ISBN: 978-628-96613-3-0. ISSN: 2414-6390. Digital Object Identifier: https://dx.doi.org/10.18687/LACCEI2026.1.1.1186

Phytotoxicological Assessment of Perchlorate in *Zea mays*, *Phaseolus vulgaris*, and *Cymbopogon citratus* plants under greenhouse conditions

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Abstract* – Perchlorate has emerged as a contaminant of growing concern in soils due to its widespread occurrence and adverse effects on terrestrial ecosystems as an endocrine disruptor. Perchlorate affects metabolism, reproduction, and development of animal and plant species. The primary exposure pathway of ClO_4^- for humans is 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 Zea mays and Phaseolus vulgaris, and Cymbopogon citratus as evaluated species. The methodology used included three stages: [1] Design and construction of a greenhouse for in vitro growth tests; [2] Determination of the effects of perchlorate on the cultivated plants [3] Statistical analysis. The results obtained indicate species-specific differences in sensitivity to perchlorate exposure, with statistically significant effects observed in Z. mays, while C. citratus and P. vulgaris exhibited comparatively lower susceptibility. These findings highlight the heterogeneous impact of ClO_4^- exposure on C. citratus, P. vulgaris, and Z. mays plants under greenhouse conditions.

Keywords- Cymbopogon citratus, in vitro, perchlorate, plants growth, Phaseolus vulgaris, Zea mays.

I. Introduction

Perchlorate has emerged as a contaminant of growing concern in soils due to its widespread occurrence and adverse effects on terrestrial ecosystems [1]. Its presence in soil environments results from both natural processes, including atmospheric deposition and volcanic activity, and anthropogenic sources such as fertilizer application, industrial waste disposal, and military and aerospace activities [2], [3], [4]. Due to its high solubility, chemical stability, and low reactivity, perchlorate can persist in soils for long periods, facilitating its migration through soil profiles and its potential transfer to groundwater and plants [2], [5].

Relatively high concentrations of perchlorate in soils are mainly found in arid and semi-arid regions, such as the Atacama Desert, where limited precipitation restricts its mobilization and removal [2], [4], [6].

Perchlorate can be absorbed by plants from contaminated soils or irrigation water, as well as from agricultural inputs such as nitrate fertilizers, which can introduce perchlorate into the food chain, posing risks to plants, animals, and ultimately leading to human exposure and potential health effects [7], [8], [9]. Studies also show that perchlorate accumulates easily in leafy vegetables and plants such as rice, where it can inhibit plant growth [8], [10], [11]. Most vascular plants can absorb and accumulate perchlorate in their tissues, which can disrupt and negatively affect seed germination, chlorophyll content, and plant growth and development [11], [12].

The occurrence of perchlorate in agricultural products has raised concerns regarding food safety. Upon ingestion, perchlorate can interfere with thyroid function by inhibiting iodide uptake, leading to reduced thyroid hormone levels, and at sufficiently high concentrations, it may also affect bone marrow and muscle tissue [2], [4], [13], [14].

Reported dietary exposure levels of perchlorate from vegetable consumption have been shown to approach or exceed established health-based guidance values. Estimated daily intakes of perchlorate from vegetables have been reported to range from 0.32 to 0.37 μg/kg body weight (bw) per day [7]. For comparison, the European Food Safety Authority (EFSA) established a chronic tolerable daily intake (TDI) of 0.3 μg/kg bw per day [15], while the United States Environmental Protection Agency (EPA) assigned a chronic oral reference dose (RfD) of 0.7 μg/kg bw per day [10], [16].

As plant uptake represents the primary pathway for its transfer into the food chain [2], [9], [11], assessing the impact and understanding the effects of perchlorate on plant growth and accumulation are key steps in ensuring food safety. However, current knowledge regarding its influence across trophic levels remains scarce, especially with respect to plant physiological responses and toxicity mechanisms.

