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Assessing the Effects of Industrial Pollution on Plant Growth and Ecosystem Stability: A Case Study of Boron Contamination

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Abstract **-** Boron (B) toxicity in industrial effluents, particularly in produced water (PW), presents significant environmental challenges due to its detrimental effects on plant growth and ecosystem health. This study evaluated the toxicological impact of boron on Vigna radiata (mung bean), a model organism widely used in contamination studies, to better understand its environmental implications. Mung beans were exposed to artificial boron solutions (2–40 ppm) and varying concentrations of PW (5–40%) for 14 days. Observations indicated pronounced toxicity symptoms, including inhibited growth, yellowing of leaves, reduced photosynthetic pigments, and proline accumulation. Notably, mung bean seeds failed to germinate in higher PW concentrations (10–40%), suggesting the compounded effects of boron and other contaminants within PW. These findings highlight the severe ecological risks posed by boron in industrial effluents and underscore the urgent need for innovative and cost-effective treatment strategies to mitigate its environmental impact.

Keywords**:** *Vigna radiata*, Plant growth inhibition, Produced water (PW), Agricultural irrigation, Environmental contaminants, Wastewater treatment, Toxicity mitigation

1. Introduction

Boron (B) is a fundamental micronutrient vital for plant growth and physiological functions. It significantly contributes to maintaining cell wall structure, membrane integrity, hormone regulation, and overall plant metabolism stability [1], [2]. At optimal levels, boron supports efficient cell division, carbohydrate metabolism, and nutrient transportation, promoting robust growth and productivity [3]. However, plants have a narrow range of boron tolerance —typically between 0.5 and 2 mg/L. Concentrations exceeding this range cause severe phytotoxicity, significantly impairing plant growth and agricultural yields [4], [5].

Various environmental conditions can intensify boron toxicity, particularly in arid, semi-arid, and coastal regions. Limited rainfall, poor soil drainage, high evaporation rates, and seawater intrusion can substantially increase soil and groundwater boron concentrations [6], [7]. Industrial processes, especially petroleum extraction, further exacerbate boron contamination. Produced water (PW), a waste product from oil production, often contains excessively high boron concentrations ranging from 26 to 28 ppm, far surpassing crop tolerance thresholds [8], [9]. Persistent irrigation with inadequately treated PW leads to boron accumulation in soils, negatively affecting crop productivity, soil chemical balance, and vital microbial activities necessary for nutrient cycling [10], [11], [12].

At the physiological level, boron toxicity manifests through clear morphological symptoms including leaf-tip necrosis, chlorosis, reduced leaf expansion, and overall inhibited growth [13], [14]. These symptoms reflect deeper cellular disturbances, such as enzyme inhibition, membrane permeability disruption, and excessive cross-linking of pectin in cell walls [1], [15]. Consequently, vital physiological processes like water uptake, nutrient transport, and photosynthesis are severely compromised. High boron levels damage chloroplast structures, reducing chlorophyll content

and photosynthetic efficiency [16], [17]. Additionally, boron-induced oxidative stress generates reactive oxygen species (ROS), accelerating cellular damage, pigment degradation, and ultimately, plant senescence [18], [19].

Managing boron toxicity requires integrated strategies including routine monitoring of soil and irrigation water. International bodies such as the World Health Organization (WHO) provide guidelines limiting boron concentrations in water used for human consumption and agriculture. WHO recommends a maximum boron concentration of 2.4 mg/L in drinking water [20]. Certain regions, such as Oman, have even stricter guidelines, limiting natural freshwater boron levels to 0.5 mg/L and 2.4 mg/L for desalinated water [21].

Advanced remediation technologies have been extensively explored, including membrane filtration, reverse osmosis, and adsorption [22]. Bio-based adsorbents derived from agricultural wastes, such as activated carbon from date seeds, rice husks, banana peels, and eggshells, have effectively reduced boron contamination due to their cost-effectiveness, high efficiency, and eco-friendly nature [23], [24]. Furthermore, developing crop varieties genetically or physiologically adapted to high boron levels offers promising long-term solutions to sustain agricultural productivity [25], [26].

