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Research Article

Naphthalen-1-yl Naphthalene-2-Sulfonate Enhances Arsenic Uptake and Phytoremediation Efficiency in Maize by Regulating PSII Performance and Antioxidant Responses

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ABSTRACT

Arsenic (As) contamination of agricultural soils threatens crop productivity and food security globally. While chelate-assisted phytoextraction enhances metal bioavailability, conventional chelating agents often exacerbate phytotoxicity and are environmentally persistent, which limits their field application. This study evaluated naphthalene-1-yl naphthalene-2-sulfonate (NNS) as a dual-mechanism agent facilitating As accumulation while mitigating toxicity in *Zea mays* seedlings. NNS+As treatment increased As concentration by 69% compared to As alone, while reducing lipid peroxidation and hydrogen peroxide by 37% each and moderating stress-induced proline accumulation by 37%. Antioxidant enzyme profiles were fundamentally altered: superoxide dismutase activity decreased 42% while catalase (86%), ascorbate peroxidase (25%), and guaiacol peroxidase (13%) activities increased significantly, enhancing H_2O_2 detoxification capacity. Non-enzymatic antioxidants increased substantially with NNS+As treatment: epicatechin (90%), chlorogenic acid (42%), syringic acid (42%), resveratrol (39%), and rutin (17%). Photosynthetic parameters showed remarkable recovery net photosynthetic rate, transpiration rate, and stomatal conductance increased 95-96% compared to As alone treatment, while intercellular CO_2 concentration increased 63%. Chlorophyll fluorescence analysis revealed improved photosystem II efficiency, with maximum and effective quantum yields increasing 18% and 15%, respectively, while non-photochemical quenching decreased 7-17%. These findings demonstrate NNS functions as an innovative dual-action phytoremediation enhancer, simultaneously promoting As bioaccumulation while maintaining photosynthetic functionality and cellular redox homeostasis, representing a promising environmentally sustainable alternative for integrated contaminant removal and crop protection strategies.

Keywords: Antioxidants, heavy metal, photosynthesis, maize, ROS.

1. INTRODUCTION

Arsenic (As) is a highly toxic metalloid and Group 1 carcinogen that is widely distributed in groundwater, surface waters, and agricultural soils (Liu et al., 2010). Anthropogenic activities such as the use of As-based pesticides, mining, and industrial waste disposal have intensified its dissemination, increasing its transfer into the food chain and posing serious public health risks (Shankar et al., 2014). In severely contaminated regions, large quantities of As are transported annually to farmlands via irrigation water, undermining sustainable

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crop production (Zhao et al., 2010). Importantly, long-term exposure to As through drinking water and food has been strongly associated with adverse effects on human health, including characteristic skin lesions, pigmentation disorders, and increased risks of cancers such as skin, bladder, and lung cancer, as well as cardiovascular diseases, diabetes, and neurological impairments (Smith et al., 2020; World Health Organization, 2022). Chronic As ingestion has also been linked to neurotoxicity, reproductive complications, and impaired cognitive development, highlighting the extensive burden of As on public health in affected populations (Tchounwou et al., 2012; Naujokas et al., 2013).

Plants absorb As primarily via phosphate transporters (for arsenate) and aquaporin channels (for arsenite), which disrupt cellular metabolism and trigger oxidative stress (Shri et al., 2024). These effects lead to growth retardation, reduced chlorophyll synthesis, impaired photosystems, and accelerated reactive oxygen species (ROS) production, culminating in lipid peroxidation and damage to proteins and nucleic acids (Mousavi et al., 2020). Despite these challenges, phytoremediation has emerged as an environmentally sustainable strategy for As-contaminated soils. High-biomass crops such as *Zea mays* can remove significant amounts of As, however low bioavailability and toxicity limit extraction efficiency.

Phytoremediation, which involves the use of As-tolerant or accumulator plants to extract, stabilize, or immobilize As from contaminated soils, is widely recognized as an environmentally friendly approach for mitigating As pollution (Zhao et al., 2020). Classical As hyperaccumulators such as *Pteris vittata* exhibit exceptionally high As accumulation capacity; however, their relatively low biomass production and limited agronomic adaptability restrict their applicability in large-scale field remediation (Zhao et al., 2020). In contrast, high-biomass field crops have been proposed as practical alternatives for phytoremediation due to their rapid growth, extensive root systems, and broad agro-ecological distribution (Vamerali et al., 2022). Although maize is not classified as an As hyperaccumulator, its high biomass production and wide cultivation potential make it a relevant model crop for evaluating phytoremediation strategies aimed at large-scale, field-applicable scenarios (Vamerali et al., 2022). Nevertheless, the efficiency of As phytoextraction is often constrained by the low bioavailability of As in soils and its phytotoxic effects on plant growth, resulting in residual arsenic remaining in the soil even after crop cultivation (Zhao et al., 2020).

Chelate-assisted approaches can improve metal uptake but face major drawbacks. EDTA, although highly effective in solubilizing metals, persists in the environment and increases groundwater contamination risks via metal–chelate leaching (Lim et al., 2005; Shahid et al., 2014). Biodegradable organic acids such as citric acid are less harmful but exhibit weaker chelation capacity, require high concentrations, and degrade rapidly, limiting sustained remediation effectiveness (Römkens et al., 2002; Shinta et al., 2021). Ameliorative treatments with silicon or selenium can mitigate As toxicity but concurrently diminish its uptake, thereby underscoring the inherent trade-off between plant protection and phytoextraction efficiency (Das et al., 2022). Although numerous protective amendments have been tested, most interventions alleviate arsenic-induced toxicity without enhancing arsenic uptake. For instance, biochar supplementation improved maize tolerance to arsenic toxicity but did not increase metal accumulation (Rahman et al., 2023), and phytohormonal modulation similarly alleviated arsenic stress in rice while limiting arsenic uptake (Shaghaleh et al., 2025). Thus, to date, no published treatment has simultaneously achieved both enhanced As accumulation and comprehensive physiological protection, underscoring the novelty of the dual-function mechanism demonstrated by naphthalen-1-yl naphthalene-2-sulfonate (NNS) in the present study.

