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Effects of perchlorate on the growth and development of corn (*Zea mays*) and bean (*Phaseolus vulgaris*) plants

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Abstract- Perchlorate ClO_4^- is a persistent and toxic inorganic anion, from both natural and anthropogenic origin, present in all environmental matrices. It is an endocrine disruptor that affects metabolism, reproduction, and development. Humans are exposed through ingestion, inhalation, and contact, causing hypothyroidism and cardiovascular diseases. The objective of this study is to determine the effects of perchlorate on plants grown under greenhouse conditions, using corn (Zea mays) and bean (Phaseolus vulgaris) as evaluated species. The methodology involved determining the effects of perchlorate on the cultivated plants. The results obtained, supported by statistical analysis, reveal significant impacts caused by ClO_4^- exposure on both P. vulgaris and Z. mays plants. This is reflected in the growth, shape, and weakness of leaves, stems, and roots, with more pronounced effects observed in the bean species, while the corn plants exhibited higher. The statistical evidence underscores the reliability of the obtained results and strengthens the understanding of the consequences of perchlorate exposure on plant species.

Keywords-- endocrine disruptor, in vitro plants growth, perchlorate, P. vulgaris, toxicity, Z. mays.

I. INTRODUCTION

Perchlorate (ClO_4^-) is a persistent and toxic inorganic anion, from both natural and anthropogenic origin, present in all environmental matrices. Perchlorate accumulation in ecosystems is possible, thanks to its chemical stability under normal environmental conditions [1],[2],[3],[4],[5]. It is produced through different industrial processes, such as military, fireworks and explosives industry, fertilizers, and agriculture [6],[7]. Perchlorate presence has been reported with concentrations up to 35.0 μg/L in freshwater [8], 22.1 μg/L in groundwater, and 13 μg/Kg in soil [9],[10].

Perchlorate enters throughout the food chain since it has been used as an additive agent in plastic food containers [11], it reaches crops through runoff water [12], and animal products such as milk, sausages, ham, fish, and meat [11],[13],[14].

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In humans, the main exposure route for perchlorate is through the ingestion of contaminated food and water [15], inhalation of atmospheric depositions [16], and by contact. Once it enters the body it is absorbed and sent to the bloodstream where it acts as a potent endocrine disruptor, affecting thyroid function, producing hypothyroidism [3],[16],[17],[18], and generating an increase in cardiovascular diseases [19].

Perchlorate effects on wildlife have also been reported, particularly mammals, amphibians, reptiles, and fish [20],[21], where it affects the metabolism, reproduction, and development of exposed species, causing irreversible damage in many organisms [22].

Studies also show that perchlorate accumulates easily in plants such as rice, where it can inhibit plant growth [23],[24]. However, there is limited information available on the effects of perchlorate at different trophic levels, and on the physiological and toxicological effects on plants. Since it is necessary to develop toxicity bioassays in different biological models such as plants [22], this work aims to determine the effects of perchlorate on plants grown under greenhouse conditions, using corn (*Z. mays*) and bean (*P. vulgaris*) as evaluated species; which allowed us to identify the effects of perchlorate on different growth parameters of these plants; such as: plant height, root length, stem fragmentation, mortality, effects of chlorosis and necrosis on leaves.

The absorption and bioaccumulation mechanisms of contaminants that affect seed germination processes and root elongation are not yet well understood. This is due to .the synergistic or antagonistic effects of complex physicochemical or biochemical processes, such as adsorption, and binding to components present in plants, which can modify the specific properties of contaminants.

Therefore, the information obtained in this study constitutes a baseline, which can be replicated in areas with similar environmental conditions. This will lead to proposing recommendations and strategies to mitigate the effects of ClO_4^-

contamination on plant species and the environment, which will be helpful for public health and food safety decision-making, due to the ingestion of food contaminated by ClO_4^- for interested parties.

II. Material and Methods

A. Greenhouse assay

The greenhouse system for the *in-vitro* tests was located beside the Biology Laboratory at the Basic Sciences Department (Universidad Tecnológica de Bolívar). It was designed as a triangular prism structure 1.0 m width, 1.5 m height, and 3.0 m length, built with ½” PVC pipes, and covered with high-density polyethylene poly-shade mesh. The bioassays for the plants’ growth were performed in 16 Oz disposable cups, filled with garden type fertile soil, and kept under greenhouse conditions, 85 to 90% humidity, and daytime ambient temperatures ranging between 30°C and 35°C. The studies were carried out during the last semester of 2022 and mid-2023, covering the rainy season of the study site.