Plants such as corn, beans, and lemongrass play a key role as primary producers and are suitable models for toxicity assessment; their use in controlled bioassays is essential for understanding perchlorate-induced effects. This work aims to determine the effects of perchlorate on plants grown under greenhouse conditions, using *Z. mays*, *C. citratus*, and *P. vulgaris* as evaluated species.

II. Material and Methods

A. Controlled greenhouse assay

The *in-vitro* experiments were conducted following the methodology described by Acevedo-Barrios *et al.* [11]. The greenhouse system was in a facility adjacent to the Biology Laboratory of the Department of Basic Sciences at Universidad Tecnológica de Bolívar. The structure consisted of a triangular prism measuring 1.0 m in width, 1.5 m in height, and 3.0 m in length, constructed using ½” PVC tubing and enclosed with high-density polyethylene shade mesh.

Plants’ growth bioassays were carried out using 16-oz disposable plastic containers filled with fertile garden soil. Throughout the experiment, greenhouse conditions were maintained with relative humidity between 85 and 90% and daytime temperatures ranging from 30 to 35 °C. The samples were studied during the rainy seasons of 2025 April to Jun and from September to November.

B. Evaluation of the effects of perchlorate on cultivated plants

The cultivated plants: *Z. mays*, *C. citratus*, and *P. vulgaris*; were exposed to three perchlorate concentrations (10 ppm, 100 ppm, and 200 ppm) applied via foliar spraying, following a modified protocol adapted from previous studies [11], [17], once plants had developed a minimum of four fully expanded leaves.

Experimental design followed a randomized block distribution for the three treatment concentrations with 15 replicates per plant species with their control groups [11].

Data were systematically recorded using binary matrices. The measured endpoints were defined as follows: root length (after 45 days); stem length (Every 24 hours, up to 45 days), as quantitative growth parameters. Additionally, weakening and stem breakage were recorded as indicators of structural damage. Additional qualitative parameters were evaluated on days 15 and 45, including plant mortality and visible phytotoxic responses such as leaf chlorosis and necrosis [11].

C. Statistical analysis

Descriptive and inferential statistical analyses were conducted to evaluate the effects of perchlorate exposure on the studied plant species. Descriptive statistics were used to summarize growth responses and visible phytotoxic effects. Data distribution was assessed using normality tests, which indicated non-normal behavior ($p < 0.05$).

Consequently, a non-parametric approach was adopted. Differences in the distributions of stem length between control plants and perchlorate-treated groups (10, 100, and 200 ppm ClO_4^-) were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). All statistical analyses were performed using R software (version 4.5.2).

III. Results

A. Early Germination Outcomes Under Greenhouse Conditions

Germination of the three cultivated plants was successfully supported under the greenhouse system implemented. Within seven days, *P. vulgaris* and *Z. mays* reached germination rates ranging from 50% to 90%. Meanwhile, *C. Citratus* required 15 days to reach around 60% of germination rate.

For subsequent experimental evaluations, a total of 60 plants per species were selected and allocated into 15 replicates for each perchlorate treatment level (10, 100, and 200 ppm), along with an additional set of 15 replicates serving as the control group.

B. Evaluation of the effects of perchlorate on cultivated plants

Stem length

A decrease in stem length was observed in perchlorate-exposed plants (Fig 1), especially beans and corn, which showed a marked reduction compared to the control group *P. vulgaris* and *Z. mays* showed a pronounced inhibitory response and a concentration-dependent decrease in stem elongation (Fig 2, Fig 3), indicating a perchlorate-induced endocrine disruption on the stem development.

For *Z. mays* no data were recorded at day 45 of exposure due to complete plant mortality prior to the final evaluation.

