

# Oxidative and Biochemical Responses of Water Hyacinth (*Eichhornia crassipes*) in Drilling Mud-Contaminated Aquatic Systems

Adeyemi, Oyeyemi<sup>1\*</sup> & Opia, Benjamin Chukwudi<sup>2</sup>

<sup>1,2</sup>Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, PMB 1221, Effurun, Delta State, Nigeria. Corresponding Author (Adeyemi, Oyeyemi) Email: adeyemi.oyeyemi@fupre.edu.ng\*



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## ABSTRACT

Water-based drilling muds (WBM) used in oil and gas operations are a significant source of aquatic pollution due to their complex mixture of heavy metals, hydrocarbons, and chemical additives. *Eichhornia crassipes* (water hyacinth), a fast-growing aquatic macrophyte, has been widely studied for its phytoremediation potential. However, the biochemical and oxidative stress responses of *E. crassipes* in WBM-contaminated aquatic environments remain underexplored. This study investigates the morphological, physiological, and enzymatic responses of *E. crassipes* to varying concentrations of WBM to evaluate its remediation performance and stress tolerance. A six-week experimental study was conducted using six treatment groups exposed to 0%, 20%, 40%, 60%, 80%, and 100% WBM concentrations. Total dissolved solids (TDS) and electrical conductivity (EC) were measured at weeks 0 and 6 to assess ionic mobilization. Plant growth parameters (height and stem girth), chlorophyll content (SPAD units), and antioxidative biomarkers, including catalase (CAT), superoxide dismutase (SOD), and vitamin C (ascorbic acid), were analyzed in leaf, stem, and root tissues. Data were subjected to ANOVA with significance set at  $p < 0.05$ . TDS and EC significantly increased across all WBM treatments post-exposure, indicating enhanced solubilization of drilling mud constituents. Plant height and stem girth exhibited concentration-dependent responses, with moderate WBM levels (40–60%) supporting partial adaptive growth, while extreme concentrations (100% and 20%) resulted in growth suppression. Chlorophyll content declined markedly in WBM-exposed plants, with the most severe reduction observed at higher contaminant loads. Catalase and SOD activities showed tissue-specific and concentration-dependent variations, with peak responses at 60–80% WBM in stems and roots, reflecting oxidative stress mitigation. Vitamin C accumulation exhibited a biphasic trend, with moderate WBM exposure stimulating ascorbate synthesis, while excessive contamination inhibited antioxidant capacity. *Eichhornia crassipes* demonstrates a nuanced phytoremediation response in WBM-contaminated aquatic systems, characterized by adaptive oxidative stress management and morphological plasticity. However, the plant's remediation efficacy is contingent on contaminant concentration, with high pollutant loads overwhelming its detoxification mechanisms. The findings emphasize the need for optimized phytoremediation strategies that consider dilution, exposure duration, and supplemental treatment measures to enhance the plant's performance in drilling waste management.

**Keywords:** Phytoremediation; Water-Based Drilling Mud; *Eichhornia crassipes*; Oxidative Stress; Antioxidant Enzymes; Catalase; Superoxide Dismutase; Vitamin C; Aquatic Pollution; Heavy Metals; Hydrocarbons; Environmental Remediation.

## 1. Introduction

The increasing scale of oil and gas exploration has amplified concerns over the environmental impacts of drilling operations, particularly the disposal of drilling fluids and cuttings. Water-based muds (WBM), although considered less toxic than oil-based formulations, are complex colloidal systems comprising clays, polymers, lubricants, and chemical additives that can introduce significant pollutant loads into aquatic ecosystems when improperly managed (Adeyemi & Opia, 2025; Omotosho et al., 2022).

These pollutants include heavy metals such as lead, chromium, arsenic, and nickel, as well as hydrocarbons and volatile organic compounds (VOCs) known for their persistence and bioaccumulative potential (Iwegbue et al., 2019; Mohammed et al., 2018; Otokunefor et al., 2020).

Environmental contamination from WBM leachates is particularly problematic in ecologically sensitive regions like the Niger Delta, where regulatory enforcement is inconsistent (Gaurav et al., 2020; Adewuyi & Azeez, 2020). Studies have highlighted that trace metals and hydrocarbons from drilling fluids can leach into surface and groundwater, posing ecotoxicological threats to aquatic organisms and human health (Li et al., 2020; Pampanin & Sydnese, 2013).

Phytoremediation, which leverages the natural absorptive and metabolic capabilities of plants, has emerged as a promising green technology for remediating polluted water bodies. Among aquatic macrophytes, *Eichhornia crassipes* (water hyacinth) has received considerable attention due to its rapid growth, high biomass yield, and exceptional capacity for heavy metal and organic pollutant uptake (Singh & Kalamdhad, 2022; Zhou et al., 2021). The plant's phytoremediation efficacy has been documented in diverse contexts, including removal of cadmium, lead, nickel, polycyclic aromatic hydrocarbons, and VOCs from contaminated waters (Anago et al., 2020; Owabor & Oaikhena, 2020).

However, recent findings indicate that the interaction between *E. crassipes* and WBM-contaminated waters is complex, with the potential for root-mediated mobilisation of bound ions and organic compounds, resulting in increased solubilisation rather than sequestration of contaminants (Adeyemi & Opia, 2025). This phenomenon has been attributed to exudation of organic acids, alteration of rhizospheric pH, and stimulation of microbial communities that can enhance desorption of contaminants from the drilling mud matrix (Chakraborty & Veeramani, 2022; Suthar et al., 2020).

Moreover, the physiological responses of *E. crassipes* to contaminant-induced stress are modulated by various biochemical mechanisms, notably the activation of antioxidant defence systems. Enzymes such as catalase (CAT) and superoxide dismutase (SOD), alongside non-enzymatic antioxidants like ascorbic acid (vitamin C), play pivotal roles in neutralising reactive oxygen species (ROS) generated under pollutant stress (Gill & Tuteja, 2010; Maestri et al., 2010). Variations in chlorophyll content and morphological traits such as stem girth and plant height also serve as reliable indicators of phytotoxicity and stress adaptation (Akinbile et al., 2020; Sharma & Agrawal, 2019).

The hormetic response of *E. crassipes*—where low to moderate pollutant exposure stimulates adaptive physiological responses, while excessive loads suppress detoxification pathways—has been observed in several studies (Calabrese, 2019; Bose et al., 2014). Understanding this biphasic behaviour is critical for optimising phytoremediation strategies involving drilling waste, as it underscores the need for concentration-specific management and exposure time calibration (Foyer & Noctor, 2005; Gallie, 2013). Furthermore, studies suggest that the bioavailability of contaminants, rather than their absolute concentration, determines phytotoxic outcomes, highlighting the importance of chemical speciation and matrix interactions in remediation studies (Reddy et al., 2012; Pourrut et al., 2011). Regulatory frameworks like WHO and NESREA standards provide essential benchmarks for evaluating remediation efficacy, yet gaps persist in addressing emerging contaminants such as thallium and uranium, whose environmental behaviours remain inadequately characterised (ATSDR, 2021; Luo et al., 2022).

Given these complexities, there is a pressing need to elucidate the oxidative and biochemical responses of *E. crassipes* in WBM-contaminated aquatic systems, focusing on its tolerance mechanisms, pollutant uptake dynamics, and physiological thresholds. This study aims to assess these parameters through a comprehensive evaluation of stress biomarkers, including TDS, EC, chlorophyll content, antioxidant enzyme activities, and ascorbate levels, to advance the understanding of *E. crassipes* as a viable phytoremediator for drilling waste pollution control.

### 1.1. Study Objectives

The objectives of this study were to:

- 1) Evaluate the morphological responses (plant height and stem girth) of *Eichhornia crassipes* under varying concentrations of water-based drilling mud (WBM) contamination.
- 2) Assess the physiological impact of WBM exposure on chlorophyll content as an indicator of photosynthetic performance.
- 3) Determine changes in water quality parameters, specifically total dissolved solids (TDS) and electrical conductivity (EC), during phytoremediation by *E. crassipes*.
- 4) Investigate tissue-specific variations in antioxidant enzyme activities, including catalase (CAT) and superoxide dismutase (SOD), in response to WBM-induced oxidative stress.
- 5) Quantify vitamin C (ascorbic acid) content in different plant tissues as a measure of non-enzymatic antioxidant defence under pollutant stress.
- 6) Analyse the relationship between WBM concentration and the phytoremediation capacity of *E. crassipes*, highlighting optimal conditions and stress thresholds for effective remediation.