B. Determination of the effects of perchlorate on the cultivated plants

Three perchlorate concentrations (10 ppm, 100 ppm, and 200 ppm) were evaluated in both corn (*Z. mays*) and bean (*P. vulgaris*) plants. The perchlorate solutions were incorporated by the spray method (modified and adapted from [25]) when the plants had at least four visible leaflets. Experimental design was a random block distribution of 3 concentrations with 15 repetitions for each plant species, with their respective negative controls (modified and adapted from [26],[27],[28]).

The variables evaluated after perchlorate application included: stem length (measured every 24 hours, up to 30 days); root length (measured after 30 days); apical buds overgrowth, susceptibility to pests (ants and aphids), stem weakening and break, generalized death of the plant, and signs of toxic effects (chlorosis and necrosis) in leaves (each observed at days 15 and 30). Binary matrices were used for data collection and acquisition.

C. Statistical analysis

For bean (*P. vulgaris*) and corn (*Z. mays*) plants, we conducted a comprehensive statistical analysis. Normality tests were performed first, and subsequently, the robust Kruskal-Wallis test [1], [25] was employed. This examination was pivotal in discerning variations among control and perchlorate-exposed plants, providing a nuanced understanding of how these botanical entities respond to environmental stressors.

This ensured the reliability of the results, allowing for a more accurate assessment of variations between control and perchlorate-exposed plants. The combined use of normality tests and the Kruskal-Wallis analysis provided nuanced insights into how these botanical entities respond to environmental stressors. The statistical evidence supports their observations on the effects of ClO_4^- exposure in plants, forming a robust foundation for the conclusions.

III. Results

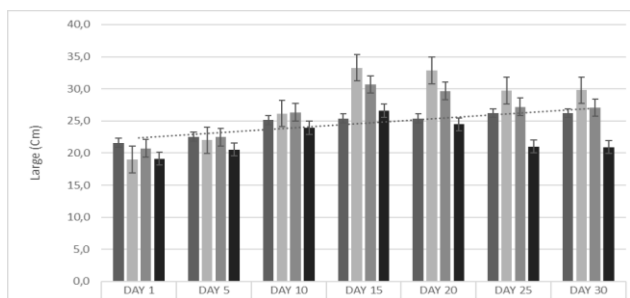
A. Bean (Phaseolus vulgaris) and corn (Zea mays) plants germination.

The greenhouse device designed allowed the germination of both corn (*Z. mays*) and bean *(P. vulgaris)* in seven days, with germination rates of 50% and 90% respectively. For further assays 60 plants of each specie were used, distributed in 15 replicas for each perchlorate concentration (10, 100, and 200 ppm) and an additional control test.

B. Determination of the effects of perchlorate on the cultivated plants.

For the bean (*P. vulgaris*) growth tests, figure 1 shows the effects of endocrine disruption caused by perchlorate; the 10 and 100 ppm concentration samples present higher stem growth when compared to the control. This effect can be influenced by the perchlorate addition to the substrate where the plants are cultivated. The higher growth in plants exposed to a concentration of 10 ppm is evidenced since the 10th day of the bioassay, confirming the effect of ClO_4^- on the growth and plant’s ability to take up nutrients [22],[29].

Meanwhile, the stem growth in the plants with 200 ppm ClO_4^- concentration, presented a greater effect in the same period, while the plant’s growth was lower than the control in figure 1.



	DAY 1	DAY 5	DAY 10	DAY 15	DAY 20	DAY 25	DAY 30
CONTROL	21,6	22,5	25,1	25,4	25,4	26,2	26,2
10ppm	19,0	22,0	26,1	33,3	32,9	29,7	29,8
100ppm	20,7	22,5	26,3	30,7	29,7	27,2	27,1
200ppm	19,1	20,5	23,9	26,6	24,5	21,0	20,9

Figure 1. Average stem growth in bean plants (*P. vulgaris*)

After the 30-day observation period, the bean (*P. vulgaris*) plants were removed from their containers for root measurement. The plants exhibited signs of stress, evident through the presence of symbiotic nodes and indications of an inability to acquire nutrients from the soil. That effect is closely related to the low quality of the roots, which presented weakness, and higher growth for the perchlorate concentrations of 10 and 200 ppm, when compared to the control tests, thus evidencing endocrine disruption effect caused by ClO_4^- exposure on bean (*P. vulgaris*) plants (figure 2).

