed a substantial reduction in mass due to the concentrations of Pb used with decreases of 26.6, 31.2 and 37.2% in root dry mass, respectively. The same was observed for aerial dry mass with linear reductions with greater damage to *D. virgatus* plants due to the action of Pb.

In corn plants (Huang; Cuningham, 1996), *Cedrela fissilis* (cedar our *cedro*) and *Tabebuia impetiginosa* (*ipê-roxo*) (Paiva et al., 2000) also observed mass loss with linear and quadratic reduction, respectively, for a dry matter of aerial part as Pb doses increased. In the second study, the researchers obtained a drastic reduction of 77 and 59% for seedlings in both species evaluated.

These, among other authors such as Kumar et al. (1995) and Huang & Cuningham (1996) highlight that groups of vegetables react to different forms and stimuli in the presence of toxic metals such as Pb. This is confirmed by Eltrop et al. (1991) where grasses and herbaceous species demonstrate a tendency to be tolerant to excess toxic elements compared to woody species as they present more efficient physiological and biochemical mechanisms to reduce the toxicity of toxic metals in their tissues. Furthermore, Oliveira (2008) and Silva et al. (2015) discuss the level of Pb toxicity for plants, which can vary between 30-300 mg kg<sup>-1</sup>.

The toxic element Cr has an affinity for roots where it is found in greater quantities, although a small amount can be translocated to higher parts of the plant and its bioaccumulation from the soil in these parts is unlikely. Although there are distinct groups of vegetables with little, moderate, and high metal accumulation capacity, among these groups with little accumulation we have legumes (soybeans) (Mortvedt, 2001; Wang et al., 2002). Our results demonstrated that the applied concentrations showed linear regression for AFM and ADM ( $p < 0.05$ ) and for the other vegetative parameters there was no significant difference ( $p > 0.05$ ). Still in this study, the highest concentration 125 mg L<sup>-1</sup> did not present toxicity, as no threshold demonstrated a decrease in both parameters. Controversial results were found by Castilho et al. (2001) evaluating doses of Cr<sup>3+</sup> in soybean crops. The researchers observed that at 16 days after germination, soybean plants showed chlorosis and apical rot grown in concentrations similar to that of our experiment (80-160 mg L<sup>-1</sup>), and in doses lower than 20-40 mg L<sup>-1</sup> they did not observe any type of visual symptom.

These authors also show that doses of 20 mg Cr<sup>3+</sup> L<sup>-1</sup> did not affect the dry matter content of soybean shoots and roots. Our results demonstrated that Cr stimulated the production of AFM and ADM ( $p < 0.05$ ). This stimulatory effect of Cr is also discussed by Grubinger et al. (1993) and Marchiori Jr et al. (1999) where they demonstrated that Cr has stimulating effects on plant

growth, however, its sensitivity has not been proven. We observed that the maximum concentration of 125 mg L<sup>-1</sup> continues to stimulate the production of AFM and ADM, however, it does not influence PL.

According to Warington (1946) and Huffman & Allaway (1973), the benefits of Cr for vegetables may be in replacing Molybdenum (Mo), as Mo influences N fixation, although this effect is still difficult to explain. According to Castilhos et al. (2001), Cr occurs in the ecosystem as a result of the weathering of soil source material or can be introduced through the deposition of tailings and residues of industrial origin such as tanneries and steelmaking. Its trivalent form (Cr<sup>3+</sup>) is an essential nutrient for human nutrition according to Mertz (1969) and is considered a stable element in the soil. The occurrence of toxicity in plants is rare, probably due to Cr<sup>3+</sup> having low mobility and restricted movement across the cell membrane. Its harmful action on plants occurs in the form of chlorosis, reduction of leaf and root growth and death of seeds or seedlings (Mertz, 1969).

Some groups of vegetables such as vegetables demonstrated the toxicity effects investigated in lettuce plants (*Lactuca sativa*) by Figliolia et al. (1992) where they found that in soil supplied with 200 mg kg<sup>-1</sup> Cr<sup>3+</sup>, they presented, after 60 days, a content of 11.1 mg kg<sup>-1</sup> in the tissue and a 60% reduction in dry mass about the control.