## 2. Materials and Methods

### 2.1. Study Design

A six-week experimental study was conducted to evaluate the oxidative and biochemical responses of *Eichhornia crassipes* cultivated in water contaminated with varying concentrations of water-based drilling mud (WBM). The experiment employed a Completely Randomized Design (CRD) with six treatment groups exposed to WBM dilutions of 0% (control), 20%, 40%, 60%, 80%, and 100%, respectively. Each treatment was replicated five times ( $n = 5$ ), resulting in a total of 30 experimental units.

### 2.2. Collection of Water Hyacinth Samples

Fresh, healthy *Eichhornia crassipes* plants were collected from the Ekpan River, Uvwie Local Government Area, Delta State, Nigeria (5°33'29"N, 5°44'29"E). The sampling site was chosen due to its relatively undisturbed ecological status. Sampling was carried out on a clear morning at 07:47 a.m., with ambient temperature at 25°C, relative humidity of 90%, and zero precipitation in the preceding 24 hours. Plants were carefully uprooted to preserve root integrity, placed in polyethylene bags, and transported in ice-cooled containers to the laboratory to maintain physiological freshness (Adeyemi & Opia, 2025).

### 2.3. Preparation of Water-Based Mud (WBM) Leachate

WBM leachate was simulated following a modified ASTM extraction protocol. A composite sample of dried, homogenized WBM (100 g) was mixed with 1 L of deionized water (1:10 w/v ratio) and agitated at 150 rpm for 18 hours at 25°C  $\pm$  2°C. The suspension was filtered using Whatman No. 42 filter paper to obtain a 100% WBM leachate stock solution. Serial dilutions were prepared to obtain 80%, 60%, 40%, and 20% leachate solutions using

deionized water. Leachates were stored in acid-washed polyethylene containers at 4°C prior to use (Adeyemi & Opia, 2025).

## 2.4. Experimental Setup

Standardized *E. crassipes* plants (20–25 cm in height) were transplanted into plastic pots (30 cm depth × 25 cm width), each containing 3 L of the respective treatment solutions. Three plants were introduced per pot. Pots were arranged in an open-air environment under natural light conditions and shielded from rainfall to ensure uniform exposure. The experimental duration spanned six weeks, with periodic monitoring and no nutrient supplementation applied. Environmental parameters were recorded daily.

## 2.5. Determination of Total Dissolved Solids (TDS) and Electrical Conductivity (EC)

TDS and EC of the water samples were measured at week 0 and week 6 using a multi-parameter water quality meter (Hanna Instruments, HI 98312). Measurements were conducted in triplicates per replicate, and results were expressed in mg/dL for TDS and µS/cm for EC.

## 2.6. Morphological Assessments

Plant height (cm) and stem girth (cm) were measured weekly using a calibrated ruler and Vernier calipers, respectively. Percentage height increase was calculated relative to initial measurements.

## 2.7. Chlorophyll Content Determination

Chlorophyll content was estimated non-destructively using a SPAD-502 Plus Chlorophyll Meter (Konica Minolta, Japan). Five SPAD readings were taken from fully expanded leaves of each replicate, and the mean was recorded.

## 2.8. Biochemical Assays

At the end of the six-week exposure period, plant tissues (leaf, stem, and root) were harvested and processed for enzymatic assays. Tissues were homogenized in ice-cold phosphate buffer (0.1 M, pH 7.0) and centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was used for subsequent analyses.

- **Catalase (CAT) Activity** was measured by monitoring the decomposition rate of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at 240 nm, as described by Aebi (1984), and expressed in U/mg protein.
- **Superoxide Dismutase (SOD) Activity** was determined following the method of Beauchamp and Fridovich (1971), based on the inhibition of nitroblue tetrazolium (NBT) reduction, and expressed in U/mg protein.
- **Vitamin C (Ascorbic Acid) Content** was quantified using the Roe and Kuether (1943) method, involving the colorimetric reaction with 2,4-dinitrophenylhydrazine (DNPH), and expressed as mg/100 mL fresh weight (FW).

## 2.9. Protein Quantification

Protein concentration in tissue extracts was determined using the Bradford assay, with bovine serum albumin (BSA) as the standard.

## 2.10. Statistical Analysis

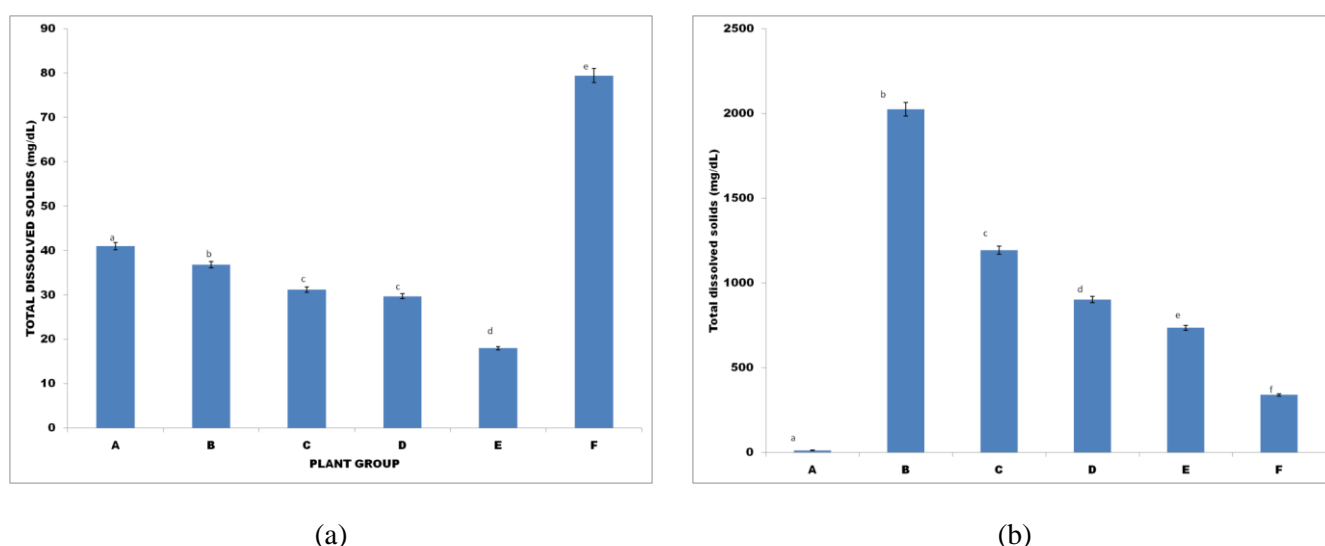
All data were expressed as mean  $\pm$  Standard Error of Mean (SEM). Statistical analyses were performed using SPSS version 27.0 (IBM Corp., USA) and GraphPad Prism version 9.0. One-way Analysis of Variance (ANOVA) was conducted to assess significant differences among treatment groups. Tukey's Honest Significant Difference (HSD) test was applied for post hoc multiple comparisons at a significance level of  $p < 0.05$ . Normality and homoscedasticity of data were confirmed using the Shapiro–Wilk and Levene's tests, respectively. Non-parametric tests (Kruskal–Wallis and Dunn's test) were employed where assumptions were violated.

### 3. Results and Discussion

#### 3.1. Total Dissolved Solids (TDS)

Figure 1(a) represents the total dissolved solids (TDS) at the start of the experiment (week 0). Among the treatment groups, the TDS levels varied considerably despite differences in WBM concentration. Interestingly, Group F (20% WBM) had the highest initial TDS ( $\sim 81$  mg/dL), which contrasts with expectations based on its lower WBM content. This suggests that at lower concentrations, the finer dispersion or breakdown of WBM components may have led to greater solubility of ions. Groups B, C, and D had moderate TDS levels ( $\sim 30$ – $36$  mg/dL), while Group E (40% WBM) had the lowest initial value ( $\sim 19$  mg/dL). The control (Group A) had a TDS of  $\sim 41$  mg/dL, slightly higher than several WBM treatments, possibly reflecting background ionic content of the tap water used.