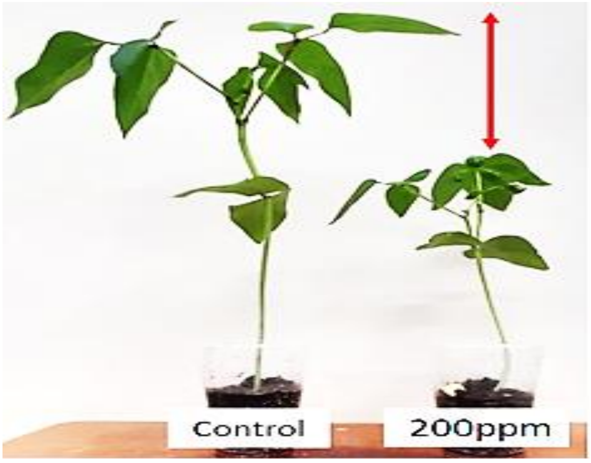


Figure 2. Effects of Perchlorate on bean plants (*Phaseolus vulgaris)* evidenced for the size of the control plant with respect to a study plant with 200 ppm ClO_4^- concentration after 30 days.

The ClO_4^- absorption by the roots affects their volume, because of the soil dynamics which causes stress levels and inability to capture nutrients by the bean plants (*P. vulgaris*), as evidenced in the root's growth average.

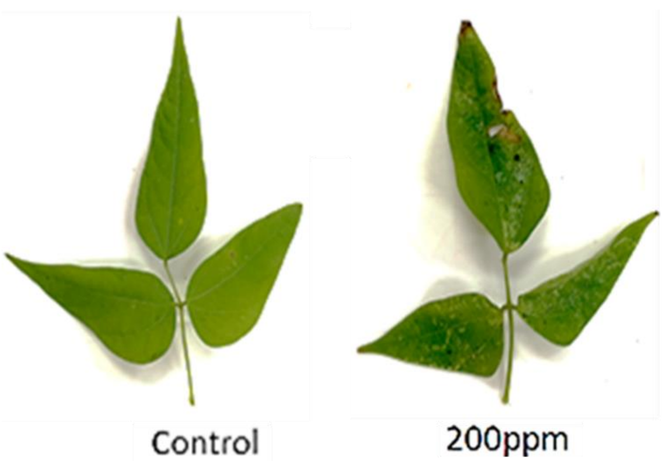


Figure 3. Comparison between bean leaves *(Phaseolus vulgaris),* for the control plant (left), and a 200 ppm ClO_4^- concentration plant (right).

Figures 3, 4, and 5 show how bean plants are affected by symptoms associated with ClO_4^-. All the plants exposed to the contaminant show stem weakness, and, for most of them, chlorosis is the predominant symptom, reflected in the leaves' coloration and associated with perchlorate exposure stress (figure 3). Additionally, high indexes of abnormal growths were observed in all the components of the exposed plants, agreeing with results reported in other biological models [22].

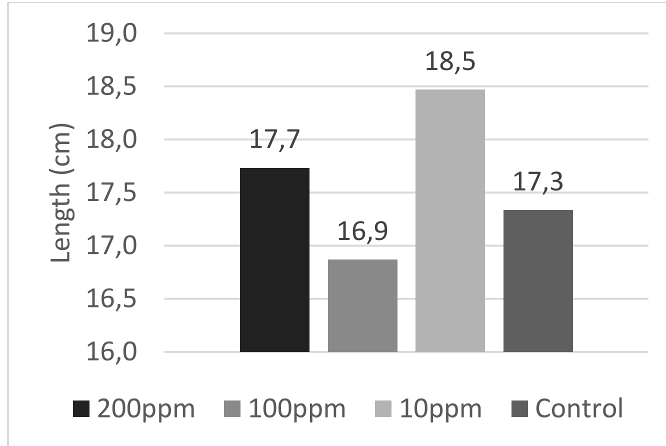


Figure 4. Average roots growth in bean (*Phaseolus vulgaris*) plants after 30 days.