NNS was designed as a potential dual-function compound linking two hydrophobic naphthalene rings with a polar sulfonate group to facilitate membrane passage (Figure 1), interact with As ions, and neutralize ROS. Related naphthalene–sulfonate derivatives have previously reduced hydrogen peroxide and TBARS in maize seedlings under drought stress (Yetişsin and Korkmaz, 2023). We hypothesize that NNS can act as a dual-action agent in maize, increasing As phytoextraction efficiency while sustaining photosynthesis and antioxidant defenses. To test this hypothesis, maize seedlings were treated with 100 µM AsV with or without 0.25 mM NNS and evaluated for As accumulation, oxidative damage markers, antioxidant responses, and photosynthetic performance to determine whether NNS can serve as a practical, sustainable aid for phytoremediation of As-contaminated soils.

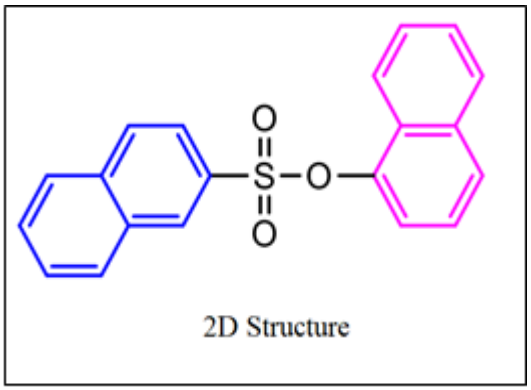

Figure 1. 2D Structure of naphthalen-1-yl naphthalene-2-sulfonate (NNS)

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2. EXPERIMENTAL

2.1 Plant material and growth conditions

Seeds of *Zea mays* L. cv. ADA-523 were obtained from Sakarya Maize Research Institute, Turkey. Seeds were surface-sterilized with 2% sodium hypochlorite solution for 10 min, rinsed thoroughly five times with sterile distilled water, and germinated on autoclaved vermiculite moistened with half-strength Hoagland's nutrient solution. Seedlings were maintained in a controlled-environment growth chamber under the following conditions: temperature 23±2°C, photoperiod 16 h light/8 h dark, photosynthetic photon flux density (PPFD) 350 µmol $m^{-2}s^{-1}$, and relative humidity 60±5%. After seedling emergence, plants were irrigated twice weekly with full-strength Hoagland's solution for 21 d following the standard formulation (Arnon, 1950). This semi-hydroponic cultivation system ensured uniform nutrition across all experimental units while minimizing soil-related variability.

2.2. Experimental design and treatment application

After 21 days of growth, maize shoots were excised 2 cm above the vermiculite substrate, placed in distilled water for 1 h equilibration to minimize handling stress, and subsequently transferred to treatment solutions for 18 h exposure under identical environmental conditions. The NNS concentration (0.25 mM) was selected based on preliminary optimization experiments demonstrating effective oxidative stress reduction without phytotoxicity (Yetişsin and Korkmaz, 2023). The arsenic concentration (100 µM) was chosen to induce significant physiological stress while allowing measurable plant responses (Arikan Abdulveli, 2025).

Four treatment groups were established in a randomized complete block design: (i) Control (distilled water), (ii) NNS alone (0.25 mM), (iii) As alone (100 µM), and (iv) NNS+As (plants pre-treated with 0.25 mM NNS for 1 h, then transferred to combined 0.25 mM NNS+100 µM As solution for 18 h). Each treatment included three biological replicates (independent plant populations) with three technical replicates per biological replicate, yielding nine total measurements per treatment.

At 18 h post-treatment initiation (between 10:00-12:00 h to minimize diurnal variation effects), gas exchange and chlorophyll fluorescence measurements were conducted *in situ* on attached, fully expanded third leaves. Immediately following physiological measurements, plant shoot tissues were harvested, rapidly frozen in liquid nitrogen, and stored at -80°C until biochemical and elemental analyses.

2.3. Determination of as uptake by ICP-MS

Shoot As was quantified by inductively coupled plasma–mass spectrometry (ICP-MS; Agilent 7900, Santa Clara, CA, USA). Full instrument settings are given in Table S1. Approximately 1 g oven-dried tissue was digested (microwave system, Anton Paar) with 65% HNO_3 and 35% H_2O_2, diluted to 50 mL, and analyzed using a concentric nebulizer, quartz torch/injector, and cyclonic spray chamber. Three biological replicates with three technical replicates each were used.

2.4. Oxidative stress marker analyses

Lipid Peroxidation (TBARS Assay): TBARS (MDA equivalents), an indicator of lipid peroxidation, was determined using the thiobarbituric acid reactive substances (TBARS) assay (Heath and Packer, 1968). The values measured in this study reflect the TBARS level equivalent to MDA. Fresh leaf tissue (0.1 g) was homogenized in 2 mL of 0.1% (w/v) trichloroacetic acid (TCA) using a pre-chilled mortar and pestle on ice. The homogenate was centrifuged at 10,000 × g for 10 min at 4°C. An aliquot of the supernatant (1 mL) was mixed with 4 mL of reaction solution containing 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA. The mixture was incubated in a temperature-controlled laboratory oven at 95 °C for 25 min, immediately cooled on ice to terminate the reaction, and centrifuged again at 10,000 × g for 5 min. Absorbance of the resulting supernatant was recorded at 532 nm and 600 nm using a UV–Vis spectrophotometer (Cary 3500, Agilent Technologies, USA).
MDA concentration was calculated using the extinction coefficient ε = 155 mM^{-1} cm^{-1} after correction for non-specific absorbance at 600 nm, and the results were expressed as nmol MDA g^{-1} fresh weight (FW). The analysis was performed using three independent biological replicates, each measured in three technical replicates (n = 3×3).

Hydrogen Peroxide (H_2O_2) Content: Fresh leaf tissue (0.1 g) was homogenized in 2 ml of 0.1% TCA and centrifuged at 12,000 × *g* for 15 min at 4°C. Supernatant (500 µl) was mixed with 500 µl of 10 mM potassium phosphate buffer (pH 7.0) and 750 µl of 1 M potassium iodide (KI). The reaction mixture was incubated for 1 h in darkness at room temperature to allow complete iodide oxidation. Absorbance was measured at 390 nm using a UV-Vis spectrophotometer. H_2O_2 concentration was determined using a standard calibration curve prepared with known concentrations of H_2O_2 (0-100 µM), and results were expressed as ng H_2O_2 g^{-1} FW (Velikova et al. 2000).