In summary, boron, while essential at trace levels, quickly becomes toxic above narrow thresholds. Excessive concentrations profoundly affect plant physiology, crop productivity, soil chemistry, and ecosystem health. Comprehensive management approaches integrating regular monitoring, advanced remediation techniques, and crop tolerance enhancement are essential to address boron toxicity sustainably in agriculture [27]. This study aim to evaluate the environmental impact of boron toxicity using mung bean (Vigna radiata) as an indicator species to understand the ecological implications and inform the development of efficient and practical treatment solutions for contaminated wastewater.

2. Methodology

2.1 Boric acid solution preparation

A stock solution of 100 ppm of boron was prepared by dissolving 0.572 g of boric acid salt (H_3BO_3) (USB, H_3BO_3 Ultrapure, ACS Reagent Grade, MB Grade) in 1 L of distilled water to prepare different dilutions (2-40 ppm) of boron-contaminated solutions.

$$\frac{0.572\text{g } (H_3BO_3) \times \ 1000\frac{\text{mg}}{\text{g}} \times 0.175}{1\ L} = 100\frac{mg}{l} of\ (B)$$

2.2 Seeds germination

Mung bean seeds (*Vigna radiata*) were germinated in pots (9 cm diameter, 8 cm height) containing 100 g compost: sandy loam soil (1:4 ratio). Seedlings were grown at 22 ± 2°C with a 12-h photoperiod. Treatments included irrigation with distilled water (control, 0 ppm B) or boron solutions (5, 10, 20, and 40 ppm as boric acid), applied weekly (75-100 mL). Each treatment had three replicates (5 plants each). After 14 days, seedlings were assessed for growth, photosynthetic activity, and proline content. Additionally, effects of diluted produced water (PW) (DW, 5, 10, 20, 50, and 75%) were evaluated under identical conditions after observing complete germination inhibition at 100% PW.

2.2.1 Measurement of growth parameters

Plants were harvested, carefully washed with distilled water to remove adhering soil particles, and gently blotted dry using tissue paper. Root lengths (cm) and fresh weights of individual plants (n = 15; 5 plants per treatment × 3 replicates) were recorded using an analytical balance. Subsequently, samples were stored at 4°C prior to further analysis.

2.2.2 Measurement of photosynthetic pigments

Chlorophyll (*a*, *b*, total chlorophyll) and carotenoid contents were measured in fully expanded young leaves following the method of Lichtenthaler and Buschmann (2001). Briefly, leaf tissues (0.1 g) were ground with 10 mL of 80% acetone, filtered through Whatman filter paper (No. 41, ashless), and stored in amber vials. Absorbance was measured spectrophotometrically at 663 nm (chlorophyll *a*), 646 nm (chlorophyll *b*), and 470

nm (carotenoids). Pigment concentrations were calculated using equations from Arnon (1949) for chlorophyll and Kirk and Allen (1965) for carotenoids, with results expressed as mg/g fresh weight.

$$\text{Chl } a = 1\ 2.21\ (A_{663}) - 2.81\ (A_{646}) * V / 1000 * W \quad (1)$$

$$\text{Chl } b = 20.13\ (A_{646}) - 5.03\ (A_{663}) * V / 1000 * W \quad (2)$$

$$C_x+c = (1000(A_{470}) - 3.27\ C_a - 104\ C_b)/ 229 \quad (3)$$

2.2.3 Measurement of proline content

Proline content was determined using the method of Bates et al. (1973). Approximately 0.5 g of plant tissues (stem and root) were homogenized in 5 mL of 3% aqueous sulfosalicylic acid. The homogenate was vacuum-filtered, and 2 mL of filtrate was reacted with 2 mL acid-ninhydrin solution (prepared with ninhydrin, glacial acetic acid, and orthophosphoric acid) in a boiling water bath (96–100 °C) for 1 hour. After cooling, the proline chromophore was extracted with 4 mL toluene and separated using a separatory funnel. Absorbance was measured spectrophotometrically at 520 nm. Proline concentrations were calculated using a standard calibration curve (0–20 ppm), and results were expressed in mg/g fresh weight.

µg proline/ g of fresh weight of plant material= [(µg proline/mL * mL of toluene)/ 115.5 µg/ µmoles]/ [(g of sample)/5] * 115.5 (4)

2.3 Produced water samples collection

Produced water (PW) from oil and gas extraction was collected from X company in Oman from the main outlet without any pre-treatment. The sample collection and preservation were done following water and wastewater methods.