Both findings coincide with previous studies [11]. In contrast, lemongrass stem length (Fig 4) remained relatively constant, with no evident changes across perchlorate concentrations

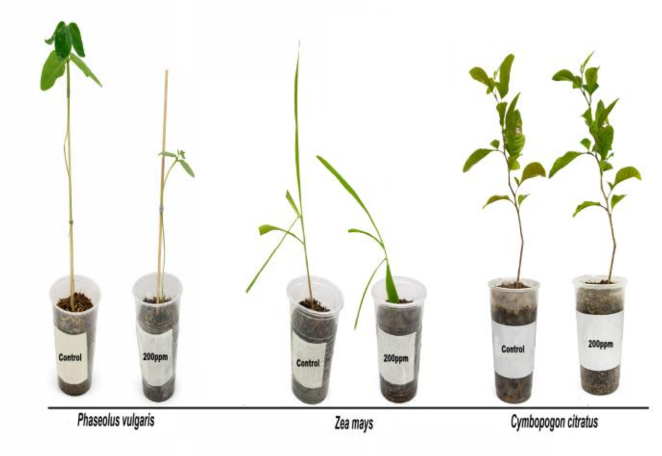

Fig 1. Effects of Perchlorate on *P. vulgaris*, *Z. mays*, and *C. Citratus* plants are shown as the size of the control plant compared to the study plant with 200 ppm perchlorate concentration after 15 and 45 days.

At a lower concentration (10 ppm), beans and corn exhibited a growth pattern comparable to or slightly higher than the control, which may be associated with a hormetic response or with changes in nutrient availability induced by perchlorate in the substrate [18].

Contrarily, intermediate concentrations (100 ppm) showed variable effects, suggesting the onset of physiological stress that may interfere with normal growth processes [19], [20].

At the highest concentration (200 ppm), a reduction or stagnation in stem growth was evident, indicating a threshold beyond which perchlorate exerts phytotoxic effects.

These contrasting responses may be influenced by factors such as perchlorate-induced disruption of nutrient uptake, alterations in hormonal balance, species-specific tolerance, and the duration of exposure under greenhouse conditions [8], [17], [21].

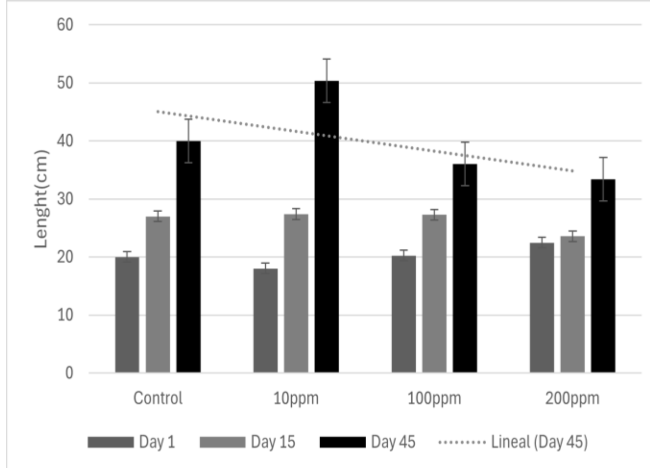

Fig 2. Average stem growth in plants *P. vulgaris*.

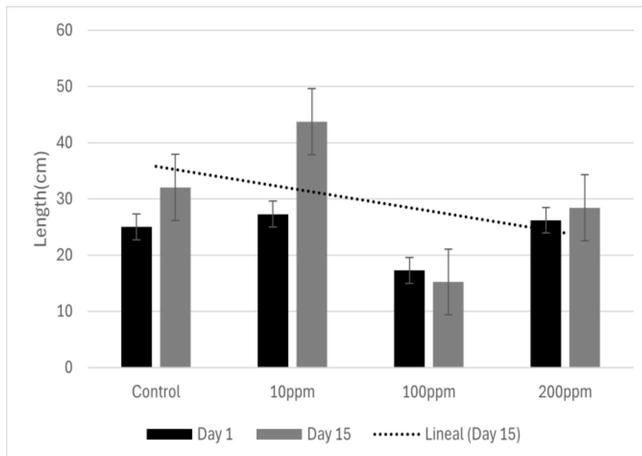

Fig 3. Average stem growth in plants *Z. mays*.

C. citratus shows a higher tolerance to perchlorate stress, possibly due to its robust root system and efficient physiological mechanisms that maintain nutrient uptake and growth. As a result, stem elongation remained stable, and no significant changes were observed under the evaluated concentrations.