Figure 1(b) shows the TDS values after six weeks of water hyacinth cultivation. A clear and substantial increase in TDS was observed across all WBM-treated groups. The highest TDS was recorded in Group B (100% WBM) at  $\sim 2050$  mg/dL, followed by Group C (80%) at  $\sim 1250$  mg/dL, Group D (60%) at  $\sim 950$  mg/dL, Group E (40%) at  $\sim 750$  mg/dL, and Group F (20%) at  $\sim 300$  mg/dL. The control group A maintained a very low TDS ( $\sim 50$  mg/dL), confirming the absence of external contaminants.



**Figure 1.** Total dissolved solids of WBM-contaminated water at (a) week 0 and (b) week 6 of water hyacinth plant cultivation. Results are means of 5 determinations  $\pm$  SEM. Bars bearing different superscripts are significantly different ( $p < 0.05$ )

The progressive increase in TDS from week 0 to week 6, particularly in the WBM-exposed groups, indicates that instead of reducing the dissolved solids, the water hyacinth likely facilitated further solubilisation of WBM

constituents. This could be due to root-induced breakdown of particles, release of organic compounds, or mobilisation of ions from the WBM matrix. The trend shows a direct relationship between WBM concentration and the extent of TDS accumulation over time. The statistical differences (denoted by different superscripts) confirm that these changes are significant across treatment groups.

The trends observed in Figure 1(a&b) challenge the expected phytoremediation outcome typically associated with water hyacinth (*Eichhornia crassipes*), revealing a complex interaction between plant activity and water-based mud (WBM) components. Instead of a reduction in total dissolved solids (TDS) over time, there was a notable increase across WBM-treated groups, suggesting that the plant may have contributed to the mobilisation rather than sequestration of dissolved materials.

This phenomenon can be attributed to several factors. First, the metabolic activity of water hyacinth in contaminated environments often involves exudation of organic acids and enzymes, which can chelate or dissolve bound minerals, thereby increasing the soluble ion content of the surrounding medium (Zhou et al., 2020). Moreover, the physical disturbance caused by root penetration and oxygen release into the rhizosphere may enhance desorption and leaching of ions from sedimented WBM particles into the water column (Ogugbue & Sawidis, 2011). Such rhizosphere-driven solubilisation is well documented in aquatic macrophyte systems exposed to complex effluents (Zhang et al., 2020).

The disproportionate rise in TDS at higher WBM concentrations suggests a concentration-dependent release of ions and possibly accelerated breakdown of colloidal components, catalysed by enzymatic or microbial interactions associated with the plant's root zone (Onojake & Osuji, 2021). Interestingly, the trend also reveals that even at lower WBM concentrations, such as 20%, there was a marked increase in TDS over time, indicating that dilution does not necessarily translate to reduced ionic exposure. This finding highlights the non-linear behaviour of drilling muds in aquatic systems, where lower viscosity or better dispersion may enhance solute availability.

Although water hyacinth is widely reported to absorb and bioaccumulate ions and metals, its remediation potential is often constrained by the chemical nature and concentration of the contaminants, as well as the duration of exposure (Bello et al., 2022). In high-contaminant scenarios, the plant's capacity to retain or metabolise dissolved substances may be overwhelmed, or it may contribute indirectly to increased solubilisation through rhizosphere effects. Therefore, the observed rise in TDS should not be interpreted solely as an indication of phytoremediation failure, but rather as a symptom of ongoing chemical and biological transformation in the system.

In practical terms, these findings underscore the importance of pre-treatment or staged phytoremediation approaches when using *Eichhornia crassipes* in WBM-contaminated environments. The mobilisation of dissolved solids during the early stages of plant exposure might be followed by gradual uptake over a longer period or under optimised environmental conditions. Additional strategies, such as integrating sediment traps or using plant consortia, could help mitigate initial solubilisation effects while maximising long-term removal efficiency.

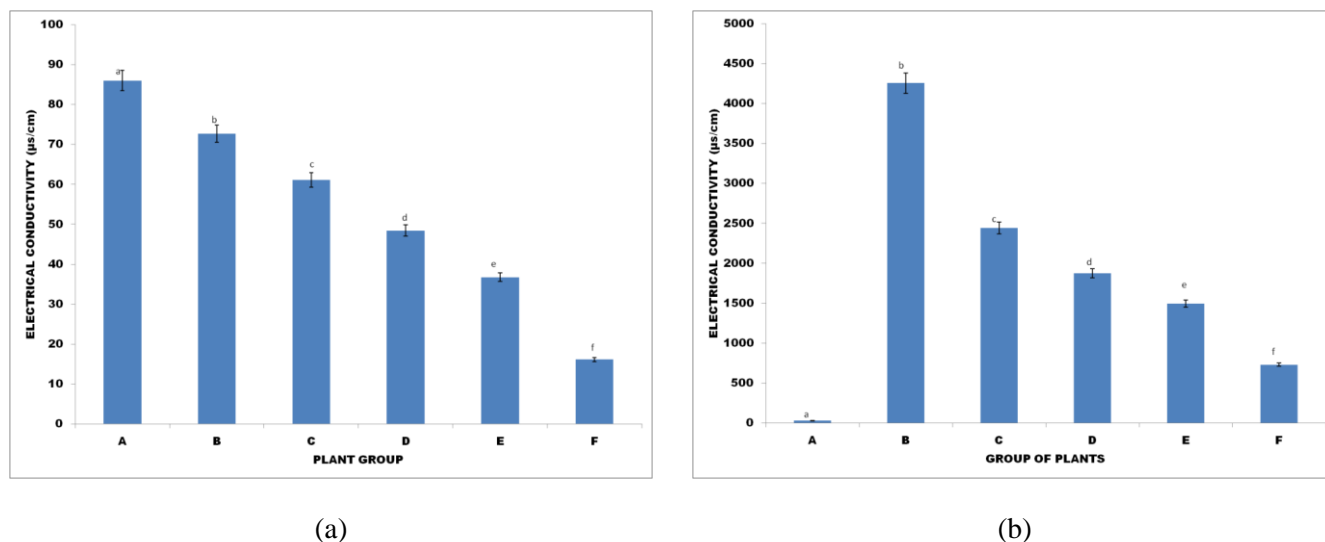
### 3.2. Electrical Conductivity (EC)

Figure 2 presents the electrical conductivity (EC) of the water medium used in the six experimental groups at week 0 (prior to water hyacinth cultivation) and at week 6 (post-cultivation). At week 0 (Figure 2a), the EC was highest in



Group A (control, tap water only) and progressively decreased from Group B (100% WBM) to Group F (20% WBM + 80% tap water). This pattern indicates that the initial ionic composition of WBM was lower than that of tap water, and that increasing dilution with tap water proportionally reduced conductivity. The significant differences among the groups, denoted by distinct superscripts (a–f), confirm that each treatment had a statistically distinct ionic environment at the onset of the experiment ( $p < 0.05$ ).

In contrast, the EC values recorded at week 6 (Figure 2b) show a markedly different trend. Group B exhibited the highest EC (approximately 4300  $\mu\text{S}/\text{cm}$ ), followed by Groups C through F, with Group A maintaining the lowest EC value. This reversal suggests a time-dependent ionic release or transformation within the WBM matrix during the course of water hyacinth cultivation. The increase in EC in the WBM treatments may be attributed to the solubilization of previously bound ions, microbial degradation of organic compounds within the mud, or the release of metabolites and exudates from water hyacinth roots that altered the chemical equilibrium. The statistically significant differences observed among all groups at week 6 (again denoted by distinct superscripts) underscore the active biochemical interactions occurring in the WBM-contaminated media.



**Figure 2.** Electrical conductivity of WBM-contaminated water at (a) week 0 and (b) week 6 of water hyacinth plant cultivation. Results are means of 5 determinations  $\pm$  SEM. Bars bearing different superscripts are significantly different ( $p < 0.05$ )

The shift in electrical conductivity (EC) observed between week 0 and week 6 in the experimental setup (Figures 2a & b) reflects critical biogeochemical interactions within the WBM-contaminated aquatic environment mediated by *Eichhornia crassipes*. While EC is fundamentally a proxy for the concentration of dissolved ionic species, its temporal transformation in contaminated systems often indicates underlying processes such as ion release, nutrient cycling, and pollutant mobilization (Suthar et al., 2020).