Figures 6 to 10 show the results of corn plants (*Z. mays*) growth tests. Unlike bean plants (*P. vulgaris*), in the corn species, growth altering disruption effects were evident for the 100 ppm ClO_4^- concentration tests, concerning the control. For the 10 and 200 ppm concentration tests, a reduction in the average stem size was observed in corn plants (*Z. mays*) at the end of the bioassays.

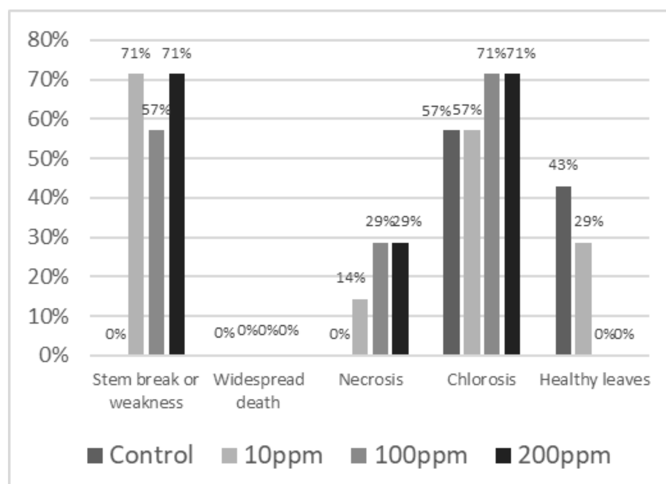


Figure 5. Effects of perchlorate exposure on bean (*Phaseolus vulgaris*) plants after 30 days.

When compared to the control tests, corn plants (*Z. mays*) did not present higher growth for any of the concentrations tested. However, they showed a reduction in the specimens' size, when exposed to 200 ppm concentrations (figure 9), mainly after 20 days of the bioassay, presenting effects on the stem, such as weakness, chlorosis, and necrosis.

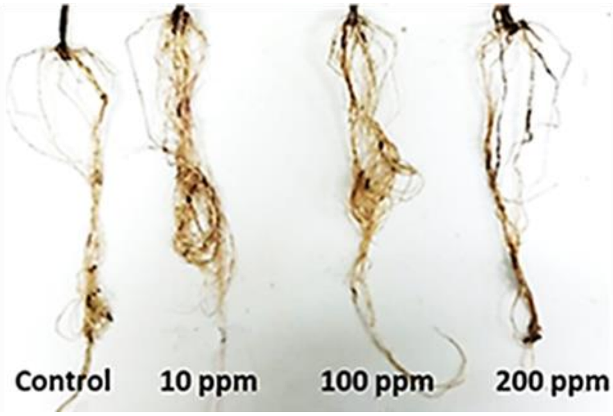


Figure 6. Average root growth in corn plants (*Zea mays*)

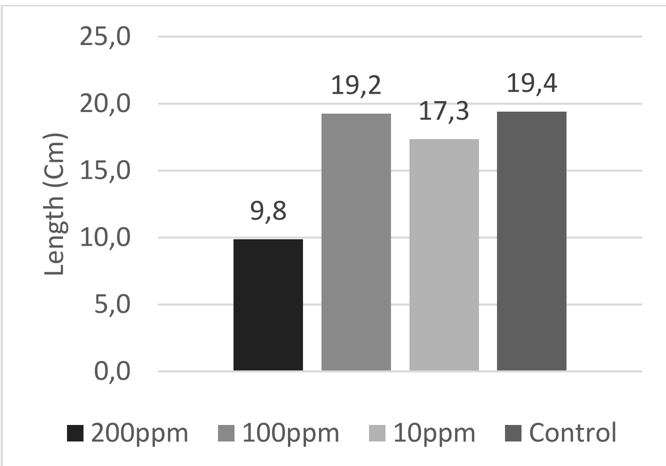


Figure 7. Length of corn roots (*Zea mays*) at the end of the study, from left to right: 200ppm, 100ppm, 10ppm, and control.