2.3.1 Produced water characterization

Oil-field produced water (PW) was characterized by measuring key parameters, including pH, dissolved oxygen (DO), salinity, electrical conductivity (EC), total suspended solids (TSS), phenols, volatile fatty acids (VFA), and total petroleum hydrocarbons (TPH). Measurements of DO, pH, salinity, and EC were conducted directly using calibrated instruments (Milwaukee MW605 MAX DO/Temp Meter and HANNA Instrument 2210 pH meter). Sodium and potassium concentrations in PW samples were quantified using a flame photometer (JENWAY, PFP7)

2.3.1 Volatile fatty acids (VFA)

Volatile fatty acids (VFA) in PW samples were determined by placing 2 mL aliquots in centrifuge tubes, centrifuging at 2000 rpm for 15 min at 4°C, and extracting the supernatant. Each sample was treated with a 25% metaphosphoric acid solution (1:5 ratio), followed by homogenization for 30 min. Approximately 1 mL of the clear supernatant was transferred into GC vials and stored at low temperature prior to analysis. VFA concentrations were quantified using gas chromatography (GC, Agilent 6890 N) equipped with a flame ionization detector (FID).

2.3.2 Total petroleum hydrocarbons (TPH)

Total petroleum hydrocarbons (TPH) in PW were determined via liquid-liquid extraction using hexane. Briefly, 500 mL PW samples were extracted three times with 30 mL hexane in a separatory funnel, shaken vigorously, and filtered through cotton and sodium sulfate to eliminate residual water. Hexane was evaporated using a rotary evaporator (G3, Heidolph, Germany), and the extracted oil weight was recorded by comparing flask weights before and after extraction. The obtained extract was further analyzed using gas chromatography–mass spectrometry (GC-MS) at 250 °C and 57.8 kPa to identify hydrocarbon

components. TPH was calculated based on the weight difference (e.g., 0.2 g TPH per 500 mL PW). TPH was calculated/estimated as follows:

Empty round bottom flask weight= 156.2 g

Round bottom flask with extracted oil weight=156.4 g

Extracted oil weight (g)= Round bottom flask with extracted oil weight - Empty round bottom flask weight= 0.2 g/ 500 mL of PW.

2.3.3 Total suspended solids (TSS) determination

Total suspended solids (TSS) in produced water were determined by filtering 50 mL samples through pre-weighed 0.45 µm filter paper (previously dried at 120 °C for 20 min, cooled in a desiccator, and weighed as weight A). After filtration, the filter paper was dried again at 103 °C for 3 hours, cooled in a desiccator, and weighed to obtain the final weight (weight B). TSS was calculated from the weight difference (B–A). The below equation 8 was used to calculate the TSS in the produced water:

$$\text{TSS}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{B} - \text{A} \times 1{,}000{,}000}{v} \quad (8)$$

here B and A is the weight of filter paper in (g) and v is the volume of PW in mL.

3. Results

3.1 Boron toxicity to *V. radiata*

Figure 1 shows germinated *V. radiata* seedlings irrigated with boron solutions (5, 10, 20 and 40 ppm) and control irrigated with distilled water. After 14 days of B exposure, leaves chlorosis was noticed.

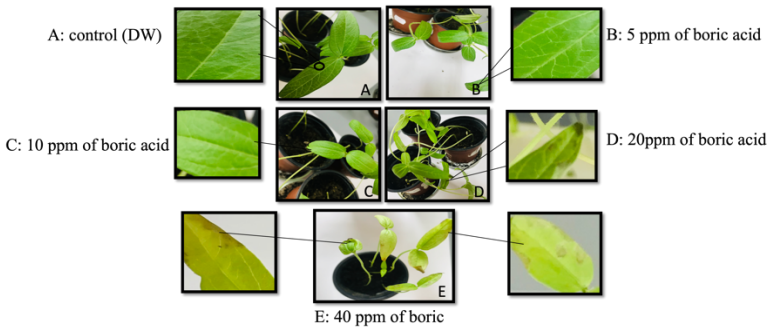

Fig.1: The visual symptoms of boron on V. radiata growth parameters and leaves after 14-days exposure to B solutions. A: control (distilled water), B: 5 ppm of boron, C: 10 ppm of boron, D: 20 ppm of boron, and E: 40 ppm of boron. All treatments were in triplicates with at least (n = 3-5 seedlings).