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2.4. Determination of proline content

Fresh tissue (0.1 g) was extracted in 40% ethanol overnight at 4°C, supernatant (1 mL) reacted with ninhydrin reagent, incubated at 95°C for 20 min, and read at 520 nm. Proline content was calculate from L-proline standarts (Bates et al., 1973).

2.5. Enzymatic antioxidant activities

Fresh tissue (0.1 g) homogenized on ice in enzyme-specific buffers containing EDTA and PVPP. For SOD, CAT, and GPX: 50 mM potassium phosphate (pH 7.8), 1 mM EDTA, 2% PVPP. For APX: 50 mM sodium phosphate (pH 7), 0.1 mM EDTA, 2% PVPP, 2 mM L-ascorbate. Homogenates were centrifuged at 15,000 rpm for 15 min at 4°C.

SOD Activity (EC 1.15.1.1): Determined according to Beauchamp and Fridovich (1971). Reaction mixtures contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 75 µM NBT, 13 mM methionine, 50 µL enzyme extract, and 2 µM riboflavin. Reactions were initiated by white light exposure (375 µmol $m^{-2}s^{-1}$) for 10 min, with absorbance recorded at 560 nm.

CAT Activity (EC 1.11.1.1): Measured following Bergmeyer and Graßl (1983). Assay mixtures contained 50 mM potassium phosphate buffer (pH 7), 30 mM H_2O_2, and 50 µL enzyme extract. H_2O_2 decomposition was monitored at 240 nm for 3 min using ε = 39.4 $mM^{-1}cm^{-1}$.

APX Activity (EC 1.11.1.11): Assayed according to Nakano and Asada (1987). Reaction mixtures contained 50 mM potassium phosphate buffer (pH 7), 1 mM H_2O_2, 5 mM ascorbate, 1 mM EDTA, and 100 µL enzyme extract. Absorbance decrease was measured at 290 nm using ε = 2.8 $mM^{-1}cm^{-1}$.

GPX Activity (EC 1.11.1.1): Determined using modified Urbanek et al. (1991) method. Reaction mixtures (1 mL) contained 100 mM potassium phosphate buffer (pH 7), 15 mM H_2O_2, 5 mM guaiacol, 0.1 mM EDTA, and 50 µL enzyme extract. Absorbance increases were monitored at 470 nm for 1 min.

2.6. Determination of non-enzymatic antioxidants (HPLC-DAD)

2.6.1. Chromatographic conditions

Phenolic profiles were analyzed on an Agilent 1260 Infinity II HPLC system (ACE 5 C18 column) with two gradient methods for ascorbic acid, epicatechin, rutin, gallic acid, chlorogenic acid, syringic acid, resveratrol, and chrysin (Table 1). Detection wavelengths ranged from 250 to 535 nm. Calibration curves were prepared from 25-300 µg mL^{-1} standards. 20 g of dried plant powder was extracted by ultrasonic-assisted methanol extraction (1:10 w/v, 600 W, 30 min) followed by 24 h shaking at 150 rpm (dark) and filtration (Whatman No. 1 and 0.45 µm PTFE). Extracts were stored at 4°C until analysis (Akbulut et al., 2021).

2.7. Photosynthetic performance

Gas Exchange: Net photosynthetic rate (P_n), transpiration (T_r), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured using LI-6800 Portable Photosynthesis System (LI-COR Biosciences). Measurements were taken on fully expanded third leaves after ≥ 30 min acclimation until CO_2 signals stabilized at 400 µmol mol^{-1}. Conditions were: 250 µmol $m^{-2}s^{-1}$ PPFD, 25°C chamber and leaf temperature, 60% relative humidity.

Chlorophyll Fluorescence: Chlorophyll fluorescence measurements were conducted using a Multi-Mode Chlorophyll Fluorometer (OS5p; Opti-Sciences, Inc., Hudson, NJ, USA). Three fresh maize seedlings per treatment were randomly selected, and fresh leaves were dark-adapted for 20 min using a dark leaf clip to standardize baseline fluorescence. Minimal fluorescence (F_0) was recorded using a low-intensity red light (0.1 µmol $m^{-2}s^{-1}$). Maximum fluorescence (F_m) was subsequently determined using an 8-second saturating pulse at 8000 µmol $m^{-2}s^{-1}$ (Nar et al. 2009). For plants exposed to actinic light (500 µmol $m^{-2}s^{-1}$) (Krause, 1991), maximum fluorescence in the light-adapted state (F_m') and steady-state fluorescence (F_s) were recorded, with F_m' representing fluorescence when all PSII reaction centers are closed.[15] Fluorescence parameters were calculated using standard formulas (Van and Snel, 1990): Maximum quantum yield of PSII (Fv/Fm): $Fv/Fm = (F_m - F_0)/F_m$; Photochemical quenching (qP): $qP = (F_m' - F_s)/(F_m' - F_0')$; Non-photochemical quenching (NPQ): $NPQ = (F_m - F_m')/F_m$ (Van and Snel, 1990); The effective quantum yield of PSII (Φ_{PSII} Yield): $\Phi_{PSII} = (F_m' - F_s)/F_m'$ (Genty et al. 1989).

2.8. Elemental composition analysis (CHNSO)

Powdered samples of dried leaves of maize plants were examined for carbon (C), hydrogen (H) nitrogen (N) and sulphur (S) contents. The organic elemental analysis was conducted using a Thermo Fisher scientific organic elemental analyzer, a Flash Smart CHNS/O. The samples were burned with a furnace temperature of 950°C and an oxygen flow of 240 ml min^{-1} in order to perform the CHNS analysis. This was done for the CHNS analysis. Utilizing gas chromatography, the generated gases (CO_2, H_2O, N_2 and SO_2) were separated at a temperature of 65 °C and a steady flow rate of 250 ml min^{-1} of helium carrier gas (Benabderrahmane et al. 2023).

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2.9. Statistical Analysis

Statistical significance was established at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Data are presented as means ± standard deviations (SD). Differences among treatments were evaluated by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. Figures and tables display treatment means with error bars representing ±SD, and different lowercase letters indicate statistically significant differences among treatments at $p < 0.05$ according to Duncan's multiple range test.