Interesting fact highlighted by Losi et al. (1994) and by Moral et al. (1995) is that Cr has a preference for roots, where they form barriers that reduce its translocation to the aerial part of plants. However, our concentrations did not demonstrate any results on the development (positive or negative) of the root system ( $p > 0.05$ ). Moral et al. (1995), evaluating different concentrations of Cr in tomato plants, found low translocation to branches and fruits grown in a solution containing 100 mg L<sup>-1</sup> of Cr<sup>3+</sup>. Another important point for nitrogen (N) fixation by plants is the excessive amount of Cr that competes for N channels in the root system, which increases toxicity in plants and problems in biochemical processes in microorganisms responsible for the Nitrogen Cycle (Hungria et al., 1994).

The Fe element is influenced by pH in the soil. As seen, in alkaline soils or those that have been limed, the increase in pH can lead to lower availability of micronutrients for the vegetable and Fe is one of the examples. Our results showed a significant difference only for the RL and RDM variables ( $p < 0.05$ ). For RL and RDM, doses greater than 85 mg L<sup>-1</sup> demonstrated toxicity in this study.

Souza et al. (2010) evaluated soybean plants for Fe content and observed that the lower pH favored the accumulation of this element in plants grown in clayey soil after 28 days of germination. The researchers found that the greatest accumulation of Fe was in the root



system. In vegetables, Fe is metabolically controlled and can be absorbed in the form of  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$ , or Fe-chelate and needs to be reduced before entering the cells. Fe toxicity is well discussed for rice cultivation (Schmidt et al., 2013), wherein flooded areas during rice production, soil microorganisms reduce insoluble  $\text{Fe}^{3+}$  to soluble  $\text{Fe}^{2+}$ . Dobermann & Fairhurst (2000) describe that in rice planting areas before submergence the concentration of soluble Fe rarely exceeds  $0.1 \text{ mg L}^{-1}$ , after flooding, in acidic soils this content can be up to  $600 \text{ mg L}^{-1}$ .

The toxic effects of Fe are extensively studied, as they influence the formation of reactive oxygen species, such as hydrogen peroxide, superoxide anion, and hydroxyl radical. In vegetables, the symptoms of poisoning are easily visible as they promote the formation of tiny brown spots from the tips that spread throughout the leaf blade. Several symptoms are observed in the roots, such as scarce, thick, short, and dark brown roots (Sahrawat et al., 2004; Silveira et al., 2007).

This micronutrient in ideal concentrations acts in a large complex of reactions, including the electron transfer chains of respiration and photosynthesis, the biosynthesis of DNA, lipids, and hormones (Rout; Sahoo, 2015), it also in the detoxification of reactive species of oxygen (superoxide dismutase), as well as in the assimilation of N (nitrate and nitrate reductase (Curie et al. (2009).

Liang et al. (2006) and Jiang et al. (2009) demonstrated in studies that the element Fe at a toxic level negatively influences the absorption of nutrients such as P, Zinc (Zn), and Copper (Cu). Our results corroborate Lux et al. (2011) and Li et al. (2015) where they found that Fe toxicity inhibited root growth, where it promoted a reduction in cell elongation and cell division. Wu et al. (2014) and Tavares et al. (2020) argue that the high content of  $\text{Fe}^{2+}$  causes symptoms of toxicity in plants that can be observed throughout the entire vegetative cycle.

## 5. Conclusion

The increase in doses of the elements Al, Cd, Pb, Cr, and Fe to toxic levels in the soil presented interesting results. Al and Cd negatively influenced some vegetative parameters where they were present. However, positive effects were observed for Pb and Cr on other morphological parameters, and the Fe element, it was possible to observe double suitability as a toxic element at high doses ( $> 85 \text{ mg L}^{-1}$ ) and at doses lower than  $85 \text{ mg L}^{-1}$  stimulatory effect on vegetative parameters in soybean cultivar NEO 790 IPRO. The NEO 790 IPRO cultivar proved to be resistant in most of the evaluated parameters and applied doses.

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## **Author's Contributions**

*Jerônimo Luiz da Silva Neto*: research design, experiment setup, data analysis, article writing. *Porshia Sharma*: translation, grammatical, and textual corrections. *Aurélio Ferreira Melo*: data analysis, study writing and publication. *Antonio Carlos Pereira de Menezes Filho*: data analysis, study writing and publication. *Matheus Vinícius Abadia Ventura*: advisor, data analysis, statistics, final corrections.

## **Ethics**

Not applicable.