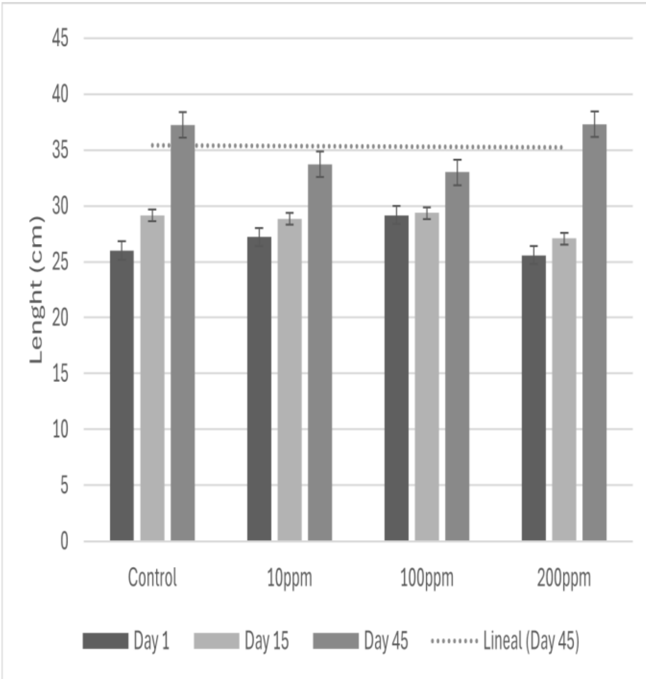

Fig 4. Average stem growth in plants *C. citratus.*

Root length

Clear species-specific responses in root growth to increasing perchlorate concentrations were observed (Fig. 5). *Z. mays* exhibited a concentration-dependent reduction in growth, with the strongest inhibition at 200 ppm. *P. vulgaris* also showed decreased vigor at higher concentrations, while intermediate levels (10 and 100 ppm) produced more variable responses, suggesting sub-lethal stress.

In contrast, *C. citratus* displayed minimal morphological changes across concentrations, indicating a higher tolerance under the evaluated conditions.

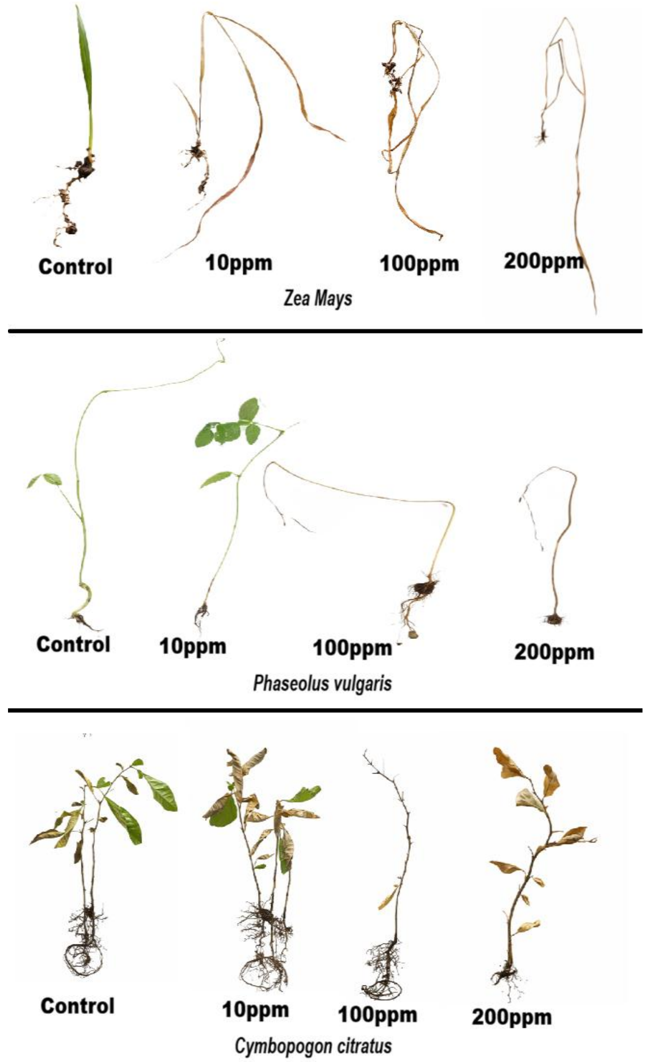

Fig 5. Average root growth in plants Zea mays, *P. vulgaris* and *C. citratus*.