3. RESULTS

3.1. As accumulation

As or NNS treatment alone increased ($p < 0.05$) shoot As content by 90% and %49, respectively, compared with the control, confirming substantial metal translocation under the applied conditions (Figure 2). Pre-treatment with NNS enhanced As accumulation by an additional 69% compared to As alone while maintaining tissue integrity ($p < 0.05$). This indicates that NNS substantially increases As uptake compared with conventional treatment groups.

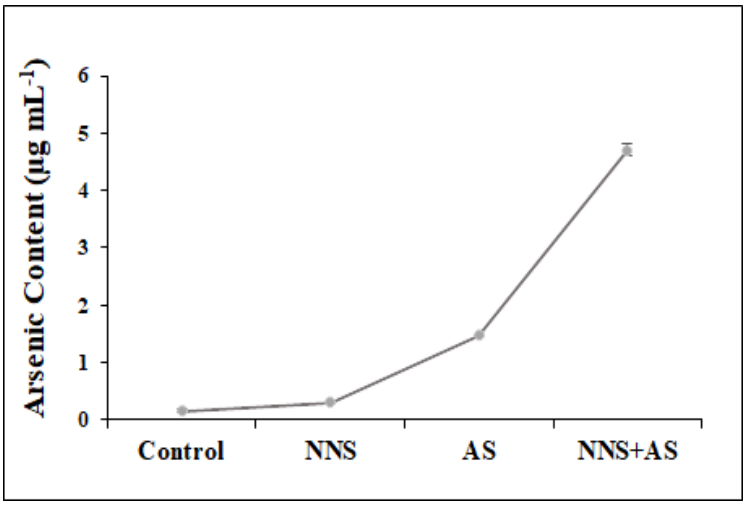

Figure 2. Effect of NNS on arsenic accumulation in maize seedlings under As stress. NNS+As treatment resulted in a 69% increase in As accumulation compared to As treatment alone. Values represent means ± SDs of three biological replicates (n=3). Different lowercase letters indicate significant differences among treatments based on Duncan's multiple range test at $p < 0.05$.

3.2. Oxidative stress parameters

TBARS levels showed no significant difference between control and NNS treatments ($p > 0.05$). As alone increased TBARS by 95% compared to control ($p < 0.05$). The NNS+As combination decreased TBARS by 37% compared with As alone ($p < 0.05$) (Table 1).
H_2O_2 content increased by 19% with NNS alone compared to control. As treatment significantly elevated H_2O_2 by 67% compared to controls. Pre-treatment with NNS lowered H_2O_2 by 37% compared with As alone ($p < 0.05$) (Table 1).

3.3. Proline content

Proline content varied significantly among treatments (Table 1). NNS alone increased proline by 21% compared with the control, As alone increased proline by 69% ($p < 0.05$), and the NNS+As combination further increased proline by 36% compared with As alone ($p < 0.05$).

3.4. Enzymatic antioxidant activities

SOD activity decreased by 3.4% under NNS alone compared with control. As treatment increased SOD activity by 64% ($p < 0.05$). The NNS+As combination reduced SOD activity by 42% compared to As alone ($p < 0.05$), CAT activity increased by 59% under NNS alone and by 91% under As alone compared to controls ($p < 0.05$). The NNS+As combination produced an additional 86% increase compared to As alone ($p < 0.05$) (Table 1).
APX activity increased by 15% under NNS alone and by 84% under As alone compared to control ($p < 0.05$). The NNS+As treatment raised APX by 25% compared to As alone ($p < 0.05$) (Table 1). GPX activity increased by 28% under NNS alone and by 82% As alone compared to controls ($p < 0.05$). NNS+As produced a further 13% increase compared to As alone ($p < 0.05$) (Table 1).

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Table 1. Effects of NNS on contents of thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2), proline and antioxidant enzyme activities in maize seedlings under As stress. NNS+As treatment decreased lipid peroxidation and hydrogen peroxide by 37%, superoxide dismutase (*SOD*) activity by 42%, increased proline, catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) activities by 36%, 86%, 25%, and 13%, respectively, compared to the As-only treatment.

Treatments	Control	NNS	As	NNS+As
TBARS (nmol g^{-1} FW)	1.60 ± 0.11^{c}	1.61 ± 0.23^{c}	3.12 ± 0.04^{a}	2 ± 0.3^{b}
H_2O_2 (nmol g^{-1} FW)	0.391 ± 0.0024^{d}	0.465 ± 0.0013^{b}	0.650 ± 0.0044^{a}	0.412 ± 0.0013^{c}
Proline (µM g^{-1} FW)	0.07 ± 0.016^{d}	0.09 ± 0.004^{c}	0.22 ± 0.01^{b}	0.34 ± 0.001^{a}
SOD (U mg^{-1} protein)	1.33 ± 0.12^{c}	1.29 ± 0.2^{c}	3.7 ± 0.2^{a}	2.15 ± 0.13^{b}
CAT (U mg^{-1} protein)	0.5 ± 0.08^{d}	1.21 ± 0.5^{c}	5.64 ± 0.08^{b}	10.5 ± 1.65^{a}
APX (U mg^{-1} protein)	0.33 ± 0.06^{c}	0.4 ± 0.07^{c}	2.1 ± 0.02^{b}	2.63 ± 0.16^{a}
GPX (U mg^{-1} protein)	2.87 ± 0.2^{d}	3.97 ± 0.6^{c}	15.77 ± 0.15^{b}	17.8 ± 0.7^{a}

Values represent means ± SDs of three biological replicates (n=3). Different lowercase letters indicate significant differences among treatments based on Duncan's multiple range test at $p < 0.05$.

3.5. Non-enzymatic antioxidant contents

AsA was below detection limits in control and As treatments but accumulated markedly under NNS treatment (40.24 µg/g), with NNS+As maintaining comparably elevated levels (36.45 µg/g) (Table 2). Compared with controls, As decreased epicatechin by 85% and chlorogenic acid by 63%, while increasing rutin by 92%. NNS alone increased epicatechin by 70%, rutin by 83%, and syringic acid by 65%, while producing detectable gallic acid levels (6.78 µg/g) (Table 2). NNS+As treatment restored and enhanced phenolic antioxidants compared to As-alone treatments: epicatechin increased by 90%, rutin by 17%, chlorogenic acid by 42%, syringic acid by 42%, resveratrol by 39%, and gallic acid by 38% (Table 2).

