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Synergistic Modulation of Oxidative Stress and Antioxidant Defence in *Eichhornia crassipes* During Nanozerovalent Iron–Assisted Remediation of Crude Petroleum Oil–Contaminated Water

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Abstract
This study evaluated the synergistic effects of *Eichhornia crassipes* (water hyacinth) and nanozerovalent iron (nZVI) on oxidative stress modulation during remediation of crude petroleum oil (CPO)-contaminated water. Experimental groups comprised CPO-only exposure (Group 2), combined CPO–nZVI treatments (Groups 3–5; 0.1–0.4 mg/kg), and nZVI-only systems (Groups 6–8). Biochemical endpoints including malondialdehyde (MDA), reduced glutathione (GSH), vitamin C, catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) were assessed across leaf, stem, and root tissues. CPO exposure (Group 2) significantly elevated lipid peroxidation, with leaf MDA reaching 0.000172 ± 0.000010 mg/g, indicating severe oxidative damage. The incorporation of nZVI resulted in a concentration-dependent reduction in MDA, with a 22.7% decrease observed in Group 5 (0.000133 ± 0.000006 mg/g). Antioxidant responses were markedly enhanced, as leaf GSH peaked at 0.55 ± 0.05 mg/g (Group 3), while root GSH reached 0.67 ± 0.02 mg/g in Group 7, representing the highest redox buffering capacity. Vitamin C levels were also elevated, with maximum leaf concentration of 2.80 ± 0.15 mg/100 g recorded in Group 3. Enzymatic antioxidant activities demonstrated strong upregulation under combined treatments. Leaf CAT increased over fourfold from 0.00369 ± 0.00055 U mg^{-1} protein (Group 2) to 0.01503 ± 0.00224 U mg^{-1} protein (Group 5). Root CAT and GST exhibited pronounced peaks in Group 4 (0.880 ± 0.21 and 0.880 ± 0.073 U mg^{-1} protein, respectively), indicating intense detoxification at the rhizosphere. SOD activity similarly increased, with leaf values reaching 0.01556 ± 0.01267 U mg^{-1} protein. Overall, the results demonstrate that nZVI significantly enhances phytoremediation efficiency by reducing oxidative stress ($p < 0.05$), improving antioxidant defence, and promoting physiological resilience. This study validates hybrid nano-phytoremediation as an effective and sustainable strategy for petroleum-contaminated water treatment.

Keywords: Nano-phytoremediation, *Eichhornia crassipes*, nanozerovalent iron (nZVI), crude petroleum oil contamination, oxidative stress biomarkers

Introduction

Petroleum hydrocarbon contamination of aquatic systems remains a persistent global environmental challenge, particularly in oil-producing regions where exploration, transportation, and accidental spills introduce complex mixtures of toxic compounds into water bodies. Crude petroleum oil (CPO) contains a diverse range of aliphatic and aromatic hydrocarbons, many of which are recalcitrant, bioaccumulative, and capable of inducing oxidative stress in exposed organisms (Duarte *et al*., 2024; Sharma *et al*., 2024) [2, 27]. These contaminants disrupt cellular homeostasis by generating reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, and impairment of vital physiological processes in aquatic plants and associated biota (Sefali *et al*., 2026) [24].

Phytoremediation, the use of plants to remove, transform, or stabilise environmental contaminants, has gained considerable attention as a cost-effective and environmentally sustainable remediation strategy. Among aquatic macrophytes, *Eichhornia crassipes* (water hyacinth) is particularly notable due to its rapid growth rate, extensive root system, and high capacity for uptake and accumulation of organic pollutants and heavy metals (Monroy-Licht *et al*., 2024) [17]. Its rhizosphere serves as a dynamic microenvironment where microbial activity, adsorption, and enzymatic degradation collectively contribute to contaminant removal. However, despite its effectiveness, phytoremediation alone is often limited by slow degradation kinetics and the persistence of high-molecular-weight hydrocarbon fractions.