3.1.1 *V. radiata* seed germination percentage (%)

The effect of boron concentrations (5, 10, 20, and 40 ppm) on seed germination (%) is shown in Figure 2. Germination at 10 ppm boron (93.3%) did not significantly differ from the control (86.6%). However, significant differences were observed at boron concentrations of 5 ppm (100%), 20 ppm (100%), and notably decreased germination at 40 ppm (53.3%), compared to the control.

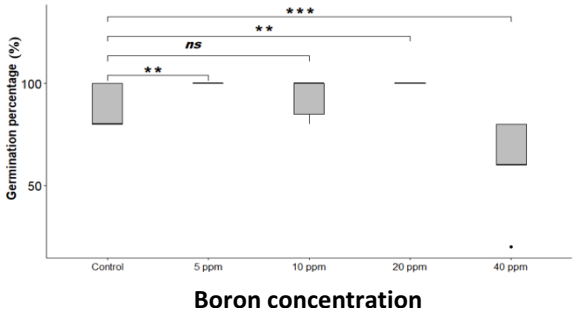

Fig.2: The effect of different boron concentrations (5, 10, 20, and 40 ppm) on seed germination of V. radiata. The boxplot shows the median, maximum, and minimum of at least 3 replicates. While ns indicates that no significant difference, the asterisks (*, ***) represent the significant differences, and ns shows no significant difference.

Table 1 presents dilutions of produced water (PW) with distilled water and their corresponding measured boron concentrations. The effects of these dilutions on *Vigna radiata* seed germination and seedling growth parameters are depicted in Figure 3. Compared to the control, exposure to diluted PW resulted in significant reductions in germination percentage, seedling growth, development, and leaf formation.

Table 1: B concentrations in PW dilutions measured in ppm using ICP-OES.

PW dilution	B concentration (ppm)
5% PW	2.07
10% PW	5.20
20% PW	11.59
50% PW	19.93
75% PW	28.74
100% PW	36.22

Figure 3: The visual symptoms of 5% PW dilution on seed germination and seedling growth of V. radiata. A: seed germination seedlings growth after 14 days irrigated with 5% PW (B= 2.1 ppm), B and C: length of seedlings. All treatments were conducted in triplicates with n =3-4 replicates. No germination was observed in other PW dilutions.

Figure 4 illustrates the impact of different dilutions of oil-field produced water (PW) on germination percentage (%) of Vigna radiata seeds compared to the distilled water control. Results indicate complete inhibition of seed

germination at PW concentrations of 10% and 20%. Additionally, a significant reduction in germination percentage was observed at 5% PW dilution (73.3%) relative to the control (93.3%).

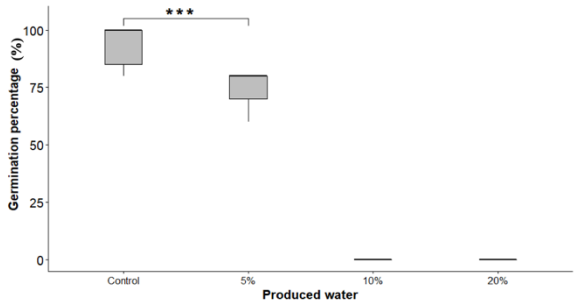

Figure 4: The effect of oil-field PW dilutions (5, 10, and 20%) on germination percentage (%) of V. radiata. the box-plot shows the median, maximum, and minimum of n = 3-5 replicates. The (***) represents the significance difference.

3.1.2 chlorophyll a content (mg/g) of *V. radiata* seedlings

Figure 5 shows the effect of different boron concentrations (5, 10, 20 and 40 ppm) used for irrigation on the chlorophyll a content (in mg/g) of *V. radiata* seedlings. The results show that photosynthetic pigments decreased noticeably under B toxicity conditions. Specifically, Chl a and b levels dropped continuously from 5 ppm (0.75 mg/g) and kept falling at 40 ppm significantly (0.52 mg/g) compared to the control (1.2 mg/g).