Table 2. Effects of NNS on phenolic compounds under As stress. NNS+As treatment increased epicatechin, chlorogenic acid, syringic acid, resveratrol and rutin by 90%, 42%, 39%, 42%, and 17%, respectively, compared to the As-only treatment (µg g^{-1} extract).

No	Compounds (µg g^{-1})	RT [min]	Control	NSS	AS	NSS + AS
			Vitamin C			
1	Ascorbic acid	3.74	N/D	40.24	N/D	36.45
			Phenolics			
2	Epicatechin	8.45	81.83	139.13	12.33	117.52
3	Rutin	11.811	32.20	58.80	61.72	74.52
4	Chrysin	52.939	N/D	N/D	145.02	148.6
			Flavonoids			
5	Gallic acid	4.837	N/D	6.78	N/D	10.92
6	Chloragenic acid	18.184	336.7	266.8	124.7	213.7
7	Syringic acid	23.228	106.5	175.7	112.4	192.1
8	Resvaratrol	37.236	40.5	14.32	36.22	59.1

Values represent means ± SDs of three biological replicates (n=3). Different lowercase letters indicate significant differences among treatments based on Duncan's multiple range test at $p < 0.05$. N/D: Not Detected

3.6. Gas exchange parameters

NNS alone showed no significant differences from controls for P_n, T_r, g_s, and C_i ($p > 0.05$). As treatment severely suppressed gas exchange, reducing P_n, T_r, and g_s by 98% and C_i by 87% compared to the control. The NNS+As combination dramatically increased P_n and g_s increased by 95%, T_r by 96%, and C_i by 63% compared to As alone (Table 3).

3.7. Chlorophyll fluorescence parameters

NNS alone did not differ significantly from controls for *Fv/Fm, ΦPSII, ETR, qN* or *NPQ* ($p > 0.05$). As exposure markedly depressed PSII performance: *Fv/Fm* declined by 16%, *ΦPSII* by 20%, and *ETR* by 18%, while energy-dissipation parameters (*qN* and *NPQ*) increased by 30% and 70%, respectively (Table 3). NNS+As treatment partially restored PSII function: *Fv/Fm* increased by 18%, *ΦPSII* by 15%, and *ETR* by 13% compared with As alone, accompanied by decreases in *qN* (7%) and *NPQ* (17%) (Table 3).

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Table 3. Effects of NNS on gas exchange parameters, and chlorophyll fluorescence in maize seedlings under As stress. NNS+As treatment increased net photosynthetic rate (P_n) increased by 95%, transpiration rate (T_r) by 96%, stomatal conductance (g_s) by 95%, and intercellular CO_2 concentration (C_i) by 63%. Regarding chlorophyll fluorescence, NNS+As increased *Fv/Fm* by 18%, *ΦPSII* by 15% while it reduced *qN* and *NPQ* by 7% and 14%.

Parameters	Control	NSS	As	NSS+As
P_n (µmol m^{-2} s^{-1})	7.73 ± 0.3^{a}	8.16 ± 1.2^{a}	0.16 ± 0.04^{c}	2.8 ± 0.11^{b}
T_r (mol m^{-2} s^{-1})	0.6 ± 0.08^{a}	0.64 ± 0.03^{a}	0.01 ± 0.004^{c}	0.24 ± 0.01^{b}
g_s (mol m^{-2} s^{-1})	0.04 ± 0.006^{a}	0.03 ± 0.01^{a}	0.0006 ± 0.0002^{c}	0.01 ± 0.0005^{b}
C_i (µmol mol^{-1})	83.01 ± 8.04^{a}	88.1 ± 8.2^{a}	10.86 ± 2.5^{c}	29.05 ± 4.1^{b}
Fv/Fm	0.63 ± 0.03^{a}	0.60 ± 0.003^{a}	0.52 ± 0.002^{b}	0.64 ± 0.04^{a}
ΦPSII	0.57 ± 0.04^{a}	0.51 ± 0.007^{c}	0.46 ± 0.04^{d}	0.54 ± 0.004^{b}
ETR	19.7 ± 1.08^{a}	19.1 ± 1.24^{b}	16.2 ± 1.23^{c}	18.3 ± 0.12^{b}
qN	0.46 ± 0.012^{c}	0.45 ± 0.023^{c}	0.6 ± 0.02^{a}	0.56 ± 0.002^{b}
NPQ	0.41 ± 0.02^{c}	0.42 ± 0.01^{c}	0.69 ± 0.04^{a}	0.59 ± 0.04^{b}

Values represent means ± SDs of three biological replicates (n=3). Different lowercase letters indicate significant differences among treatments based on Duncan's multiple range test at $p < 0.05$.

3.8. Elemental composition (CHNS analysis)

Compared to the control, NNS treatment alone did not cause any significant changes in elemental composition ($p > 0.05$). In contrast, As exposure markedly disrupted metabolic balance, leading to a 4.5% decrease in C and a 5.0% decrease in H contents, while S content dropped by approximately 2.8-fold. N content, however, increased by about 1.3-fold under As stress. The combined NNS+As treatment significantly mitigated these alterations compared to As alone, increasing C and H contents by 1.01- and 1.03-fold, respectively, and elevating S levels by nearly 2.9-fold, thereby restoring values close to those of the control (Table 4). The C:N ratio in the NNS+As group also approached the control level, indicating the reestablishment of carbon assimilation and overall metabolic equilibrium. In summary, while NNS alone maintained elemental stability, As exposure caused severe reductions, and co-application of NNS effectively counteracted these imbalances (Table 4).

Table 4. Effects of naphthalen-1-yl naphthalene-2-sulfonate on elemental composition and ratios in maize seedlings under arsenic stress.