Recent advances in nanotechnology have introduced nanozerovalent iron (nZVI) as a highly reactive material capable of reductive transformation of a wide spectrum of organic and inorganic pollutants. Owing to its large surface area and strong electron-donating capacity, nZVI facilitates rapid degradation of petroleum hydrocarbons through adsorption, reduction, and catalytic reactions (Kane *et al*., 2026) [10]. Nevertheless, the interaction between nanoparticles and plant systems is complex, as nZVI may exert both beneficial and potentially pro-oxidant effects depending on concentration and environmental conditions (Steliga *et al*., 2026) [29].

The integration of phytoremediation with nanotechnology—termed nano-phytoremediation—has emerged as a promising hybrid approach that combines the biological uptake capacity of plants with the catalytic efficiency of nanoparticles. This synergistic strategy has been reported to enhance contaminant removal rates while modulating plant physiological responses, particularly antioxidant defence systems (Varghese *et al*., 2025) [31]. Central to this interaction is the plant's oxidative stress response, which involves both non-enzymatic antioxidants such as reduced

glutathione (GSH) and ascorbic acid (vitamin C), and enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST). These biomarkers provide critical insight into the balance between ROS generation and detoxification during remediation processes.

Despite growing interest in nano-phytoremediation, there remains limited integrated understanding of how nZVI influences oxidative stress dynamics and antioxidant responses in aquatic macrophytes exposed to petroleum hydrocarbons. In particular, there is a paucity of data linking nanoparticle dosage to biochemical responses across different plant tissues, as well as the extent to which these responses reflect remediation efficiency and physiological recovery.

Therefore, this study investigates the synergistic effects of *Eichhornia crassipes* and nanozerovalent iron on the remediation of CPO-contaminated water, with a specific focus on oxidative stress biomarkers and antioxidant defence mechanisms in leaf, stem, and root tissues. By elucidating the biochemical pathways underpinning nano-phytoremediation, this work aims to provide mechanistic insight into the optimisation of sustainable remediation strategies for petroleum-contaminated aquatic environments.

Materials and Methods

Study Design and Experimental Setup

A controlled laboratory experiment was conducted to evaluate the biochemical responses of Eichhornia crassipes during remediation of crude petroleum oil (CPO)-contaminated water using nanozerovalent iron (nZVI). The study employed a completely randomised design with seven treatment groups (Groups 2–8), each replicated in triplicate. The treatment structure comprised: Group 2 (WHS + tap water + 1000 ppm CPO), Groups 3–5 (WHS + tap water + 1000 ppm CPO + nZVI at 0.1, 0.2, and 0.4 mg/kg, respectively), and Groups 6–8 (WHS + tap water + nZVI only at corresponding concentrations).

Healthy, disease-free E. crassipes plants of uniform size were collected from a freshwater body, rinsed thoroughly with distilled water to remove adhering debris, and acclimatised in dechlorinated tap water for 7 days prior to experimentation.

Preparation of Crude Petroleum Oil-Contaminated Water

Bonny Light crude petroleum oil was obtained from a certified petroleum facility. Contaminated water was prepared by dissolving crude oil in tap water to achieve a concentration of 1000 ppm (mg/L), followed by vigorous agitation using a mechanical shaker for 24 h to ensure homogeneity. The physicochemical properties of the crude oil were determined using standard methods, including density (ASTM D1298), viscosity (ASTM D445), and total petroleum hydrocarbon (TPH) characterization by gas chromatography–mass spectrometry (GC–MS) following EPA Method 8015.

Synthesis and Application of Nanozerovalent Iron (nZVI)

nZVI particles were synthesised via chemical reduction of ferric ions using sodium borohydride ($NaBH_4$) as a reducing agent, following established protocols (Zhang *et al*., 2025) [38]. Briefly, an aqueous solution of ferric sulfate ($Fe_2(SO_4)_3$) was prepared and reduced under continuous stirring by dropwise addition of freshly prepared $NaBH_4$ solution under inert conditions. The resulting black precipitate was washed repeatedly with deoxygenated distilled water and ethanol, then dried under vacuum.

The synthesised nZVI was characterised for particle size and morphology using scanning electron microscopy (SEM) and for elemental composition using energy-dispersive X-ray spectroscopy (EDX). Appropriate quantities of nZVI were introduced into treatment tanks to achieve concentrations of 0.1, 0.2, and 0.4 mg/kg.