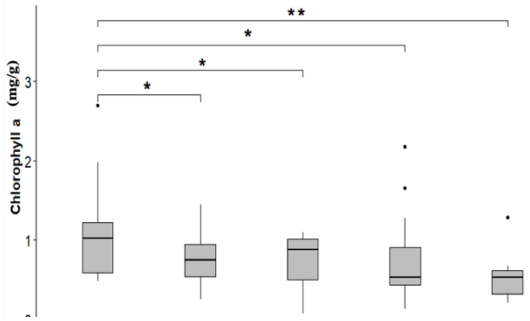

Figure 5: The effect of different boron concentrations (5, 10, 20, and 40 ppm) on chlorophyll a of V. radiata seedlings. the box-plot shows the median, maximum, and minimum of n = 3-5 replicates. The asterisks (*,**,***) represent the significant difference while (ns) means no significant difference

On chlorophyll a content (mg/g) in *Vigna radiata* seedlings. Results indicate a marked reduction in chlorophyll pigments under boron toxicity. Chlorophyll a levels progressively decreased, starting from 5 ppm (0.75 mg/g) and significantly declined to 0.52 mg/g at 40 ppm, compared to the control (1.2 mg/g).

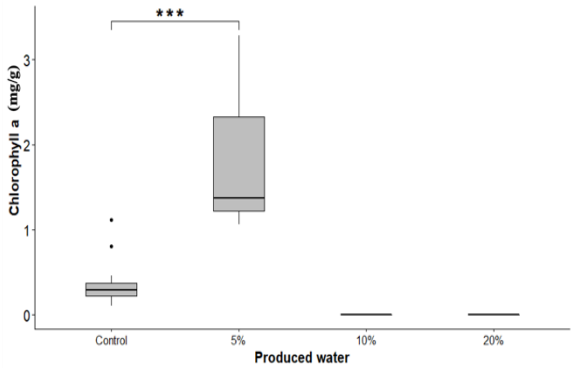

Figure 6: The effect of oil-field PW dilutions (5, 10, and 20%) on chlorophyll a content of V. radiata seedlings. The box plot shows the median, maximum, and minimum of n = 3-5 replicates. The asterisk (***) represents the significance difference, and the dots (•) are the outliers.

3.1.3 Chlorophyll b (mg/g) of *V. radiata* seedlings

Figure 7 illustrates the effect of different boron concentrations (5, 10, 20, and 40 ppm) on chlorophyll b content (mg/g) of *Vigna radiata* seedlings relative to the control. No significant difference was observed between the control (6.92 mg/g) and 5 ppm boron treatment (5.98 mg/g). However, seedlings exposed to higher boron concentrations (10, 20, and 40 ppm) demonstrated a substantial reduction in chlorophyll b content compared to control seedlings.

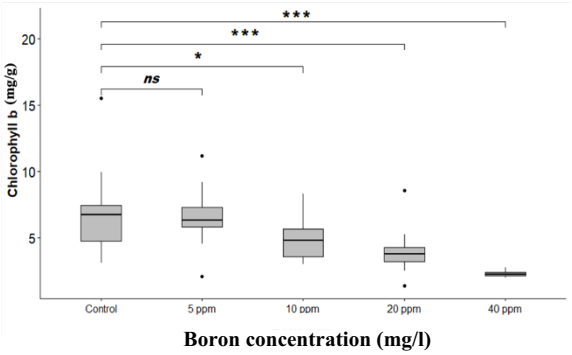

Figure 7: Effect of different boron concentrations (5, 10, 20, and 40 mg/l) on chlorophyll b (mg/g) content of V. radiata seedlings The box plot shows the median, maximum, and a minimum of n = 3-5 replicates. While (ns) indicates no significant difference between the treatments, the asterisks (*, **, ***) represent the significant difference. The dots (•) are the outliers.

Similarly, there was a significant decrease in chlorophyll b content of V. radiata seedlings irrigated with 5% PW (1.87 mg/g) compared to the control (Fig. 8). Since there was no germination at 10 and 20% PW, determination of Chl b was not possible.

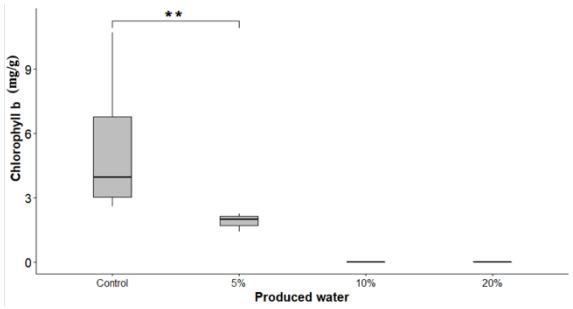

Figure 8: The effect of oil-field PW dilutions (5, 10, and 20%) on chlorophyll b content of V. radiata seedlings. The box-plot shows the median, maximum, and minimum of n= 3 replicates. The asterisks (**) represents the significance difference.