Treatments	C (%)	H (%)	N (%)	S (%)	C:N	H:C
Control	41.86 ± 0.3^{b}	5.95 ± 0.02^{b}	1.9 ± 0.2^{c}	0.88 ± 0.02^{a}	22.03 ± 0.2^{a}	0.142 ± 0.02^{b}
NNS	42.88 ± 0.5^{a}	6.1 ± 0.05^{a}	1.88 ± 0.4^{c}	2.49 ± 0.02^{b}	22.8 ± 0.3^{a}	0.142 ± 0.03^{b}
AS	39.97 ± 0.1^{d}	5.65 ± 0.01^{d}	2.47 ± 0.04^{a}	0.85 ± 0.03^{a}	16.2 ± 0.12^{d}	0.141 ± 0.001^{b}
NNS+AS	40.44 ± 0.09^{c}	5.8 ± 0.02^{c}	1.84 ± 0.03^{b}	2.44 ± 0.05^{b}	22 ± 0.35^{a}	0.143 ± 0.001^{a}

Values represent means ± SDs of three biological replicates (n=3). Different lowercase letters indicate significant differences among treatments based on Duncan's multiple range test at $p < 0.05$

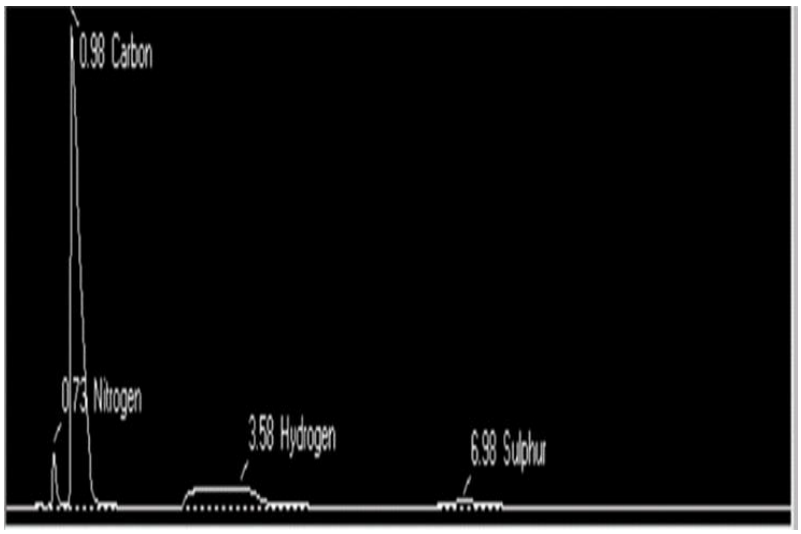

Figure 3. Chromatogram of carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) in the optimum CHNS analyzer condition.

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4.Discussion

4.1. As accumulation

As accumulation reflects metal bioavailability and phytotoxicity and is widely used to assess phytoremediation efficiency (Yan et al., 2020; Tamma et al., 2025). In our study, NNS pre-treatment produced markedly higher As uptake (Figure 2) than As-only plants while avoiding the toxicity typically observed with conventional chelators. EDTA, though effective in mobilizing metals, is environmentally persistent and poses leaching risks (Shahid et al., 2014), while citric acid is rapidly degraded by microbes and therefore limited in sustained remediation (Römkens et al., 2002). Selenium supplementation and similar protective treatments reduce As uptake while improving tolerance, illustrating the classic "protection–versus–extraction" trade-off (Malik et al., 2012). By contrast, NNS simultaneously enhanced As mobilization and maintained physiological functions, demonstrating a dual-function mechanism that resolves this trade-off and positions NNS as a promising tool for sustainable metalloid remediation.

4.2. Oxidative stress indicators (TBARS, H_2O_2)

Heavy metal stress accelerates the generation of ROS, and hydrogen peroxide (H_2O_2) serves as a reliable indicator of this redox imbalance. At low levels H_2O_2 acts in signaling, but excessive accumulation, along with elevated malondialdehyde (MDA), a lipid peroxidation marker (TBARS), indicates membrane damage and cellular injury (Mansoor et al., 2023). In our study, As exposure markedly increased MDA and H_2O_2 levels, confirming severe oxidative stress in maize seedlings (Sattar et al., 2022; Arikan et al., 2022).

Pre-treatment with NNS significantly countered this oxidative load. TBARS and H_2O_2 levels decreased by 37% compared with As-only plants, nearly restoring control values, while NNS alone produced no measurable injury (Table 2). This outcome highlights that NNS is not merely non-toxic but actively protective against As-induced ROS formation and lipid peroxidation.

The dual functionality of NNS contrasts with conventional agents. While citric acid can enhance antioxidant enzyme activities and partially lower H_2O_2 (Tahjib-Ul-Arif et al., 2021) and EDTA may reduce MDA yet sustain elevated ROS or phytotoxicity under heavy metal stress (Kamal et al., 2023), neither achieves simultaneous As mobilization and strong oxidative protection.

These findings position NNS as more than a chelator: it provides a robust antioxidant shield that maintains cellular redox homeostasis and minimizes oxidative damage. By preserving vital physiological processes under As stress, NNS represents an efficient and innovative strategy for managing heavy metal toxicity in crops.

4.3. Proline content

In our study, arsenic stress markedly increased proline content in maize seedlings, consistent with its established role in maintaining osmotic balance, stabilizing cellular macromolecules, and supporting antioxidant activity (Abbas et al., 2018). By contrast, the relatively low proline accumulation observed under NNS treatment highlights its clear distinction from conventional chelators (Table 2). Literature evidence reinforces this contrast: EDTA and citric acid treatments often substantially elevate proline levels under heavy metal stress, with EDTA showing significant effects under copper exposure (Saleem et al., 2020) and citric acid increasing proline by up to 63% under cadmium stress (Tahjib-Ul-Arif et al., 2021). This pattern reflects the high energetic demands associated with conventional defensive responses, and other protective amendments such as humic acid–silicon–biochar blends also trigger large proline increases under metal stress (Adhikari et al., 2023).

NNS, however, shifts plants from a "high stress–high cost" to a "low stress–high efficiency" paradigm. By simultaneously chelating As and attenuating ROS formation, NNS reduces the need for energy-intensive proline synthesis while maintaining osmotic adjustment. This efficient stress accommodation conserves metabolic resources, enabling greater As uptake and translocation without compromising physiological balance.