Experimental Exposure

Each treatment was conducted in plastic tanks containing a fixed volume (10 L) of prepared solution. A uniform biomass of E. crassipes (500 g fresh weight per tank) was introduced into each system. The experiment was maintained under ambient laboratory conditions (temperature: 27 ± 2°C; photoperiod: 12 h light/12 h dark) for a duration of 21 days. Water levels were maintained throughout the exposure period by periodic addition of dechlorinated water.

Sample Collection and Tissue Preparation

At the end of the exposure period, plants were harvested and separated into leaf, stem, and root tissues. Samples were rinsed with distilled water, blotted dry, and homogenised in ice-cold Tris–KCl buffer (0.1 M Tris-HCl, 0.15 M KCl, pH 7.4). The homogenates were centrifuged at 10,000 × g for 15 min at 4°C, and the supernatants were collected for biochemical analyses. Protein concentration was determined using the method of Lowry *et al*. (1951) [14].

Determination of Oxidative Stress Biomarkers

- **Malondialdehyde (MDA):** Lipid peroxidation was quantified using the thiobarbituric acid reactive substances (TBARS) assay as described by Varshney and Kale (1990) [32], with absorbance measured at 532 nm.
- **Reduced Glutathione (GSH):** GSH levels were determined using Ellman's reagent (DTNB) method (Ellman, 1959) [3], based on the formation of a yellow chromophore measured at 412 nm.
- **Vitamin C (Ascorbic Acid):** Ascorbate concentration was determined by the method of Roe and Kuether (1943) [21], involving reduction of 2,6-dichlorophenolindophenol and spectrophotometric measurement at 520 nm.

Determination of Antioxidant Enzyme Activities

- **Catalase (CAT):** CAT activity was measured according to Sinha (1972) [28], based on the decomposition of hydrogen peroxide, with absorbance recorded at 240 nm.
- **Superoxide Dismutase (SOD):** SOD activity was determined using the method of Misra and Fridovich (1972) [16], based on inhibition of adrenaline autoxidation at 480 nm.
- **Glutathione-S-Transferase (GST):** GST activity was assayed following the method of Habig *et al*. (1974) [7], using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate and monitoring absorbance at 340 nm.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences among treatment groups at $p < 0.05$. All analyses were conducted using SPSS (Version 25.0, IBM Corp., USA).

Results and Discussion

The biochemical responses of *Eichhornia crassipes* under crude petroleum oil (CPO) contamination and nanozerovalent iron (nZVI) treatment demonstrate a structured oxidative stress–antioxidant defence continuum consistent with established phytoremediation and nanoremediation paradigms. The grouping strategy—CPO-only exposure (Group 2), combined CPO–nZVI systems (Groups 3–5), and nZVI-only systems (Groups 6–8)—provides a robust framework for mechanistic interpretation of stress induction, mitigation, and physiological recovery.

MDA levels (Table 1) revealed significant ($p < 0.05$) oxidative perturbations across tissues, with Group 2 (WHS + Tap water + 1000 ppm CPO) exhibiting elevated lipid peroxidation, particularly in leaves (0.000172 ± 0.000010 mg/g) and roots (0.000108 ± 0.000008 mg/g). This is consistent with previous studies demonstrating that petroleum hydrocarbons induce excessive ROS production, leading to membrane lipid degradation and cellular dysfunction (Adewuyi *et al*., 2021; Zhang *et al*., 2022). The elevated MDA in roots further reflects the direct interface of contaminants with the rhizosphere, a phenomenon widely reported in aquatic macrophytes exposed to hydrocarbon stress (Janbazi *et al*., 2024) [8].

The introduction of nZVI (Groups 3–5) resulted in a concentration-dependent modulation of lipid peroxidation. At low nZVI dosage (Group 3: 0.1 mg/kg), a significant reduction in leaf MDA (0.000152 ± 0.000009 mg/g) was observed relative to Group 2, suggesting partial alleviation of oxidative stress. This aligns with findings by Orocio-Carrillo *et al*., (2024) [19], who reported that nZVI enhances reductive degradation of organic contaminants, thereby limiting ROS generation.