The marked elevation in EC across the WBM-treated groups at week 6 suggests that water hyacinth cultivation promoted the solubilization of ions previously immobilized within the drilling mud matrix. This phenomenon has been associated with the activity of root exudates, which can alter rhizospheric pH, release chelating agents, and facilitate desorption of metal and salt constituents (Ali et al., 2021). Water hyacinth, in particular, has been documented for its capacity to influence physicochemical properties of contaminated water through rhizofiltration

and root-associated microbial interactions, which enhance the bioavailability of both nutrients and toxicants (Rezania et al., 2016).

Moreover, the increased EC values may reflect microbial degradation of organic components within the WBM. Anaerobic and facultative microbial consortia often flourish in mud-rich substrates and can mineralize hydrocarbons or other complex organic compounds, releasing ammonium, phosphate, and other soluble ions into the water column (Chakraborty & Veeramani, 2022). The cumulative effect of these processes likely accounts for the significant ionic enrichment in WBM treatments post-cultivation, distinguishing them from the control group, where ionic input and microbial transformation were minimal.

Interestingly, this conductivity increase also underscores the dual role of *E. crassipes* in such systems—not only as a phytoremediator capable of heavy metal uptake and organic pollutant attenuation, but also as a biological agent that modifies the ionic dynamics of the medium (Yadav et al., 2020). While these alterations may enhance pollutant bioavailability, they also necessitate a careful balance in remediation strategies, as excessive ion mobilization could pose secondary ecological risks if not adequately managed.

In conclusion, the transformation in EC values over the 6-week period reflects a complex interplay of plant-mediated and microbial processes that influence ionic mobility in WBM-impacted water. These findings align with prior studies demonstrating the capacity of *E. crassipes* to alter water chemistry in polluted systems, affirming its potential utility in phytoremediation of drilling waste, while also highlighting the need for integrated assessments of its long-term ecological implications.

### 3.3. Plant Height

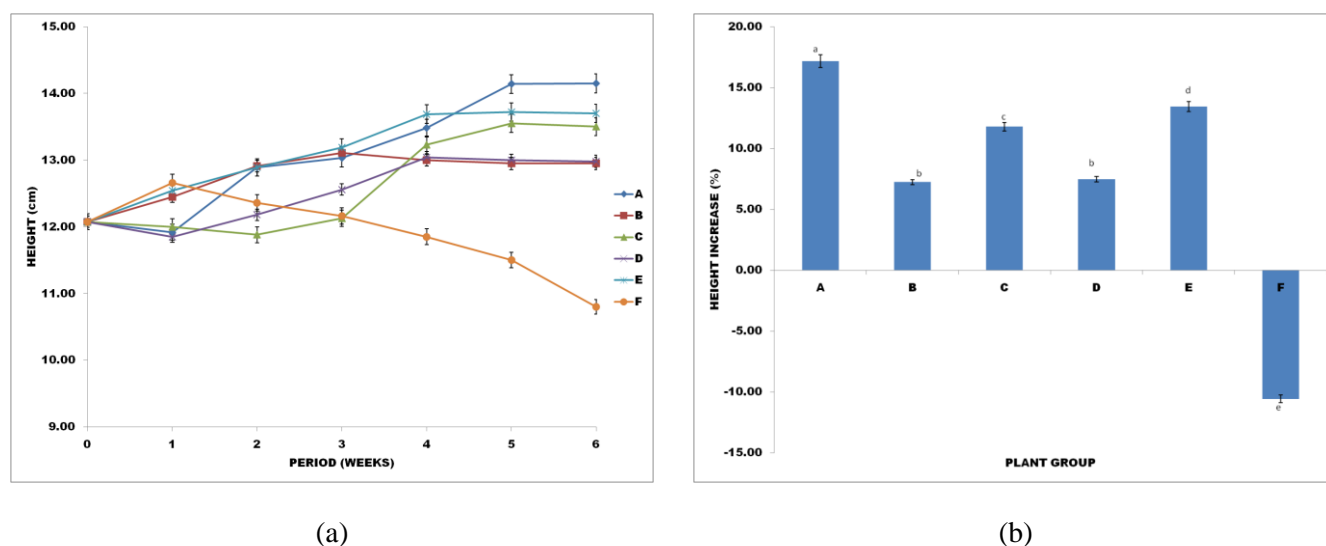
Figure 3(a) illustrates the variation in height of *Eichhornia crassipes* (water hyacinth) cultivated in water contaminated with different concentrations of water-based mud (WBM) over a period of six weeks. The control group (A), grown in tap water, demonstrated a consistent and significant increase in plant height, culminating in the highest recorded mean (~14.5 cm), indicative of optimal growth in an uncontaminated environment. In contrast, plants grown in 100% WBM (Group B) showed an early increase followed by a plateau after Week 3 (~13 cm), suggesting that undiluted WBM imposes physiological constraints that limit sustained growth. Similarly, Group D (60% WBM) showed modest and steady growth with limited final height (~13.3 cm).

Group C (80% WBM) initially exhibited stunted growth, which later improved from Week 3 onwards, suggesting delayed adaptation. Remarkably, Group E (40% WBM) displayed vigorous growth, reaching ~13.8 cm by the sixth week, second only to the control. This suggests a possible hormetic effect, where moderate levels of stress-inducing agents in the WBM may have elicited compensatory growth responses. Conversely, Group F (20% WBM) exhibited a sharp decline in height from Week 2 onwards, finishing at the lowest mean height (~10.5 cm), indicating a deleterious impact despite the relatively low WBM concentration. This could be due to disproportionate bioavailability of toxicants or disruption of ionic balance at this dilution level.

Figure 3(b) further quantifies the percentage increase in height among the treatment groups. The control group (A) had the highest percentage increase (~17%), followed by Group E (~13%) and Group C (~11%). These were significantly greater than the growth observed in Groups B and D (each 7%). Group F displayed a significant



negative growth (−12%), reflecting net height loss. Bars bearing different superscripts (a–e) indicate statistically significant differences among the treatment groups ( $p < 0.05$ ), confirming that the variation in plant response across WBM concentrations is not due to random variation but is attributable to treatment effects.



**Figure 3.** (a) Height of water hyacinth plant cultivated in WBM-contaminated water. (b) Height increase (%) of water hyacinth plant cultivated in WBM-contaminated water. Results are means of 5 determinations  $\pm$  SEM. Bars bearing different superscripts are significantly different ( $p < 0.05$ )

The observed trends in Figure 3 (a&b) highlight the complex physiological responses of *Eichhornia crassipes* to varying concentrations of water-based mud (WBM), a mixture likely containing hydrocarbons, drilling additives, and heavy metals commonly associated with oil and gas exploration (Onojake & Osuji, 2021). The differential growth responses across treatments suggest that the plant's tolerance threshold was exceeded at both the highest and lowest concentrations, supporting the principle that phytotoxicity is not always linearly dose-dependent. This aligns with the concept of **hormesis**, wherein low to moderate exposure to stressors may elicit adaptive or even stimulatory responses, while both insufficient and excessive exposures lead to stress or inhibition (Calabrese, 2019).

Water hyacinth is widely recognized for its phytoremediation potential, owing to its robust growth, high biomass production, and ability to accumulate pollutants in its tissues (Owabor & Oaikhen, 2020). However, the divergent responses observed in this study underscore that its efficacy is strongly influenced by contaminant load, chemical composition, and exposure duration. The plant's apparent capacity to adapt to moderate WBM concentrations (notably 40–80%) could indicate a threshold beyond which detoxification mechanisms such as antioxidant enzyme activation, metal chelation, or cellular compartmentalisation become effective (Zhou et al., 2020). Conversely, growth inhibition at full-strength WBM exposure could reflect overwhelmed physiological systems, reduced nutrient availability, or direct cytotoxicity of pollutants (Bello et al., 2022).

Of particular interest is the marked growth reduction at the 20% WBM treatment, suggesting that certain contaminants may exert more pronounced toxicity when diluted below a critical point, possibly due to enhanced solubility or mobility in a less viscous matrix. This finding warrants further investigation into the chemical

dynamics of WBM at lower concentrations and its interactions with aquatic macrophytes. It also supports earlier observations that pollutant speciation, rather than concentration alone, can play a dominant role in determining biological effects (Ogugbue & Sawidis, 2011).