After the 30 days observation period, the corn (*Z. mays*) plants were removed from their containers for roots measurement. Similarly, to the bean species, the exposed corn samples presented stress symptoms, reflected as the presence of symbiotic nodes, which indicates inability to obtain nutrients from the soil. That is closely related to the low quality observed in the roots, which present weakness and low growth rates, mainly at 200 ppm concentration (figure 6), which is evidence of endocrine disruption factors associated to ClO_4^- exposure.

The ClO_4^- absorption by the corn roots, similarly to the bean samples, affects their volume, due to the effect on the soil dynamics which causes stress levels and inability to capture nutrients by the corn plants (*Z. mays*), as evidenced in the roots growth average (figure 7).

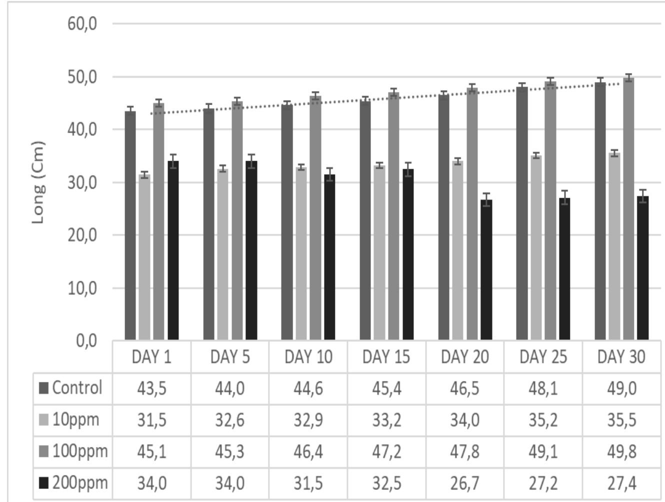


Figure 8. Average stem growth in corn (*Zea mays*) plants after 30 days.

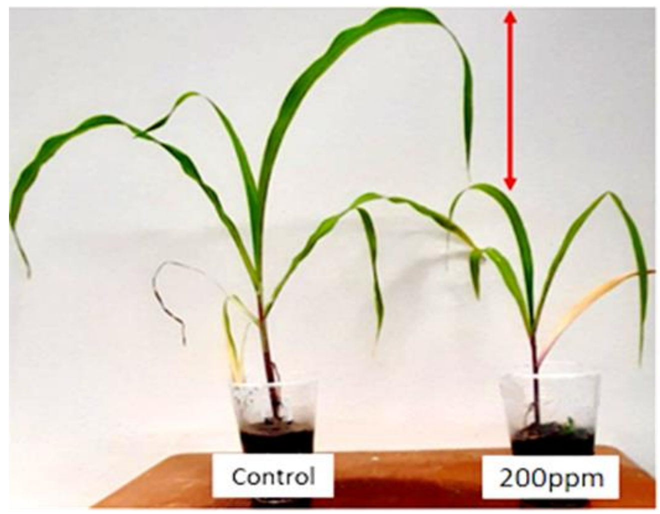


Figure 9. Effects of Perchlorate on corn plants (*Zea mays*) evidenced for the size of the control plant compared to a study plant with a concentration of 200 ppm ClO_4^- at 30 days.

The population of corn plants affected by symptoms associated with ClO_4^- is presented in figure 8. Regardless of the concentration, all the plants exposed show stem weakness, and, for most of them, chlorosis is the predominant symptom, reflected in the leaves coloration and associated with perchlorate exposure stress (figure 10). However, although perchlorate exposure symptoms were present in all 200 ppm specimens, corn plants (*Z. mays*) did not show teratogenesis, as bean plants (*P. vulgaris*) did. Additionally, necrosis was not observed in any of the 100 ppm tests which indicates endocrine disruption effects are more evident for low concentration tests.