However, the transient increase in MDA observed in Group 4 (0.2 mg/kg) indicates a dual role of nZVI, where intermediate concentrations may catalyse Fenton-like reactions, temporarily elevating oxidative stress before remediation becomes effective. Similar biphasic responses have been reported in nanoparticle–plant interactions, where moderate nanoparticle concentrations initially exacerbate oxidative stress prior to stabilisation (Mukhtar *et al*., 2026) [18].

At higher nZVI concentration (Group 5: 0.4 mg/kg), a marked decline in MDA (0.000133 ± 0.000006 mg/g) confirms effective mitigation of lipid peroxidation, indicating that enhanced catalytic degradation of hydrocarbons outweighs any pro-oxidant effects of nanoparticles. This supports reports that optimised nZVI dosing improves remediation efficiency while restoring cellular integrity (Semerád *et al*., 2020) [25].

In nanoparticle-only systems (Groups 6–8), MDA levels were consistently lower, particularly in roots (e.g., Group 6: 0.000072 ± 0.000005 mg/g), indicating minimal oxidative stress in the absence of hydrocarbons. This observation corroborates studies showing that nZVI at controlled concentrations does not induce significant oxidative toxicity in plants and may even enhance redox balance (Kİlİc *et al*., 2025).

Table 1: Malondialdehyde (MDA) Content in Water Hyacinth Tissues Across Treatment Groups Values expressed as Mean ± SEM

Group	Leaf MDA (mg/g)	Stem MDA (mg/g)	Root MDA (mg/g)
2	$0.000172 \pm 0.000010^{a}$	$0.000089 \pm 0.000005^{c}$	$0.000108 \pm 0.000008^{a}$
3	$0.000152 \pm 0.000009^{b}$	$0.000108 \pm 0.000007^{b}$	$0.000088 \pm 0.000004^{c}$
4	$0.000171 \pm 0.000008^{a}$	$0.000077 \pm 0.000004^{c}$	$0.000108 \pm 0.000003^{a}$
5	$0.000133 \pm 0.000006^{c}$	$0.000109 \pm 0.000008^{b}$	$0.000100 \pm 0.000006^{b}$
6	$0.000152 \pm 0.000011^{b}$	$0.000052 \pm 0.000005^{d}$	$0.000072 \pm 0.000005^{d}$
7	$0.000104 \pm 0.000005^{d}$	$0.000130 \pm 0.000006^{a}$	$0.000096 \pm 0.000004^{b}$
8	$0.000132 \pm 0.000007^{c}$	$0.000125 \pm 0.000006^{a}$	$0.000098 \pm 0.000006^{b}$

Different superscripts (a–d) within each column indicate significant differences ($p < 0.05$) based on Tukey's HSD.

The GSH profile (Table 2) further elucidates intracellular antioxidant responses. Elevated GSH levels in Group 2 (leaf: 0.54 ± 0.02 mg/g; stem: 0.57 ± 0.05 mg/g) indicate activation of thiol-based detoxification pathways in response to CPO-induced oxidative stress. This agrees with previous findings that glutathione plays a central role in detoxifying hydrocarbon-induced ROS and maintaining cellular redox homeostasis (Sayed *et al*., 2024) [23].

In Groups 3–5, GSH dynamics reflect adaptive modulation under nano-assisted remediation. The highest leaf GSH in Group 3 (0.55 ± 0.05 mg/g) suggests an early compensatory response, while subsequent stabilisation in Groups 4 and 5 indicates reduced oxidative burden as remediation progresses. This trend is consistent with reports that effective pollutant degradation reduces the demand for excessive antioxidant synthesis (Roy *et al*., 2023) [22].

Notably, root GSH peaked in Group 7 (0.67 ± 0.02 mg/g), highlighting the rhizosphere as the primary site of detoxification. This observation aligns with studies demonstrating enhanced glutathione-mediated detoxification in roots during phytoremediation processes, particularly in systems involving metal–nanoparticle interactions (Falak *et al*., 2024) [5].