From an applied perspective, these findings have implications for the use of water hyacinth in phytoremediation schemes involving drilling wastes. While the plant remains a promising candidate, the success of such interventions will depend on the precise characterisation of waste concentrations and the fine-tuning of environmental conditions to remain within the plant's tolerance thresholds. Moreover, the incorporation of pre-treatment steps or co-planting strategies may be necessary to improve outcomes in severely contaminated sites.

### 3.4. Stem Girth

Figure 4 presents the dynamic changes in stem girth (cm) of water hyacinth plants over a 6-week period when cultivated in various concentrations of water-based mud (WBM). The results, expressed as mean  $\pm$  SEM for five replicates, reflect the morphological plasticity of *Eichhornia crassipes* under environmentally induced stress.

At week 0, all treatment groups began with comparable stem girths ( $\sim 1.7$  cm), suggesting uniform baseline morphology. However, by week 1, all groups exhibited a sharp decline in stem girth, reaching as low as  $\sim 1.1$ – $1.4$  cm, which likely reflects an initial shock or acclimatisation response to the altered water chemistry associated with WBM exposure. This immediate reduction may be attributed to osmotic stress, reduced turgor pressure, or interference with nutrient uptake due to the presence of petroleum residues or drilling additives.

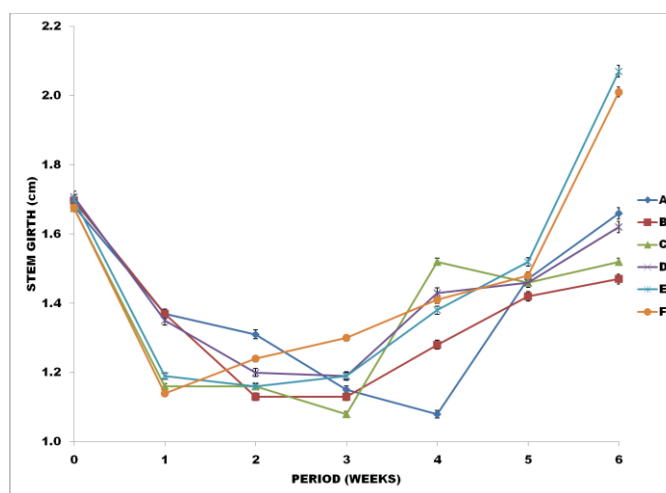
Between weeks 2 and 4, most groups maintained relatively stable girth values with minor fluctuations, although Groups A (control) and C (80% WBM) reached their lowest points in weeks 3–4, indicating a delayed response to earlier stress. Notably, Group C had the least stem girth ( $\sim 1.05$  cm) at week 3, suggesting that the 80% WBM concentration may have posed a substantial physiological constraint on stem expansion during mid-exposure.

From week 4 onwards, there was a marked recovery in stem girth across all groups. By week 6, the most significant increases were observed in Groups E (40% WBM) and F (20% WBM), both exceeding 2.0 cm, indicating not just recovery but possible overcompensation or adaptive restructuring of stem tissue. Group A (control) also showed substantial improvement, reaching  $\sim 1.75$  cm, while Groups B–D exhibited more modest increases ( $\sim 1.45$ – $1.6$  cm), indicating varying degrees of tolerance and recovery.

The pronounced improvement in Groups E and F may suggest that lower concentrations of WBM—while initially inhibitory—subsequently stimulated cellular division or tissue thickening as a compensatory growth mechanism. This aligns with earlier findings suggesting that sub-lethal concentrations of contaminants can elicit stress adaptation in aquatic macrophytes (Calabrese & Mattson, 2017). Conversely, the relatively limited recovery in the 100% WBM group (B) indicates persistent toxicity and reduced resilience, likely due to cumulative exposure to high pollutant loads.

The changes in stem girth observed in Figure 4 underscore the dynamic morphological responses of *Eichhornia crassipes* to water-based mud (WBM) exposure, reinforcing its dual nature as both sensitive and adaptive under environmental stress. Stem girth, a key proxy for mechanical support and vascular efficiency, is directly influenced

by cellular turgor, nutrient transport, and tissue integrity—all of which are susceptible to disruption by xenobiotic substances present in WBM, including hydrocarbons, heavy metals, and drilling fluid additives (Bello et al., 2022; Onojake & Osuji, 2021).



**Figure 4.** Stem girth of water hyacinth plant cultivated in WBM-contaminated water. (Results are means of 5 determinations  $\pm$  SEM)

The biphasic response—an initial decrease in stem girth followed by variable recovery—suggests a classical stress-adaptation pattern. According to Zhang et al. (2020), aquatic macrophytes often undergo structural suppression during early exposure to toxicants as resources are redirected towards detoxification and maintenance processes. The subsequent recovery observed in some treatment groups is indicative of compensatory growth, likely supported by physiological adjustments such as cell wall thickening, increased lignification, or enhanced antioxidant defence systems (Gbenou et al., 2023). Such responses are characteristic of plants with high phenotypic plasticity, particularly those like *Eichhornia crassipes* that thrive in fluctuating and polluted aquatic environments.

Interestingly, the superior stem girth expansion observed at moderate-to-low WBM concentrations may be interpreted through the lens of hormesis—a biological phenomenon where low doses of stress-inducing agents stimulate adaptive overcompensation (Calabrese & Mattson, 2017). This aligns with studies that document improved biomass allocation, vascular differentiation, and secondary growth under mild contaminant loads, where the plant shifts from a defensive to a constructive metabolic mode (Zhou et al., 2020).

From an ecological remediation standpoint, the results affirm the resilience of *E. crassipes* in environments contaminated with drilling waste, though its performance is clearly concentration-dependent. While extreme concentrations may impair structural development, sublethal exposures could still permit effective phytoremediation by enabling partial recovery and continued pollutant uptake. Therefore, integrating *Eichhornia* in constructed wetlands or polishing ponds treating WBM effluents remains promising, provided that contaminant levels are within its physiological thresholds.

### 3.5. Chlorophyll Content

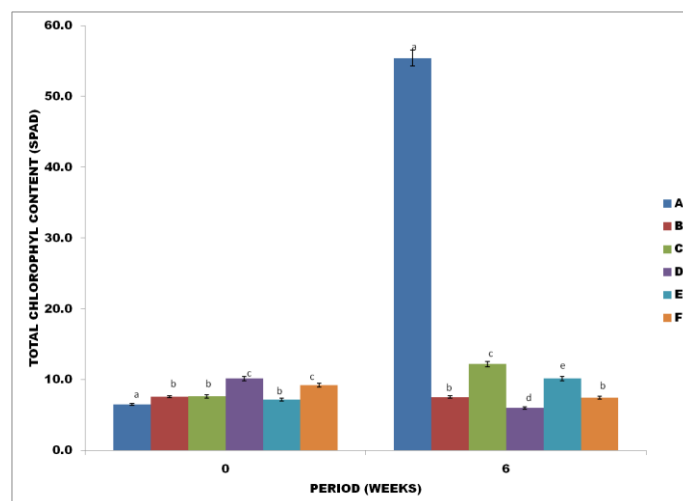
Figure 5 illustrates the changes in total chlorophyll content (measured in SPAD units) of *Eichhornia crassipes* (water hyacinth) over a 6-week period under varying concentrations of water-based drilling mud (WBM). At week

0, chlorophyll levels across all groups were relatively low, with values ranging between approximately 6 and 11 SPAD units. There were statistically significant differences among the groups (denoted by different superscripts), but the variations were modest. This similarity in baseline chlorophyll content reflects the relatively uniform physiological status of the plants prior to exposure to WBM.

By week 6, a substantial divergence in chlorophyll accumulation was observed. The control group (Group A, cultivated in 100% tap water) recorded a dramatic increase in SPAD value, peaking at approximately 55 SPAD units, which was significantly higher than all other treatments ( $p < 0.05$ ). This sharp rise suggests that under non-contaminated conditions, *E. crassipes* was able to accumulate chlorophyll effectively, reflecting optimal photosynthetic development.

In contrast, WBM-treated groups (B to F) showed significantly lower chlorophyll levels at week 6. Among them, Group C (80% WBM + 20% tap water) had the highest chlorophyll content (~18 SPAD units), followed by Group E, Group F, Group B, and Group D, which had the lowest (~6 SPAD units). These reductions suggest that WBM exposure inhibited chlorophyll synthesis or promoted chlorophyll degradation, possibly due to the presence of phytotoxic constituents such as heavy metals, hydrocarbons, or elevated ionic concentrations in the drilling mud.