Visible phytotoxic responses

Different effects of perchlorate exposure were evaluated to assess the plant health and structural integrity at the three concentrations (Fig 6). In *P. vulgaris*, plants exposed to 10 ppm showed a higher proportion of healthy leaves (33%) and chlorosis (56%), with no widespread mortality observed. At 100 and 200 ppm, an increase in adverse effects was detected (Fig 7), including stem weakness (11%), widespread plant death (44%), and necrosis (33%), indicating a concentration-dependent increase in damage.

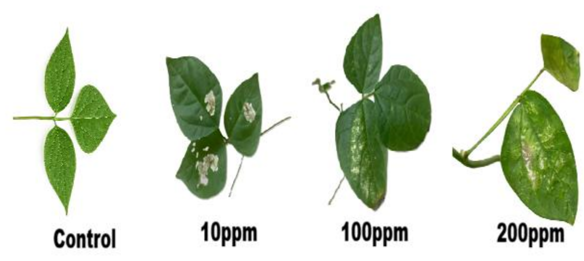

Fig 6. Comparison of the effect of perchlorate on bean leaves *P. vulgaris* at different concentrations (Control, 10 ppm, 100 ppm, and 200 ppm).

In *Z. mays*, perchlorate exposure resulted in a marked increase in stem breakage or weakening across all treatments. The highest incidence of stem damage was observed at 100 ppm (57%), while widespread death ranged from 14% to 29% as concentration increased. Chlorosis remained present across all concentrations, reaching 43% at both 10 and 200 ppm.

For *C. citratus*, stem weakness was the predominant response to perchlorate exposure, increasing from 43% at 10 ppm to 86% at 100 ppm, followed by a decrease to 57% at 200 ppm. Necrosis and chlorosis were less frequent in this species, although fungal infection was detected at the highest concentration (14%).

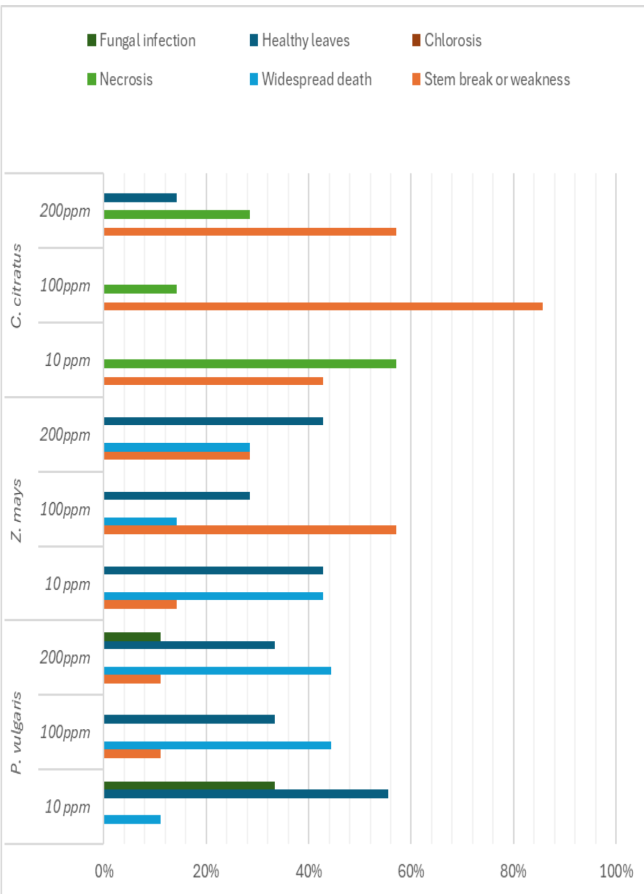

Figure 7. Effects of perchlorate exposure on *P. vulgaris*, *Z. mays* and *C.citratus* plants at different concentrations.