3.1.4 Carotenoid content of *V. radiata* seedlings

Higher boron concentrations (20 and 40 ppm) significantly decreased carotenoid pigment levels in Vigna radiata seedlings compared to control plants, whereas carotenoid contents at lower boron concentrations (5 and 10 ppm) were not significantly different from the control (Fig. 9). Additionally, irrigation with 5% PW notably reduced carotenoid levels (171.2 mg/g) compared to control (440.7 mg/g). Due to the absence of seed germination at 10% and 20% PW, carotenoid determination was not conducted for these treatments (Fig. 10).

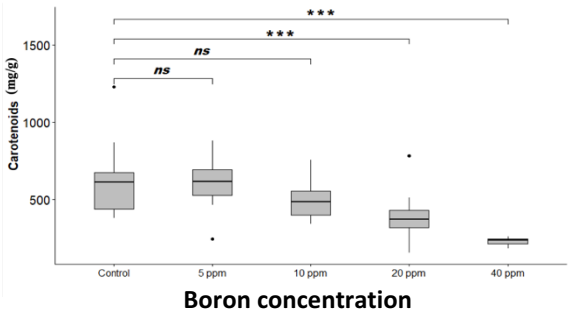

Figure 9: The effect of different boron concentrations (5, 10, 20, and 40 ppm) on carotenoid content of V. radiata seedlings. The box-plot shows the median, maximum, and minimum of n = 5 replicates. While (ns) indicates no significant difference between the treatments, the asterisks (***) represent the significant difference. The dots (•) are the outliers.

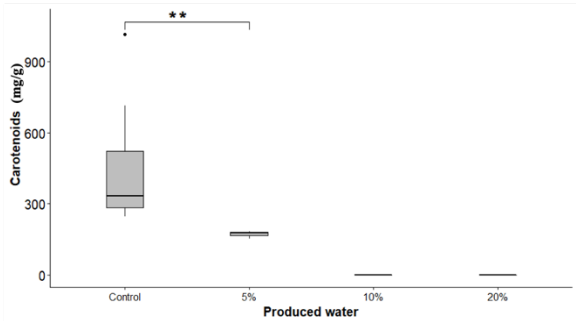

Figure 10: The effect of different oil-field PW dilutions (5, 10, and 20 %) on carotenoid content of V. radiata seedlings. The box-plot shows the median, maximum, and minimum of n = 3 replicates. The asterisks (**) represent the significance difference. The dot (•) is an outlier.

3.1.5 Proline content (µg/g) of *V. radiata* seedlings

The effect of increasing boron concentrations (5, 10, 20, and 40 ppm) used for irrigation on the proline content of Vigna radiata seedlings is shown in Figure 11. There were no significant differences between the control, and 5 and 10 ppm treatments.

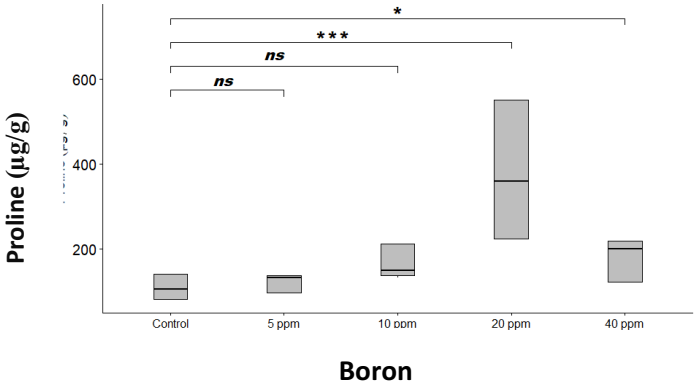

Figure 11: The effect of different boron concentrations (5, 10, 20, and 40 ppm) on proline content (µg/g) of V. radiata seedlings. The box-plot shows the median, maximum, and minimum of n = 3-5 replicates. While ns indicates that, no significant difference between the treatments, the asterisks (*, **, ***) represent the significant difference.