The pattern of chlorophyll accumulation at week 6 implies a dose-dependent toxicological effect: as the proportion of WBM increased, chlorophyll content generally declined. This trend supports the hypothesis that prolonged exposure to WBM impairs photosynthetic capacity in water hyacinth, likely through oxidative stress, nutrient imbalance, or structural damage to chloroplasts.



**Figure 5.** Chlorophyll content of water hyacinth plant cultivated in WBM-contaminated water at week 0 and week 6. Results are means of 5 determinations  $\pm$  SEM. Bars bearing different superscripts are significantly different ( $p < 0.05$ )

Chlorophyll content is a critical physiological indicator of plant health, photosynthetic performance, and environmental stress tolerance. The patterns observed in Figure 5, reflecting chlorophyll responses of *Eichhornia crassipes* after six weeks of exposure to varying concentrations of water-based drilling mud (WBM), point to the phytotoxic effects of drilling waste on aquatic macrophytes. While water hyacinth is known for its resilience and remediation potential, its photosynthetic pigment synthesis is evidently compromised under drilling mud stress,

affirming previous reports on the sensitivity of chlorophyll metabolism to pollutant-induced oxidative and nutritional imbalances (Akinbile et al., 2020; Sharma & Agrawal, 2019).

The suppression of chlorophyll accumulation under WBM treatments may be linked to several interacting mechanisms. First, elevated concentrations of heavy metals such as cadmium, lead, or chromium—often associated with drilling fluids—can disrupt chloroplast integrity, inhibit  $\delta$ -aminolevulinic acid synthesis, and interfere with the enzymatic steps of chlorophyll biosynthesis (Khoshgoftarmanesh et al., 2021). Second, the presence of oil-based residues and dispersants in WBM may coat root surfaces and reduce nutrient uptake efficiency, particularly of magnesium and nitrogen, which are core components of the chlorophyll molecule. Consequently, chlorophyll degradation is accelerated, possibly via the activation of chlorophyllase or peroxidase enzymes under stress conditions (Ali et al., 2020).

Additionally, the decline in chlorophyll content across the WBM gradient is consistent with stress-induced photoinhibition, wherein reactive oxygen species (ROS) generated under toxic exposure damage photosystems and impair light-harvesting complexes (Tripathi et al., 2021). Such oxidative stress is a hallmark of polluted aquatic environments and is exacerbated by long-term exposure to contaminants, especially when remediation thresholds of the plant are exceeded.

Notably, while *Eichhornia crassipes* demonstrated some capacity to maintain chlorophyll production under moderate WBM dilutions, the sharp contrast with the control group indicates that uncontaminated conditions are essential for optimal pigment biosynthesis and functional photosynthesis. This aligns with studies that have highlighted water hyacinth's dual role as both a sentinel species for water quality and an agent of phytoremediation, albeit with performance limitations under high contaminant loads (Rezania et al., 2016; Yadav et al., 2020).

In essence, the decline in chlorophyll content in WBM-exposed water hyacinths underscores the toxicological burden imposed by drilling wastes and highlights the need for careful management of such effluents in aquatic systems. The findings also reinforce the use of chlorophyll indices, such as SPAD values, as sensitive and reliable biomarkers for assessing contaminant stress and evaluating phytoremediative efficacy.

### 3.6. Catalase Specific Activity

Figure 6 illustrates the tissue-specific activity of catalase (CAT), an essential antioxidant enzyme, in the leaf, stem, and root tissues of *Eichhornia crassipes* following exposure to varying concentrations of water-based drilling mud (WBM). The data are expressed as specific activity (U/mg protein), and significant differences among treatments are denoted by differing superscripts ( $p < 0.05$ ).

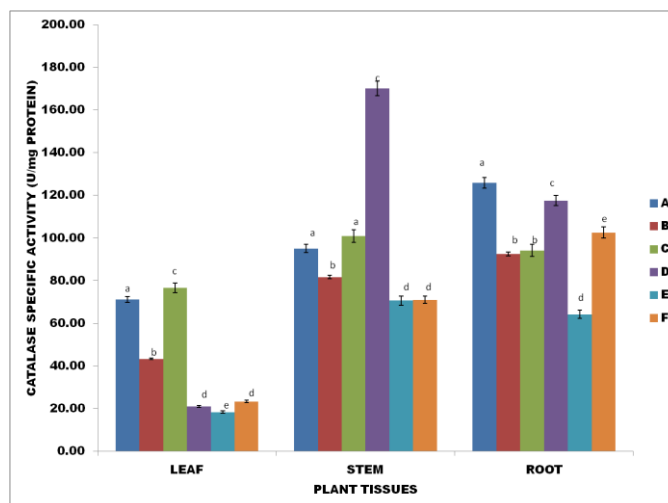
In leaf tissues, catalase activity was highest in Group C (80% WBM), followed by Group A (control), with Group B (100% WBM) showing moderate activity. The lowest activities were observed in Groups D, E, and F, with Group E recording the minimum. This pattern suggests that moderate levels of WBM induced a stronger antioxidant response in the leaf, while high or low concentrations may have either overwhelmed or inadequately stimulated catalase expression.

In stem tissues, the most pronounced catalase activity occurred in Group D (60% WBM), significantly exceeding all other groups, indicating a strong oxidative challenge and robust enzymatic defense in this tissue. Groups A and

C followed closely, while Groups B, E, and F showed significantly lower activities. The data imply that the stem is highly responsive to moderate WBM-induced oxidative stress and may serve as a key site of reactive oxygen species (ROS) detoxification.

In root tissues, catalase activity was highest in Group A (control), indicating basal antioxidant defense under non-stress conditions. This was followed by Groups C and B, suggesting a stress-induced upregulation. Group D exhibited the lowest catalase activity, which may point to enzymatic suppression or damage at higher WBM concentrations. Groups E and F showed moderate activity, possibly reflecting partial adaptive responses.

Overall, the variation in catalase activity across tissues and treatments reflects the differential oxidative stress burden and tissue-specific antioxidant responses in *E. crassipes*. While moderate WBM exposure stimulated catalase activity, excessive exposure may have led to enzyme inhibition or oxidative damage, particularly in the root and leaf tissues. These findings highlight catalase as a sensitive biochemical marker for assessing oxidative stress in aquatic macrophytes exposed to drilling waste pollutants.



**Figure 6.** Catalase Specific activity in selected tissues of water hyacinth plant cultivated in WBM-contaminated water over a period of six weeks. Results are means of 5 determinations  $\pm$  SEM. Bars bearing different superscripts are significantly different ( $p < 0.05$ )

Catalase (CAT) is a primary antioxidant enzyme involved in the detoxification of hydrogen peroxide ( $H_2O_2$ ), a reactive oxygen species (ROS) generated during environmental stress. The differential CAT activity observed in the leaf, stem, and root tissues of *Eichhornia crassipes* (Figure 6) exposed to varying concentrations of water-based drilling mud (WBM) offers important insights into the oxidative stress responses and adaptive strategies of the plant under toxic conditions. As oxidative stress is a hallmark of pollutant exposure, catalase activity serves as a reliable biomarker for evaluating cellular responses to environmental contaminants (Gill & Tuteja, 2010).

The modulation of CAT activity across plant tissues in response to WBM exposure suggests a tissue-specific defense mechanism driven by the intensity and localization of ROS generation. Enhanced CAT activity in certain tissues under moderate WBM concentrations is consistent with a compensatory response aimed at neutralizing excess  $H_2O_2$ , thereby protecting cellular structures and maintaining redox homeostasis. This adaptive enzymatic upregulation is widely documented in macrophytes exposed to heavy metals and petroleum-based contaminants,



where increased ROS scavenging is vital for sustaining physiological functions (Ali et al., 2020; Maestri et al., 2010).

Interestingly, the peak catalase response in the stem tissue at moderate WBM exposure may reflect the stem's central role in metabolite transport and signaling, which makes it particularly responsive to systemic stress signals. Stems may also act as translocation channels for both contaminants and signaling molecules, prompting enhanced antioxidant defenses in these tissues (Foyer & Noctor, 2011). Conversely, the suppression or insufficient induction of CAT activity in other tissues under higher WBM concentrations may indicate oxidative damage beyond the enzymatic coping threshold. Such a decline has been attributed to enzyme inactivation, metal binding to active sites, or downregulation of antioxidant gene expression under chronic stress conditions (Singh et al., 2016).