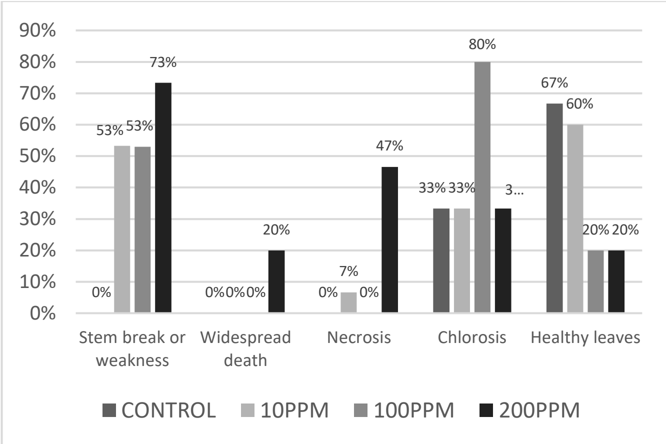


Figure 10. Effects of perchlorate exposure on corn (*Zea mays*) plants after 30 days

C. Statistical analysis of the data

For bean plants (*P. vulgaris*), the Kruskal-Wallis analysis yielded a p-valor of 0.06 Although this value hovers just above the conventional significance threshold of 0.05, its presence suggests a noteworthy trend. Interpreting these findings cautiously, we chose not to reject the null hypothesis, suggesting that no statistically significant differences exist between the experimental groups exposed to perchlorate and the control group. This decision prompts us to delve deeper into the subtleties of bean plant responses to perchlorate exposure and the implications for their overall ecological resilience.

Conversely, the analysis of corn plants (*Z. mays*) revealed a strikingly low p-valor of 6.01e-16. This diminutive p-valor led us to decisively reject the null hypothesis, indicating clear and statistically significant differences among the experimental groups. This marked divergence in statistical outcomes between bean and corn plants underscores the importance of species-specific responses to perchlorate concentrations, a facet demanding attention for comprehensive ecological assessments and targeted mitigation strategies, in Table 1.

Table 1. Analysis of both species through Kruskall-Wallis test.

Plant species	P-valor	Decision
P. vulgaris	0.062	No rejection of H_0
Z. mays	6.01^{-16}	Rejection of H_0

This statistical exploration aligns with qualitative botanical assessments, contributing a multifaceted perspective to our understanding of perchlorate's influence on plant physiology. The divergent statistical outcomes for bean and corn plants reflect the nuanced intricacies of species-specific responses, a phenomenon substantiated by previous studies [22, 23, 29]. Notably, Lee et al. [14] and Calderón et al. [10] have previously explored the species-dependent nature of perchlorate accumulation, emphasizing the need for tailored ecological management strategies.

Moreover, the inclusion of statistical rigor enhances the relevance of our findings, offering insights into the broader implications for ecological resilience and sustainability in agricultural systems [16] highlighted the pervasive presence of perchlorate in the food chain, our results underscore the importance of understanding how different plant species contribute to the bioaccumulation of this contaminant. This aligns with broader concerns raised by Maffini et al. [11] and Calderón et al. [17] regarding perchlorate's impact on human health through the food chain.

IV. Discussion

Based on the previous results, it can be affirmed that growth and development of both bean (*P. vulgaris*) and corn (*Z. mays*) plants behaved as expected. However, bean plants (*P. vulgaris*) showed a greater tendency to be affected by the pollutant than corn plants (*Z. mays*) which exhibit a higher resistance to the effects of perchlorate.

Chlorosis presence in the control plants for both plant species could be attributed to the presence of rain in the study area. The increased volume of water received daily by the plants, combined with other environmental factors, may have contributed to the manifestation of this symptom. For both plant species, chlorosis affected the quality of the leaves as expected, fully covering the scale for leaf quality and stem structure. Importantly, it should be noted that factors such as rain and container size did not impact the statistical analysis conducted.

The overgrowth effect found in bean (P. vulgaris) plants exposed to perchlorate affected leaves in the same way, causing alterations in plant development. Both the leaves and the stem grew without a defined shape, size, or normal thickness, when compared to the control plants, and they presented a break tendency due to their fragility. On the other hand, corn plants (*Z. mays*) presented stem weakness defined by their thickness.

When analyzing the quality of the roots, in bean plants (*P. vulgaris*) the presence of nodules was notorious, when compared to control tests, it was possible to observe that the 10 ppm and 100 ppm exposed plants could double this quantity, and the loss of secondary roots was appreciated. These results contrasted with corn plants (*Z. mays*), which managed to maintain their roots in better conditions at these concentrations.

Compared to other studies [24] performed with rice plants (*Oryza sativa L.)* it can be observed in our study that leaves and roots behavior was similar, although the presented data used a different classification method. The analysis of the relationship of the ClO_4^- exposure associated symptoms for the control line, show that the control plants present higher strength, chlorophyll content, and roots activity, with less stress, which is comparable to the data presented for the rice plants study [24].