C. Statistical analysis of the data

For common *P. vulgaris* plants, the Kruskal-Wallis test yielded a p-value of 0.062, which is slightly above the significance threshold ($\alpha = 0.05$). Similarly, Lemongrass (*C. citratus)* showed a *p*-value of 0.070. In both cases, the null hypothesis could not be rejected, indicating no statistically significant differences in stem length distributions between perchlorate-treated plants and the control group.

In contrast, *Z. mays* exhibited a highly significant response to perchlorate exposure, with a *p*-value of 5.01×10^{-16}, leading to rejection of the null hypothesis. This result indicates statistically significant differences in stem length distributions among treatments, evidencing a markedly higher sensitivity of corn to perchlorate exposure.
The contrasting outcomes among the evaluated species highlight the species-specific nature of perchlorate effects, with corn being the most sensitive species, while bean and lemongrass exhibited comparatively higher tolerance under greenhouse conditions see Table 1.

Table I. Results of the Kruskal–Wallis test applied to stem length distributions in the evaluated plant species.

Plant species	P-valor	Decision
P. vulgaris	0.062	No rejection of H_0
Z. mays	5.01×10^{-16}	Rejection of H_0
C citratus	0.07	No rejection of H_0

These findings are consistent with previous reports [2], [4], [11] and emphasize the need for species-specific ecological assessments and management strategies. Furthermore, they provide relevant insights into ecological resilience and sustainability in agricultural systems affected by perchlorate contamination [16].

IV. Discussion

These results align with and extend the existing literature on perchlorate phytotoxicity and plant stress physiology, reinforcing the clear species-specific responses to perchlorate exposure. Perchlorate is recognized as a persistent contaminant that can be readily taken up by vegetation and accumulate in above-ground tissues, leading to phytotoxic effects, including chlorosis, necrosis, and reduced growth parameters in sensitive species [7], [11], [19].

Its uptake is primarily driven by transpiration and is influenced by the presence of competing anions such as nitrate and chloride [21], [22]. Inside the plant, perchlorate is translocated via the xylem to aerial tissues, where it can accumulate in leaves, stems, and, in some species, fruits. The bioaccumulation factor varies among species and tissues, with leafy vegetables and certain crops (e.g., lettuce, spinach, corn) exhibiting higher accumulation in edible parts [11], [21], [22].

In this study, all three *Z. mays, C. citratus,* and *P. vulgaris* exhibited sensitivity to perchlorate. Increasing signs of stress and morphological impairment with higher perchlorate concentrations were observed, aligning with previous findings that perchlorate can adversely affect growth and development in crop plants under controlled conditions [20].

Perchlorate has been associated with oxidative stress and damage to photosynthetic systems in autotrophs, disrupting electron transport and downregulating key components of photosystem II, which would further impair growth under stress conditions [23]. Perchlorate-induced stress is closely associated with the generation of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radicals. Excessive ROS production overwhelms the plant's antioxidant defense system, leading to oxidative damage of lipids, proteins, and nucleic acids [24], [25]. The resulting cellular dysfunction manifests as chlorosis, necrosis, and impaired growth [2], [11], [20]. Stem weakness, widespread plant death, chlorosis, and necrosis symptoms were found in the cultivated plants. Constituting hallmark features of the hormetic response detected at low perchlorate concentrations [18].

Z. mays presented a significant reduction in stem and root growth at 200 ppm, the presence of chlorosis and stem weakness at lower concentrations (10 ppm, 100ppm) indicates impaired nutrient uptake and oxidative stress, in concordance with previous studies [11]. Overall resilience suggests a capacity for compensatory responses, possibly linked to its robust antioxidant system and efficient ion transport mechanisms [26].