Table 2: Reduced Glutathione (GSH) Content in Water Hyacinth Tissues Across Treatment Groups

Group	Leaf GSH (mg/g)	Stem GSH (mg/g)	Root GSH (mg/g)
2	0.54 ± 0.02^{ab}	0.57 ± 0.05^{a}	0.47 ± 0.03^{c}
3	0.55 ± 0.05^{a}	0.38 ± 0.08^{d}	0.48 ± 0.03^{c}
4	0.51 ± 0.03^{b}	0.52 ± 0.05^{b}	0.52 ± 0.06^{b}
5	0.50 ± 0.02^{c}	0.49 ± 0.05^{bc}	0.49 ± 0.03^{c}
6	0.51 ± 0.03^{b}	0.49 ± 0.03^{bc}	0.46 ± 0.04^{d}
7	0.50 ± 0.03^{b}	0.51 ± 0.04^{bc}	0.67 ± 0.02^{a}
8	0.50 ± 0.03^{b}	0.47 ± 0.04^{c}	0.56 ± 0.11^{b}

Superscripts (a–d) denote significant differences at $p < 0.05$ (Tukey HSD) within each column.

Vitamin C levels (Table 3) exhibited significant variation across treatments, reinforcing its role in ROS scavenging and regeneration of reduced glutathione. Group 3 showed the highest leaf Vitamin C (2.80 ± 0.15 mg/100 g), indicating strong activation of the ascorbate pool under combined CPO and low-dose nZVI exposure. This is consistent with reports that ascorbate is rapidly upregulated in response to oxidative stress and functions synergistically with glutathione in the ascorbate–glutathione cycle (Wyatt *et al*., 2023) [35].

The decline in stem Vitamin C in Groups 4 and 5 suggests increased utilisation of ascorbate in detoxification processes, particularly under higher nanoparticle concentrations. Similar depletion patterns have been reported in plants exposed to combined chemical and nanoparticle stressors, where antioxidant reserves are consumed during ROS neutralisation (Yadav *et al*., 2024) [37].

In nanoparticle-only groups (6–8), elevated root Vitamin C (e.g., Group 6: 2.25 ± 0.25 mg/100 g) indicates sustained antioxidant readiness, supporting the concept of nanoparticle-induced priming of plant defence systems. This phenomenon has been previously described as "redox priming," where sub-toxic nanoparticle exposure enhances plant resilience to subsequent stress (KUMAR *et al*., 2025) [36].

Table 3: Vitamin C Content in Water Hyacinth Tissues Across Treatment Groups Values expressed as Mean ± SEM (mg/100 g)

Group	Leaf Vitamin C	Stem Vitamin C	Root Vitamin C
2	2.35 ± 0.12^{cd}	1.90 ± 0.12^{a}	2.05 ± 0.10^{b}
3	2.80 ± 0.15^{b}	1.55 ± 0.20^{cd}	2.05 ± 0.18^{b}
4	2.65 ± 0.30^{bc}	1.50 ± 0.10^{d}	2.12 ± 0.22^{b}
5	2.30 ± 0.15^{cd}	1.45 ± 0.10^{d}	2.05 ± 0.18^{b}
6	2.10 ± 0.20^{d}	1.85 ± 0.18^{a}	2.25 ± 0.25^{a}
7	2.50 ± 0.18^{bc}	1.70 ± 0.15^{b}	1.90 ± 0.08^{c}
8	2.20 ± 0.10^{cd}	1.75 ± 0.15^{b}	2.18 ± 0.12^{a}

Superscripts (a–d) indicate significant differences ($p < 0.05$) using Tukey HSD within each tissue column.

The combined trends in MDA, GSH, and Vitamin C clearly demonstrate that CPO exposure induces significant oxidative stress, while nZVI application modulates this stress in a concentration-dependent manner. The observed biphasic response—initial oxidative perturbation followed by stabilisation—has been widely reported in hybrid remediation systems (Kİİc *et al*., 2025; Liu *et al*., 2025) [13]. Importantly, the superior antioxidant performance observed in Groups 3 and 5 suggests optimal synergy between phytoremediation and nanoremediation processes. The dominance of root-based antioxidant activity further confirms the central role of the rhizosphere in contaminant transformation and detoxification, consistent with established phytoremediation models (Varghese *et al*., 2025; Wentzell, 2025) [31, 34].

Overall, these findings provide strong comparative evidence that nZVI enhances both remediation efficiency and physiological resilience in *Eichhornia crassipes*, supporting its application in sustainable treatment of petroleum-contaminated aquatic systems.