Other research studies [22],[24] used different ClO_4^- concentrations in different biological models, also showing that lower perchlorate concentrations present higher effects after exposure when compared to the evaluated models.

On the other hand, [29] suggested that the perchlorate ClO_4^- effects are highly dependent on the species, which coincides with other results presented [22],[23] as well as in this study, where each plant species reacted in different ways to the perchlorate concentrations, while each specie concentrated the contaminant in a different place. Plants such as corn and lettuce mainly concentrate the ClO_4^- in the fruit (*Z. mays*) and leaves (*Lactuca sativa L* and other varieties) [10],[30], while studies performed in *Nicotiana tabacum* variety K326 plants [31] also concluded that the plants present the capacity to accumulate the contaminant in the leaves, affecting the final product from the tobacco plant with concentration of the ClO_4^- contaminant.

Although fruits were not obtained in the present research in any of the plants (*P. vulgaris* or *Z. mays)*, figures 4 and 7 present the comparison of the percentage of plants effect associated to the exposure to ClO_4^- which indicates that the evaluated plants: can accumulate perchlorate contaminant concentrations in the leaves and stem, producing weakness and stem break, as well as necrosis and chlorosis in the leaves, results that are in agreement with other studies [24],[29].

The impact of perchlorate in agriculture has been studied in different models [10],[11],[24],[29],[30],[31],[32],[33] and their results showed the capacity of this contaminant to be persistent and bioacumulable in plants as well as in different environmental matrixes such as soil. Some studies [16],[18],[33],[34],[35],[36] indicate perchlorate presence in the food chain, while food ingestion in some Chinese populations demonstrated to contain this contaminant [7], thus, affecting the population's health when perchlorate contaminated foods are consumed, producing hypothyroidism [17],[22] and showing the impact that this contaminant may have.

The presence of ClO_4^- in the trophic chain [33], is associated to the distribution of environmental matrixes, as shown in studies performed in soil and water samples [2],[4],[12],[14],[23] that proved that, at different seasons, perchlorate traces are found in food due to wrong agricultural practices that make excessive use of fertilizers based in Cl compounds [10], as well as the high incidence of anthropogenic activities that promote the introduction of this contaminant in ecosystems through water and soil.

The contamination of water bodies used for agricultural irrigation increases the contact between the perchlorate contaminant and the foods to be processed [12],[14], also increasing the effects on human health, as highlighted in other studies regarding hypothyroidism cases [17],[22] and the increase in cardiovascular diseases [19].

V. CONCLUSIONS

The evaluation of the effects of perchlorate contamination on plants grown under greenhouse conditions allowed us to conclude that both corn (*Z. mays*) and bean (*P vulgaris*) plants are affected by perchlorate exposure, which is reflected in the growth distribution and contamination symptoms detected in the plants. The statistical analysis revealed significant differences between the groups, highlighting the impact of perchlorate and leading to the rejection of the null hypothesis. However, the effects were not similar for both species, as bean specimens presented a higher tendency to be affected by the pollutant, while corn plants showed higher resistance. These findings underscore the importance of considering the statistical insights in understanding the nuanced responses of different plant species to perchlorate contamination.

The presence of chlorosis effects was detected in both plant species, reflected in the quality of the leaves and the stem structure. The exposed bean plants (*P. vulgaris)* also showed alterations in their growth and development, reflected in the shape, size, and thickness of the leaves, as well as a break tendency due to their fragility, while corn plants (*Z. mays*) were affected in the stem thickness. For the analysis of the roots, bean plants (*P. vulgaris*) presented notorious nodules and the loss of secondary roots, while corn plants (*Z. mays*), presented better quality roots at the same contaminant concentrations.

The results obtained in this research allow the evaluation of the impact caused by ClO_4^- exposure on *P. vulgaris* and *Z. mays* plants and explain the effects of this contaminant on the trophic chain and how it can affect the environment and the health of the species that consume the plants evaluated. These results likely also provide evidence of the existence of a linear relationship between perchlorate concentration and bioaccumulation rate, causing an inhibitory effect on growth in vegetal systems.

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