Figure 12 shows the effect of the oil-field PW containing different B concentrations on the proline content of V. radiata seedlings. There was no germination at 10 and 20% and therefore, estimating proline content was not possible. There was a significant reduction in the proline content of V. radiata seedlings exposed to 5% of PW relative to the control counterparts.

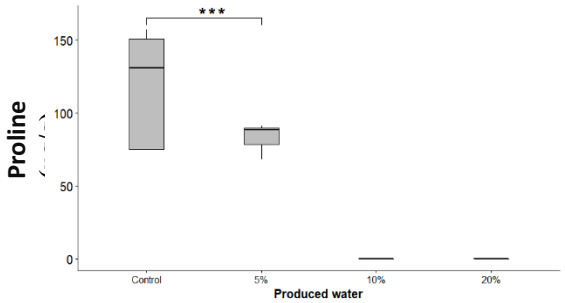

Figure 12: The effect of different oil-field PW dilutions (5, 10, and 20 %) on proline content (µg/g) of V. radiata seedlings. The box-plot shows the median, maximum, and minimum of n = 3 replicates. The asterisks (***) represent the significant difference.

4. Discussion

Boron (B) enters plants primarily through passive diffusion of boric acid ($B(OH)_3$) across root cell membranes [28], [29], [30]. Once absorbed, it is transported upwards via xylem, facilitated by transpiration [31]. Under excess boron conditions, its accumulation occurs primarily in leaves and stems, resulting in significant tissue damage and metabolic dysfunction [32]. The irrigation water quality significantly influences the severity of boron toxicity. Produced water (PW), a major waste stream from oil and gas extraction processes, often contains elevated concentrations of boron along with other contaminants such as salts and hydrocarbons [33], [34]. High salinity associated with PW further exacerbates

the detrimental effects of boron, reducing seedling vigor and overall crop yield. Effective treatment to remove organic contaminants, salts, and excess boron from PW is essential prior to its agricultural reuse [35].

At a physiological level, excess boron negatively impacts photosynthetic pigments by damaging chloroplast thylakoid membranes, impairing electron transport, and limiting ATP and NADPH production [36], [37]. High boron availability in the root zone promotes its accumulation in leaf tissues, affecting sensitive biochemical pathways involved in photosynthesis [38]. Plants have evolved mechanisms to tolerate excess boron, including specialized transporters that sequester or expel boron, reducing its accumulation and mitigating toxicity [39], [40]. However, in sensitive species such as *Vigna radiata* (mung bean), high boron levels consistently lead to reductions in chlorophyll a, chlorophyll b, and carotenoid concentrations, resulting in diminished photosynthetic capacity [41], [42]. Boron toxicity also induces the formation of reactive oxygen species (ROS), accelerating pigment degradation and leading to premature leaf senescence [43], [44]. Interestingly, at low PW dilutions, temporary increases in chlorophyll content suggest an adaptive physiological response; however, prolonged exposure results in overall pigment decline [45], [46].

Proline accumulation frequently serves as a biochemical indicator of plant stress tolerance, functioning as both an Osmo protectant and a ROS scavenger [47], [48]. Elevated proline levels typically correspond to increased severity of stress conditions, including boron toxicity, reflecting a protective adaptation by plants [49]. Nevertheless, reports on proline accumulation under boron stress are inconsistent; some studies demonstrate clear increases, while others report minimal or even reduced proline accumulation, highlighting variability among plant species and experimental conditions [50], [51], [52]. For *V. radiata*, proline accumulation remained relatively stable under low boron concentrations, indicating optimal growth conditions. Intermediate boron concentrations (20–40 ppm) increased proline levels significantly, reflecting an active stress response. However, at higher boron levels (e.g., 5% PW), proline accumulation sharply declined, potentially due to disruptions in proline biosynthesis pathways or enhanced proline degradation under extreme stress conditions [53].

5. Conclusion

Boron (B) is a vital micronutrient for plants but becomes toxic at elevated concentrations, adversely affecting plant growth and physiological processes. This study highlighted the toxic effects of B on plants, including inhibited growth, chlorosis, and reduced productivity. In the case of produced water (PW), the observed plant toxicity was influenced not only by B but also by other co-existing contaminants, emphasizing the complexity of PW treatment. Effective removal of boron and associated pollutants is crucial to reduce their ecological and agricultural impact and enhance the potential reuse of PW for irrigation.

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