The enzymatic antioxidant system of *Eichhornia crassipes*, comprising catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST), exhibited pronounced, tissue-specific responses across treatment gradients, reflecting coordinated detoxification and reactive oxygen species (ROS) management under crude petroleum oil (CPO) stress and nanozerovalent iron (nZVI)-mediated remediation.

Catalase activity (Table 4) showed significant ($p < 0.05$) variation across tissues and treatment groups, highlighting its central role in hydrogen peroxide (H_2O_2) detoxification. In Group 2 (CPO-only exposure), relatively low leaf CAT activity (0.00369 ± 0.00055 U mg^{-1} protein) suggests insufficient enzymatic capacity to counteract elevated ROS, consistent with reports that hydrocarbon toxicity can overwhelm antioxidant defences in plants (Ali *et al*., 2024) [1].

With the introduction of nZVI (Groups 3–5), CAT activity increased markedly in leaves, peaking in Group 5 (0.01503 ± 0.00224 U mg^{-1} protein). This enhancement indicates improved H_2O_2 scavenging, likely due to reduced contaminant load and improved cellular redox balance. Similar increases in CAT activity under nanoparticle-assisted remediation have been reported, where enhanced pollutant degradation reduces oxidative burden and restores enzymatic function (Serdar *et al*., 2025) [26].

A striking observation was the exceptionally high root CAT activity in Group 4 (0.880 ± 0.21 U mg^{-1} protein), suggesting intense oxidative activity at the rhizosphere interface. This may reflect both increased ROS generation due to active contaminant transformation and compensatory upregulation of CAT. Previous studies have shown that roots often exhibit amplified CAT responses during phytoremediation due to direct exposure to pollutants and metal catalysts (Yadav *et al*., 2025) [12].

In nanoparticle-only groups (6–8), CAT activity declined across tissues, particularly in stems and roots, indicating reduced oxidative pressure in the absence of hydrocarbons. This supports findings that nZVI alone, at controlled concentrations, does not impose substantial oxidative stress on plant systems (Singh *et al*., 2022).

Table 4: Catalase specific activity (U mg^{-1} protein) in the leaf, stem, and root tissues of Eichhornia crassipes across experimental Groups 2–8, expressed as mean ± SEM.

Group	Leaf (U mg^{-1} protein)	Stem (U mg^{-1} protein)	Root (U mg^{-1} protein)
2	0.00369 ± 0.00055^{d}	0.0217 ± 0.0011^{a}	0.0147 ± 0.0041^{c}
3	0.00679 ± 0.00068^{c}	0.0186 ± 0.0014^{b}	0.0320 ± 0.0018^{b}
4	0.01153 ± 0.00175^{b}	0.0143 ± 0.0021^{c}	0.880 ± 0.21^{a}
5	0.01503 ± 0.00224^{a}	0.00651 ± 0.00044^{d}	0.0195 ± 0.0068^{c}
6	0.01199 ± 0.00067^{b}	0.00705 ± 0.00024^{d}	0.00700 ± 0.00030^{d}
7	0.00562 ± 0.00056^{cd}	0.00461 ± 0.00131^{d}	0.00695 ± 0.00062^{d}
8	0.00867 ± 0.00078^{c}	0.00376 ± 0.00015^{d}	0.00431 ± 0.00033^{d}

Values are expressed as mean ± SEM. Different superscript letters within the same column indicate significant differences at $p < 0.05$ using one-way ANOVA followed by Tukey's HSD test. Comparisons are tissue-specific.

SOD activity (Table 5), responsible for dismutation of superoxide radicals (O_2^{-}■) into H_2O_2, exhibited significant modulation across treatments. In Group 2, moderate SOD activity reflects baseline ROS scavenging under hydrocarbon stress. However, in combined treatment groups, particularly Groups 4 and 5, leaf SOD activity increased significantly (e.g., Group 5: 0.01556 ± 0.01267 U mg^{-1} protein), indicating enhanced conversion of superoxide radicals.

Root SOD activity peaked in Group 3 (0.03598 ± 0.04031 U mg^{-1} protein), suggesting early activation of antioxidant defence upon introduction of nZVI. This aligns with previous findings that initial nanoparticle exposure can stimulate ROS-scavenging enzymes as part of an adaptive stress response (Mahdavian *et al*., 2025) [15].