Furthermore, the relatively high CAT activity observed in the roots under some treatments is consistent with their primary interface with contaminants. As roots are the initial contact point with pollutants in the growth medium, they often experience elevated ROS production and consequently activate robust enzymatic defenses. However, the variability in root catalase activity suggests that prolonged or intense exposure to toxicants, such as those present in WBM, may impair root function and enzyme stability (Pourrut et al., 2011).

The findings in this study underscore the complexity of oxidative stress management in aquatic plants exposed to petroleum-derived wastes. They also reinforce the role of *E. crassipes* as a sentinel species for ecotoxicological monitoring due to its measurable biochemical responses to pollutant gradients. Importantly, these insights align with broader literature on plant antioxidant responses, highlighting the need to consider tissue-specific dynamics when evaluating phytotoxicity and phytoremediation potential.

### 3.7. Superoxide Dismutase Specific Activity

Figure 7 presents the tissue-specific activities of superoxide dismutase (SOD), a key antioxidative enzyme, in the leaf, stem, and root tissues of *Eichhornia crassipes* after six weeks of cultivation in water contaminated with varying concentrations of water-based drilling mud (WBM). The enzyme activity is expressed in units per milligram of protein (U/mg protein), and statistically significant differences ( $p < 0.05$ ) among treatment groups are indicated by different superscripts.

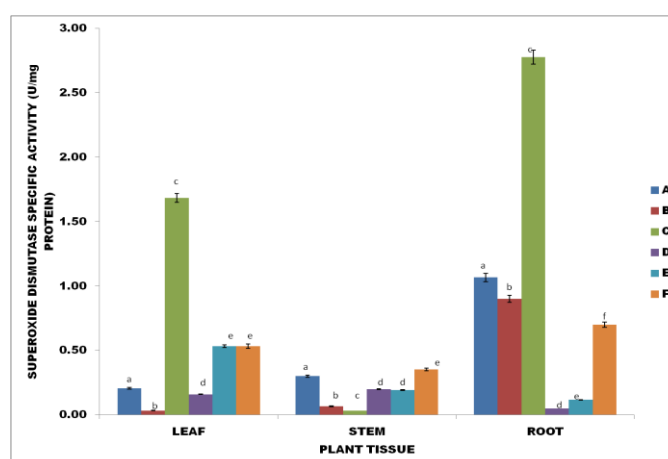
In leaf tissues, SOD activity was significantly elevated in Group C (80% WBM + 20% tap water), indicating a heightened response to oxidative stress, likely triggered by the accumulation of superoxide radicals in moderately contaminated conditions. Group C's leaf tissues recorded the highest activity (~1.6 U/mg protein), suggesting that this concentration of WBM induced an oxidative challenge that effectively activated the enzymatic defense system. Groups E and F followed with moderate activities, while Groups A, B, and especially D exhibited significantly lower SOD levels, implying either a lower level of oxidative induction or potential enzyme inhibition under more toxic or less stimulating conditions.

In stem tissues, SOD activity was more subdued across all groups. Group A (control) and Group F exhibited slightly elevated activities, while other treatments (especially Group C) showed very low enzyme levels. This suggests that SOD-based antioxidant defense in the stem was either minimally induced or insufficiently maintained

across the WBM treatments, possibly due to tissue-specific differences in ROS generation or the compartmentalization of oxidative stress responses.

In root tissues, a pronounced peak in SOD activity was again observed in Group C, reaching approximately 2.8 U/mg protein, significantly higher than all other groups. This indicates a robust oxidative stress response in roots exposed to 80% WBM, likely reflecting their role as the primary site of contaminant interaction and uptake. Groups A and B showed intermediate activities, while Groups D, E, and F exhibited significantly lower responses, suggesting either low stress levels or impaired antioxidant activation under those specific exposure conditions.

Overall, the data underscore the tissue- and concentration-dependent variation in SOD activity in *Eichhornia crassipes* under WBM-induced stress. The elevated enzyme activity in Group C across all tissues implies that moderate WBM contamination triggers an optimal defense response, whereas higher or lower concentrations may either overwhelm or inadequately stimulate antioxidant pathways. The root tissue appeared most responsive, likely due to its direct interface with contaminants, reinforcing the notion of spatially distinct stress responses within the plant. These findings position SOD activity as a sensitive and dynamic biomarker for evaluating oxidative responses in phytoremediation studies involving petroleum-derived contaminants.



**Figure 7.** Superoxide dismutase specific activity in selected tissues of water hyacinth plant cultivated in WBM-contaminated water over a period of six weeks. Results are means of 5 determinations  $\pm$  SEM.

Bars bearing different superscripts are significantly different ( $p < 0.05$ )

Superoxide dismutase (SOD) plays a pivotal role in the antioxidant defense system of plants by catalysing the dismutation of superoxide radicals ( $O_2^-$ ) into molecular oxygen and hydrogen peroxide, thereby forming the first line of defense against oxidative stress. The tissue-specific activity patterns observed in *Eichhornia crassipes* exposed to water-based drilling mud (WBM) (Figure 7) reflect a dynamic and compartmentalized response to the varying intensities of oxidative burden induced by the contaminants present in the medium. These responses align with previous findings that describe SOD as highly responsive to environmental stressors, particularly heavy metals, petroleum hydrocarbons, and xenobiotic compounds, which are prevalent in drilling waste (Mittler, 2017; Zhang et al., 2022).

The heightened SOD activity, particularly in the roots under moderate WBM exposure, may be attributed to the fact that root tissues are the primary interface between the plant and the contaminated environment. Roots are often the

first to encounter elevated ROS production due to the uptake of toxicants, leading to upregulation of SOD as an early protective mechanism (Pourrut et al., 2011). Such localized stress responses have been reported in other aquatic macrophytes and terrestrial plants exposed to petroleum effluents and heavy metal-rich wastewater (Kumar et al., 2020). However, excessive exposure, particularly at higher concentrations of WBM, can surpass the antioxidant threshold, resulting in enzyme suppression, as has been noted in studies where toxicants bind directly to enzyme active sites or disrupt gene expression of antioxidant proteins (Sharma et al., 2021).

Interestingly, while roots demonstrate strong enzymatic responses, leaf and stem tissues show more variable patterns. This may be due to differences in tissue-specific sensitivity, metabolic activity, and ROS-scavenging requirements. For instance, leaves are metabolically active sites of photosynthesis and are prone to photo-oxidative stress; however, their antioxidant response is often moderated by other enzymes such as ascorbate peroxidase and glutathione reductase (Foyer & Noctor, 2005). In contrast, the relatively lower and inconsistent SOD activity in stems may reflect their intermediary physiological role and lesser exposure to direct oxidative insults.

The observed upregulation of SOD in response to moderate WBM levels supports the notion of a hormetic response, where low-to-intermediate levels of stress elicit adaptive antioxidant defense, while extreme exposure leads to oxidative damage and enzymatic dysfunction (Calabrese & Mattson, 2017). This biphasic behavior has been widely reported in stress physiology, reinforcing the complexity of interpreting antioxidant dynamics in polluted environments.

### 3.8. Vitamin C Content

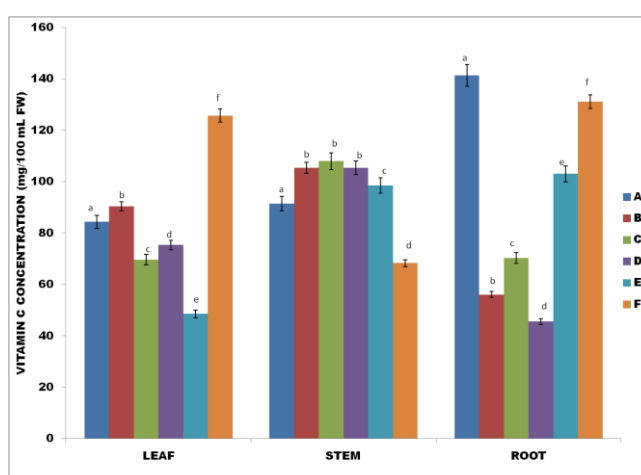
Figure 8 illustrates the tissue-specific concentrations of vitamin C (ascorbic acid) in the leaf, stem, and root of *Eichhornia crassipes* after six weeks of exposure to varying levels of water-based drilling mud (WBM). The data are presented in mg/100 mL fresh weight (FW) and are the mean of five determinations  $\pm$  SEM. Statistically significant differences ( $p < 0.05$ ) among groups are denoted by differing superscripts.