4.4. Enzymatic antioxidants

Heavy metal exposure strongly activates the plant antioxidant defense network, typically elevating SOD, CAT, APX, and GPX to counter reactive oxygen species (ROS) (Hasanuzzaman et al., 2020; Mansoor et al., 2023). Mechanistically, SOD converts $O_2^{\cdot-}$ to H_2O_2, whereas CAT and APX decompose H_2O_2 into water and oxygen, and GPX further detoxifies peroxides using phenolic substrates (Mittler, 2002; Sharma, 2012). In our study, As stress alone increased SOD by 64% and CAT/APX/GPX by 80–91% (Table 2), consistent with previous reports of As-induced antioxidant activation (Mylona et al., 1998; Finnegan & Chen, 2012).

Pre-treatment with NNS fundamentally reshaped this enzymatic profile. Relative to As alone, SOD decreased by 42% (approaching control values), whereas CAT/APX/GPX rose by 86%, 25%, and 13%, respectively (Table 2). This combination of lower superoxide production demand and stronger peroxidase-mediated H_2O_2 detoxification indicates that NNS

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enhances redox efficiency rather than merely triggering generalized upregulation. Similar patterns of optimized antioxidant enzyme activities have been reported under other protective treatments, such as thiamine combined with indole-3-acetic acid (IAA) or biochar in maize under As stress (Atif et al., 2022; Rahman et al., 2023). However, NNS is distinctive in uniting metal interaction and direct ROS control within a single molecule.

This dual mechanism clearly differentiates NNS from conventional chelators. EDTA, although effective at mobilizing metals, is persistent in the environment and can elevate oxidative burden and phytotoxicity through stable metal–EDTA complexes and leaching risks (Evangelou et al., 2007; Kamal et al., 2023). Citric acid provides weaker and short-lived chelation, with effects that are species- and dose-dependent (Tahjib-Ul-Arif et al., 2021). By contrast, the structural features of NNS, aromatic naphthalene rings together with sulfonate groups, enable dual functionality: sulfonate moieties chelate As ions, lowering intracellular burden and superoxide generation, while aromatic rings scavenge ROS and support peroxidase-mediated H_2O_2 removal. This interpretation is consistent with reports on related naphthalene–sulfonate derivatives reducing H_2O_2 and TBARS in maize under stress (Yetişsin and Korkmaz, 2023) and with broader evidence that reinforcing peroxidase pathways is central to maintaining redox homeostasis under metalloid stress (Mittler, 2002; Hasanuzzaman et al., 2020).

Taken together, these findings demonstrate that NNS does more than mobilize As. It rebalances the antioxidant machinery from a high-cost, generalized upregulation typical of EDTA or weak organic acids to a targeted, metabolically efficient configuration that minimizes superoxide pressure and accelerates H_2O_2 detoxification. This dual action preserves physiological function under As stress and positions NNS as a next-generation aid for phytoremediation that overcomes the usual protection-versus-extraction trade-off.

4.5. Non-enzymatic antioxidants

Non-enzymatic antioxidants such as AsA, flavonoids, and phenolic acids are key components of the plant defense system against ROS under abiotic stress (Gill and Tuteja, 2010; Hasanuzzaman and Fujita, 2013). By donating electrons or hydrogen atoms, these metabolites neutralize radicals and protect cellular membranes and metabolism from oxidative injury (Foyer and Noctor, 2011). In our study, As stress suppressed many of these antioxidant pools (Table 3), indicating disruption of the intrinsic non-enzymatic defense network.

NNS co-treatment markedly restored and even enhanced these compounds. Rutin and gallic acid rose above control levels, epicatechin increased by 89%, and resveratrol, which declined under NNS alone, increased by 39% with NNS+As. Notably, AsA was detected only in NNS-treated plants (40.24 µg/g), highlighting selective activation of the AsA–GSH cycle for H_2O_2 detoxification (Smirnoff, 2000; Akram et al., 2017). The exclusive appearance of gallic acid under NNS further suggests induction of specific metabolic pathways rather than mere preservation of baseline pools (Table 3).

These shifts are consistent with activation of the phenylpropanoid pathway. NNS likely stimulates MYB- and Bhlh-regulated phenolic biosynthesis (Liu et al., 2015; Xu et al., 2015), resulting in increased accumulation of compounds such as rutin, chlorogenic acid, and resveratrol (Vogt, 2010; Fraser and Chapple, 2011). This dual mechanism, As chelation together with elicitation of antioxidant pathways, clearly distinguishes NNS from conventional chelators. Unlike EDTA, which indiscriminately mobilizes metals, or weak organic acids with limited chelating capacity, NNS achieves an optimal balance by binding As while simultaneously triggering beneficial metabolic responses without depleting cellular reserves.

Taken together, these findings show that NNS reinforces both enzymatic and non-enzymatic antioxidant defenses while chelating As. This coordinated action increases total antioxidant capacity, reduces metal toxicity, and explains the superior oxidative stress tolerance observed in maize under As stress. By maintaining cellular redox homeostasis and minimizing oxidative injury, NNS preserves vital physiological functions and represents an efficient, innovative approach to heavy metal toxicity management in crops (Hasanuzzaman et al., 2017; Gill and Tuteja, 2010).

4.6. Photosynthesis, gas exchange parameters

Heavy metal stress strongly restricts photosynthesis by inducing stomatal closure and impairing mesophyll metabolism (Singh et al., 2015). In our study, arsenic drastically reduced P_n, T_r, g_s, and C_i, confirming severe inhibition of CO_2 assimilation. By contrast, NNS combined with arsenic dramatically restored these parameters toward control values, indicating protection of both stomatal function and chloroplast activity (Table 4).

This response can be explained by the dual-function chemistry of NNS. Its sulfonate groups chelate free arsenic ions, reducing their direct toxicity, while its aromatic rings scavenge ROS, thereby preserving guard cell function and mesophyll photosynthetic machinery. Conventional chelators such as EDTA, although enhancing arsenic uptake, frequently aggravate phytotoxic effects and suppress photosynthesis due to persistent metal–EDTA complexes (Evangelou et al., 2007; Kamal et al., 2023). Citric acid can partly restore photosynthetic activity by boosting antioxidant capacity,

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but its weak and short-lived chelation limits effectiveness under high arsenic stress (Chen et al., 2015; Tahjib-Ul-Arif et al., 2021).