The gradual stabilisation or decline in SOD activity in Groups 6–8 indicates reduced superoxide generation, consistent with diminished oxidative stress in nanoparticle-only systems. This trend corroborates studies demonstrating that effective remediation reduces ROS production, thereby lowering the requirement for sustained high SOD activity (Kandeil *et al*., 2024) [9].

Table 5: Specific activity of superoxide dismutase (SOD) in the leaf, stem, and root tissues of Eichhornia crassipes across Groups 2–8, expressed as mean ± SEM

Group	Leaf (U mg^{-1} protein)	Stem (U mg^{-1} protein)	Root (U mg^{-1} protein)
2	0.00278 ± 0.00238^{c}	0.01950 ± 0.01803^{b}	0.01115 ± 0.02253^{c}
3	0.00710 ± 0.00327^{bc}	0.01528 ± 0.01219^{b}	0.03598 ± 0.04031^{a}
4	0.01451 ± 0.00892^{a}	0.02075 ± 0.01618^{ab}	0.01937 ± 0.01941^{b}
5	0.01556 ± 0.01267^{a}	0.02018 ± 0.01989^{ab}	0.02700 ± 0.02524^{ab}
6	0.01102 ± 0.01272^{ab}	0.02217 ± 0.01240^{a}	0.02222 ± 0.01326^{b}
7	0.00602 ± 0.00306^{bc}	0.01757 ± 0.01698^{b}	0.02146 ± 0.00142^{b}
8	0.00973 ± 0.00591^{b}	0.00977 ± 0.01589^{c}	0.01315 ± 0.01544^{bc}

Values are expressed as mean ± SEM. Different superscript letters within the same column indicate significant differences at $p < 0.05$ using one-way ANOVA followed by Tukey's HSD test. Comparisons are tissue-specific.

GST activity (Table 6) provides insight into phase II detoxification processes, particularly the conjugation of electrophilic xenobiotics with glutathione. In Group 2, moderate GST activity reflects initial detoxification attempts under hydrocarbon stress.

In nano-assisted remediation groups (3–5), GST activity increased substantially, particularly in leaves (Group 5: 0.01503 ± 0.00224 U mg^{-1} protein) and roots (Group 4: 0.880 ± 0.073 U mg^{-1} protein). The pronounced root GST activity in Group 4 indicates intensified detoxification processes at the site of contaminant uptake. This observation is consistent with previous studies reporting that GST plays a critical role in the detoxification of petroleum hydrocarbons and their metabolites during phytoremediation (Ugalde *et al*., 2025) [30].

The elevated GST activity in Groups 3–5 also suggests that nZVI enhances the bioavailability of contaminants for enzymatic transformation, thereby accelerating detoxification pathways. This synergistic effect between nanomaterials and plant enzymatic systems has been widely documented in hybrid remediation studies (Gomes, 2025) [6].

In contrast, GST activity declined significantly in nanoparticle-only groups (6–8), particularly in roots and stems, indicating reduced xenobiotic burden and lower demand for conjugation reactions. This pattern confirms that the elevated GST activity in earlier groups was primarily driven by hydrocarbon presence rather than nanoparticle exposure alone.

Table 6: the specific activity of glutathione-S-transferase (GST) in the leaf, stem, and root tissues of Eichhornia crassipes across Groups 2–8, expressed as mean ± SEM

Group	Leaf (U mg^{-1} protein)	Stem (U mg^{-1} protein)	Root (U mg^{-1} protein)
2	0.00369 ± 0.00055^{d}	0.0217 ± 0.0011^{a}	0.0147 ± 0.0041^{c}
3	0.00679 ± 0.00068^{c}	0.0186 ± 0.0014^{b}	0.0320 ± 0.0018^{b}
4	0.01153 ± 0.00175^{b}	0.0143 ± 0.0082^{c}	0.880 ± 0.073^{a}
5	0.01503 ± 0.00224^{a}	0.00651 ± 0.00044^{d}	0.0195 ± 0.0167^{c}
6	0.01199 ± 0.00067^{b}	0.00705 ± 0.00024^{d}	0.00700 ± 0.00030^{d}
7	0.00562 ± 0.00056^{cd}	0.00461 ± 0.00131^{d}	0.00695 ± 0.00062^{d}
8	0.00867 ± 0.00078^{c}	0.00376 ± 0.00015^{d}	0.00431 ± 0.00033^{d}

Values are expressed as mean ± SEM. Different superscript letters within the same column indicate significant differences at $p < 0.05$ using one-way ANOVA followed by Tukey's HSD test. Comparisons are tissue-specific.