P. vulgaris exhibited a marked sensitivity to perchlorate, with pronounced growth inhibition reflected in the shape, size, and thickness of the leaves, as well as a break tendency due to their fragility, chlorosis, necrosis, and loss of secondary roots at higher concentrations [11]. The disruption of symbiotic nodulation and nutrient acquisition further exacerbated the stress response, leading to reduced biomass and increased fragility of stems and leaves. As a legume, bean relies on effective root symbiosis for nitrogen fixation, making them particularly vulnerable to environmental stressors that disrupt this relationship [17], [24], [27].

C. citratus showed a relatively stable morphology across perchlorate concentrations (10ppm, 100ppm, 200ppm), with no marked reduction in stem growth compared to the more sensitive responses seen in both *Zea mays* and *P. vulgaris*. However, qualitative indicators of stress, such as stem weakness and localized necrosis, were observed. This suggests that sublethal physiological damage occurred. The apparent tolerance in stem growth may be attributed to the well-developed and robust root system characteristic of *C. citratus*, which has been reported to enhance nutrient acquisition and confer resilience under adverse environmental conditions [25], [28].

Statistical analysis using the Kruskal-Wallis test revealed

Statistical analysis using the Kruskal–Wallis test revealed statistically significant differences in stem length only in *Z. mays*, indicating a species-specific response to perchlorate exposure. Although no significant differences were detected for *P. vulgaris* and *C. citratus*, the observed plant responses support existing evidence on the persistence and bioaccumulation of perchlorate in plants, which have important implications for food safety and human health. Consumption of contaminated crops, particularly leafy vegetables and grains, is a primary route of human exposure, with potential adverse effects on thyroid function, especially in vulnerable populations such as infants, children, and pregnant women [2], [3], [4], [11], [12], [14].

Future research should prioritize elucidating the molecular mechanisms through which perchlorate affects plant physiology. A deeper understanding of its impact on agricultural crops is essential to accurately assess potential risks and to support the development of effective bioremediation strategies [13], [29],[30], [31].

V. CONCLUSIONS

The assessment of perchlorate exposure under controlled greenhouse conditions indicated that all three *Z. mays*, *C. citratus*, and *P. vulgaris* plants were adversely affected, as evidenced by alterations in growth patterns and the manifestation of phytotoxic symptoms associated with contamination.

The evaluation of the effects of perchlorate contamination on plants grown under greenhouse conditions concluded that *Z. mays*, *C. citratus*, and *P. vulgaris* plants are all affected by perchlorate exposure. This is reflected in the distribution of growth and the contamination symptoms detected in the plants, such as chlorosis, necrosis, stem fragmentation, and effects on the roots, which exhibited noticeable nodules and loss of secondary roots, with statistical analysis indicating a species-dependent response, characterized by significant differences in stem length only for *Z. mays*.

Statistical analysis revealed species-specific differences between the groups of plants evaluated. The effects were not uniform across species, as common bean and lemongrass specimens showed a lower tendency to be affected by the contaminant, while corn plants showed a statistically significant reduction in stem length and lower resistance to perchlorate exposure.

These findings underscore the importance of understanding the responses of the evaluated plant species to different concentrations of perchlorate.

The results obtained in this research allow us to determine the impact of exposure to different concentrations of perchlorate on *P. vulgaris*, *C. citratus*, and *Z. mays* plants, and contribute to explaining the effects of this contaminant on the food chain; particularly in terms of its potential influence on the environmental health of species that consume the evaluated plants. These results also likely demonstrate the existence of a relationship between perchlorate concentration and its bioaccumulation, based on observed plant responses and supported by existing literature, as well as the toxic effects on the evaluated species.

Acknowledgment

This work was supported by a grant from the Universidad Tecnológica de Bolívar, with the help of researchers from the Chemical and Biological Studies Research Group (GEQB) and Gravitation and Applied Mathematics Research Group.

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