By contrast, NNS combines strong metal binding with physiological protection, enabling sustained gas exchange even under severe arsenic stress. These findings are consistent with previous reports that strengthening antioxidant systems helps maintain stomatal aperture and CO_2 assimilation under heavy metal stress (Finnegan and Chen, 2012; Anjum et al., 2016). Maintaining P_n, T_r, g_s, and C_i under stress is particularly critical for phytoremediation, because it sustains transpiration-driven arsenic uptake and continuous biomass production, thereby enhancing phytoextraction efficiency.

4.7. Chlorophyll fluorescence

As stress impaired PSII photochemistry and energy-use efficiency, as commonly reported under heavy metal stress (Gururani et al., 2015; Singh et al., 2015). In our maize leaves, arsenic exposure reduced *Fv/Fm*, *ΦPSII*, and *ETR* while increasing *qN* and *NPQ* (Table 4), confirming that arsenic imposes photochemical limitations and forces excess energy to be dissipated as heat, likely due to oxidative damage to thylakoid membranes (Stoeva and Bineva, 2003; Finnegan and Chen, 2012).

Co-treatment with NNS partially reversed these effects. *Fv/Fm*, *ΦPSII*, and *ETR* increased by 22%, 17%, and 13% compared to arsenic-only plants, while *qN* and *NPQ* declined by 7% and 15%, respectively (Table 4). This improvement suggests that NNS reduces photoinhibition and stabilizes PSII reaction centers. Mechanistically, NNS sulfonate groups likely chelate free arsenic, reducing its inhibition of PSII, while its aromatic rings scavenge ROS, protecting PSII complexes and thylakoid membranes from oxidative damage.

This dual functionality parallels the protective effects reported for polyphenolic compounds and antioxidant inducers under heavy-metal stress (Michalak, 2006; Rudrapal et al., 2022). In summary, NNS mitigates the arsenic-induced decline in chlorophyll fluorescence parameters (*Fv/Fm*, *ΦPSII*, *ETR*, *qN*, *NPQ*) and sustains PSII efficiency through combined chelation and antioxidative mechanisms. This dual role is especially significant for phytoremediation, ensuring enhanced metal uptake without compromising photosynthetic capacity.

4.8. CHNS analysis

As exposure induced oxidative stress and disrupted metabolic homeostasis, which was clearly reflected in the CHNS profiles (Table 4). The decline in C content corresponded with a sharp reduction in photosynthetic parameters (P_n, *ΦPSII*, and *ETR*), indicating suppressed C assimilation and weakened photosynthetic C flow. Similar patterns of elemental imbalance under As stress have been reported in *Pteris cretica* and *Spinacia oleracea* and in other plants under heavy metal toxicity (Mansoor et al., 2023).

The increase in N% paralleled the elevated levels of oxidative stress markers (H_2O_2 and TBARS). This suggests that under As toxicity, N is diverted toward the biosynthesis of amino acids, proline, and other N-rich stress metabolites. Consequently, the reduced C:N ratio observed here represents a biochemical signature of stress adaptation.

S content exhibited a distinctive pattern across treatments. As exposure decreased S content compared to NNS-treated plants, consistent with sulfur's known involvement in thiol-containing antioxidant systems such as glutathione and phytochelatins. In contrast, the co-application of NNS markedly restored S levels close to the control values, indicating the reactivation of S-dependent detoxification pathways. Enhanced S assimilation under NNS+As treatment may therefore reflect the recovery of glutathione metabolism and the reinforcement of antioxidant capacity, a trend also supported by previous findings in metal-stressed plants (Benabderrahmane et al., 2023).

Co-application of NNS effectively mitigated these imbalances. The C:N ratio in the NNS+As treatment approached the control level, implying restoration of C assimilation and metabolic equilibrium. These elemental improvements are consistent with the recovery of gas-exchange parameters (P_n, g_s, T_r, C_i) and chlorophyll-fluorescence indices, reflecting re-established photosynthetic efficiency. The reduction in ROS markers following NNS treatment likely protected chloroplast membranes and maintained enzymatic function, thus facilitating the stabilization of both C and N metabolism.

H content remained nearly constant across treatments, and the H:C ratio exhibited minimal variation, signifying preserved lipid and hydrocarbon structures. This chemical stability aligns with the decline in TBARS values, supporting the conclusion that NNS suppressed lipid peroxidation and maintained membrane integrity. Studies on antioxidant compounds under heavy metal stress have similarly demonstrated that reduced oxidative lipid degradation supports structural stability. In conclusion, the CHNS and sulfur data confirm and strengthen the physiological findings: NNS alleviates arsenic-induced oxidative damage, sustains photosynthetic function, and restores elemental homeostasis. By maintaining balanced carbon, nitrogen, and sulfur levels and preventing hydrogen loss from membrane structures, NNS ensures coordinated redox

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regulation, photosynthetic efficiency, and metabolic resilience under arsenic stress.

5. CONCLUSION

Naphthalen-1-yl naphthalene-2-sulfonate effectively resolved the long-standing trade-off between arsenic accumulation and oxidative protection in maize. It enhanced As uptake by nearly 70% without inducing toxicity, while reducing H_2O_2 and lipid peroxidation by over 40%. NNS restructured the antioxidant system through SOD suppression and selective activation of CAT, APX, and GPX, promoting efficient peroxide detoxification. Simultaneously, it maintained photosynthetic performance, stomatal conductance, and PSII efficiency, sustaining carbon assimilation and C–N balance under As stress. CHNS analysis revealed restored C and H contents and a nearly threefold rise in sulfur, reflecting reactivation of thiol-based antioxidant systems. These findings highlight NNS as a multifunctional chelating antioxidant that integrates metal mobilization with redox stability, offering a mechanistic and practical foundation for next-generation, sustainable phytoremediation strategies.

Authors' Contributions

AG and CA conceived and designed the present study and have supervised this work. AG and CA performed the experiments. The analysis and interpretation of the results were carried out by AG and CA. The drafting of the manuscript was carried out by AG with the assistance of CA. All the authors contributed and reviewed the results and approved the final manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

Authors declare that there is no a conflict of interest with any person.

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