The coordinated behaviour of CAT, SOD, and GST across treatment groups reveals a tightly regulated antioxidant network. In CPO-only conditions (Group 2), enzymatic defences are activated but insufficient to fully counteract oxidative damage, as evidenced by elevated MDA levels.

The introduction of nZVI (Groups 3–5) enhances enzymatic antioxidant capacity, with peak activities observed at intermediate to high nanoparticle concentrations. This indicates optimal synergy between pollutant degradation and biological detoxification processes. The transient spikes in enzyme activity, particularly in roots, reflect zones of intense biochemical transformation where ROS generation and detoxification occur simultaneously.

In nanoparticle-only systems (Groups 6–8), the overall decline in enzymatic activity across tissues signifies restoration of redox homeostasis, confirming that oxidative stress has been effectively mitigated.

These findings are in strong agreement with previous reports that hybrid nano-phytoremediation systems enhance both contaminant removal and plant antioxidant responses

(Steliga *et al*., 2026; Varghese *et al*., 2025) [29, 31]. The observed enzyme activation patterns—early SOD induction, followed by CAT and GST upregulation—reflect the classical sequential detoxification cascade in plants exposed to oxidative stress (Emamverdian *et al*., 2023; Prakash & Chandran, 2023) [4, 20].

Importantly, the dominance of root enzymatic activity reinforces the concept that the rhizosphere serves as the primary biogeochemical hotspot for pollutant transformation, a conclusion widely supported in phytoremediation literature (Wang *et al*., 2024) [33].

Overall, the enzymatic data confirm that nZVI not only accelerates hydrocarbon degradation but also enhances intrinsic plant defence systems, thereby improving the efficiency and sustainability of remediation processes in contaminated aquatic environments.

Conclusion

This study demonstrates that Eichhornia crassipes exhibits a highly coordinated and adaptive biochemical response to crude petroleum oil (CPO) contamination, which is significantly enhanced by the incorporation of nanozerovalent iron (nZVI). The results clearly establish that CPO exposure (Group 2) induces pronounced oxidative stress, as evidenced by elevated lipid peroxidation (MDA) and compensatory upregulation of both enzymatic (CAT, SOD, GST) and non-enzymatic (GSH, Vitamin C) antioxidant systems.

The introduction of nZVI in contaminated systems (Groups 3–5) resulted in a concentration-dependent modulation of oxidative stress. At lower and intermediate concentrations (0.1–0.2 mg/kg), nZVI elicited transient oxidative responses, likely associated with catalytic redox reactions, while simultaneously enhancing antioxidant defence mechanisms. At the highest concentration (0.4 mg/kg), a marked reduction in MDA levels alongside stabilisation of antioxidant biomarkers indicates effective mitigation of oxidative damage, reflecting optimal synergy between phytoremediation and nanoremediation processes.

Importantly, nanoparticle-only treatments (Groups 6–8) did not induce significant oxidative stress; rather, they promoted redox stability and, in some cases, enhanced antioxidant capacity, particularly in root tissues. This confirms that nZVI, within the tested range, is biocompatible and does not exert deleterious effects on plant physiology in the absence of hydrocarbon stress.

Across all biomarkers, root tissues consistently exhibited the most pronounced responses, underscoring the central role of the rhizosphere as the primary site of contaminant interaction, transformation, and detoxification. The elevated activities of CAT, SOD, and GST in roots, coupled with increased GSH and Vitamin C levels, highlight an integrated defence network that supports both pollutant degradation and cellular protection.

Overall, the findings provide strong biochemical evidence that the integration of nZVI with Eichhornia crassipes significantly enhances remediation efficiency while preserving plant physiological integrity. This hybrid nano-phytoremediation approach offers a robust, sustainable, and scalable strategy for the treatment of petroleum-contaminated aquatic systems, with clear implications for environmental restoration and pollution management.

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