In leaf tissues, vitamin C concentrations varied widely, with the highest level recorded in Group F (20% WBM + 80% tap water), followed by Groups B and A. Groups C, D, and E exhibited significantly lower ascorbate content, with Group E recording the least. This pattern indicates that moderate stress or low WBM exposure may stimulate vitamin C synthesis in leaves, likely as a protective response to oxidative stress, whereas higher concentrations (e.g., in Group E) may have suppressed biosynthesis or induced ascorbate degradation.

In the stem tissues, vitamin C content was again highest in Group F, followed closely by Groups B and C. Group A (control) maintained a moderate level, while Groups D and especially F showed the lowest concentrations. These differences suggest that the stem tissues may upregulate ascorbic acid in response to certain levels of WBM-induced oxidative stress, but the response is not linear—indicating that at higher contaminant concentrations, physiological functions related to vitamin C biosynthesis may become compromised.

In root tissues, the highest ascorbic acid content was recorded in Group A (control), with substantial levels also seen in Group F and E. Groups B, C, and D recorded markedly lower values, with Group D showing the minimum concentration. This trend suggests that while control plants maintained robust antioxidant defense in the roots, certain WBM concentrations may impair the root's capacity to produce or retain vitamin C.

Overall, the observed patterns of vitamin C distribution across tissues and treatments indicate that the ascorbate antioxidant system in *Eichhornia crassipes* is highly responsive to WBM exposure, but in a dose- and tissue-specific manner. Low to moderate stress (as in Group F) may stimulate vitamin C accumulation, especially in aerial parts, serving as a key non-enzymatic antioxidant to neutralise reactive oxygen species. Conversely, excessive contamination may inhibit this protective mechanism, particularly in the roots and leaves, where metabolic activity is directly impacted by pollutants. These findings confirm the modulatory role of vitamin C in oxidative stress defense and support its relevance as a sensitive biomarker in ecotoxicological evaluations of contaminated aquatic environments.



**Figure 8.** Vitamin C content of selected tissues of water hyacinth plant cultivated in WBM-contaminated water over a period of six weeks. Results are means of 5 determinations  $\pm$  SEM. Bars bearing different superscripts are significantly different ( $p < 0.05$ )

Vitamin C (ascorbic acid) is a vital non-enzymatic antioxidant that plays a central role in plant defense against oxidative stress induced by environmental contaminants. Its distribution and modulation in different tissues of *Eichhornia crassipes* cultivated in water-based drilling mud (WBM) (Figure 8) offer valuable insights into the plant's biochemical responses to petroleum-derived pollutants. The observed variability in ascorbate levels among the tissues and treatments reflects a complex interplay between stress perception, antioxidative adaptation, and metabolic disruption, consistent with findings in similar ecotoxicological studies (Smirnov, 2018; Foyer & Noctor, 2011).

Ascorbic acid functions as a scavenger of reactive oxygen species (ROS), particularly superoxide and hydrogen peroxide, and also serves as a cofactor in several key physiological processes including cell wall synthesis, hormone regulation, and photosynthetic protection (Gallie, 2013). In response to moderate environmental stress, plants often exhibit an upregulation of ascorbate biosynthesis as part of a primed defense mechanism, a phenomenon that is supported by elevated vitamin C levels in specific tissues under intermediate WBM concentrations. This aligns with the hormesis model of stress physiology, where low to moderate pollutant exposure stimulates protective pathways without causing irreversible damage (Calabrese & Mattson, 2017).

The tissue-specific responses noted in the present study may be explained by the differential sensitivity of leaf, stem, and root tissues to environmental stressors and their associated metabolic functions. Leaves, being

photosynthetically active, are typically more prone to oxidative damage from both endogenous and exogenous ROS, necessitating a rapid antioxidant response including ascorbate accumulation (Mittler, 2017). Meanwhile, root tissues, which are in direct contact with WBM contaminants, may suffer structural or metabolic impairment that suppresses antioxidant production under high stress loads, a trend previously reported in hydrocarbon-exposed macrophytes (Kumar et al., 2020). The reduced vitamin C levels observed under such conditions may be attributed to either impaired biosynthetic capacity or increased degradation rates due to sustained oxidative stress (Sharma et al., 2021).

Furthermore, the ability of *E. crassipes* to modulate its ascorbate content in response to varying degrees of WBM contamination underscores its biochemical plasticity and adaptive resilience. This antioxidant buffering capacity is a key feature supporting its application in phytoremediation. However, the non-linear trends observed—especially the decline in vitamin C content at higher contamination levels—highlight potential physiological limits beyond which antioxidant defences may become insufficient, leading to oxidative damage and compromised plant function (Bose et al., 2014).

In sum, vitamin C serves not only as an important indicator of oxidative stress management in aquatic plants but also as a functional marker of phytoremediation performance. Its fluctuating levels across plant tissues and pollutant gradients provide an integrative signal of both stress intensity and adaptive capacity, reinforcing its value in ecophysiological assessments of contaminated aquatic systems.

#### 4. Conclusion

This study provides critical insights into the oxidative and biochemical responses of *Eichhornia crassipes* when exposed to water-based drilling mud (WBM)-contaminated aquatic systems. The findings revealed that, contrary to the expected reduction of contaminants through phytoremediation, the presence of water hyacinth initially facilitated the solubilisation of WBM constituents, leading to significant increases in total dissolved solids (TDS) and electrical conductivity (EC) across treatment groups. Morphological assessments, including plant height and stem girth, displayed a concentration-dependent response, with moderate WBM exposures eliciting adaptive growth patterns, while extreme concentrations suppressed growth performance.

The decline in chlorophyll content under WBM treatments underscores the adverse impact of drilling mud-associated pollutants on the photosynthetic efficiency of *E. crassipes*. Biochemical assays further revealed tissue-specific and concentration-dependent modulations in catalase (CAT), superoxide dismutase (SOD), and vitamin C levels, indicating a complex oxidative stress response mechanism. Notably, moderate contaminant levels triggered significant upregulation of antioxidant enzymes, while excessive exposure overwhelmed the plant's defense capacity, resulting in enzyme suppression and compromised stress tolerance.

Overall, *Eichhornia crassipes* demonstrated a measurable but limited phytoremediation potential in WBM-contaminated water, with its efficacy being highly contingent on pollutant concentration, exposure duration, and the physicochemical nature of the contaminants. The study highlights the necessity for integrated remediation strategies that combine phytoremediation with pre-treatment or auxiliary methods to enhance contaminant removal efficiency and mitigate secondary environmental risks. Moreover, physiological and biochemical stress markers, as

demonstrated in this study, are essential tools for monitoring and optimising phytoremediation systems involving drilling waste effluents.

#### 4.1. Future Suggestions

- 1) Conduct long-term monitoring of *Eichhornia crassipes* in WBM-contaminated environments to determine seasonal variations in phytoremediation efficiency and oxidative stress responses.
- 2) Investigate the combined use of *E. crassipes* with other aquatic macrophytes or microbial consortia to enhance pollutant removal and reduce initial contaminant solubilisation.
- 3) Perform detailed chemical speciation analyses of heavy metals and hydrocarbons in WBM leachates to better understand bioavailability and toxicity thresholds.
- 4) Explore the application of pre-treatment methods (e.g., sedimentation, aeration, or adsorption) prior to phytoremediation to minimise early-phase contaminant mobilisation.
- 5) Evaluate the potential reuse of harvested biomass from *E. crassipes* grown in WBM-contaminated water for bioenergy or compost production, ensuring safe disposal of accumulated pollutants.

#### Declarations

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##### Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

##### Consent for publication

The authors declare that they consented to the publication of this study.

##### Authors' contributions

Both the authors made an equal contribution in the Conception and design of the work, Data collection, Drafting the article, and Critical revision of the article. Both authors have read and approved the final copy of the manuscript.

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