Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 25 | No. 3 | Article ID B4395 | 2026

Original Research Paper

https://doi.org/10.46488/NEPT.2026.v25i03.B4395

Open Access Journal

# Amelioration of Cadmium-Induced Stress in Tomato (*Solanum lycopersicum*) Using Gasotransmitters: A Combined Approach to Enhancing Antioxidant Defense and Growth Resilience

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**Abbreviation: Nat. Env. & Poll. Technol.**
**Website: www.neptjournal.com**

*Received:* 15-09-2025
*Revised:* 25-11-2025
*Accepted:* 29-11-2025

*Key Words:*

Cadmium toxicity
Oxidative stress
Nitric oxide
Hydrogen sulfide
Antioxidant defense
Tomato
Gasotransmitters

*Citation for the Paper:*

Raut, S., Sahoo, P.P., Srivastava, P.K. and Raut, S., 2026. Amelioration of cadmium-induced stress in tomato (*Solanum lycopersicum*) using gasotransmitters: A combined approach to enhancing antioxidant defense and growth resilience. *Nature Environment and Pollution Technology*, 25(3), B4395. https://doi.org/10.46488/NEPT.2026.v25i03. B4395

*Note: From 2025, the journal has adopted the use of Article IDs in citations instead of traditional consecutive page numbers. Each article is now given individual page ranges starting from page 1.*



## ABSTRACT

Cadmium (Cd) toxicity is a major environmental stressor that adversely affects plant growth, photosynthesis, and metabolism, causing oxidative damage and yield loss. This study investigates the role of nitric oxide (NO) and hydrogen sulfide ($H_2S$) in mitigating Cd-induced stress in *Solanum lycopersicum* by analyzing growth parameters, oxidative stress markers, antioxidant enzyme activity, and physiological responses. Tomato seedlings exposed to Cd (20 µM $CdCl_2$) exhibited severe growth inhibition, leaf chlorosis, chlorophyll degradation, and increased oxidative stress. Exogenous application of NO (sodium nitroprusside) and $H_2S$ (sodium hydrosulfide), individually and in combination, significantly alleviated Cd toxicity. The combined NO + $H_2S$ treatment showed the highest increase in shoot and root length (~60% over Cd-stressed plants), improved chlorophyll and carotenoid content (87% restoration to control levels), and reduced oxidative damage, indicated by lower malondialdehyde (MDA) (40%) and $H_2O_2$ (55%) accumulation. Antioxidant enzyme activities (SOD, CAT, APX, POD) were significantly upregulated, enhancing reactive oxygen species (ROS) detoxification. Additionally, proline accumulation (~4-fold increase) and protein content (~30% restoration) were improved, suggesting better osmotic balance and metabolic stability. NO and $H_2S$ mitigate Cd stress by reducing oxidative damage, boosting antioxidant defenses, and enhancing resilience. Their combined action highlights gasotransmitter-based strategies for developing Cd-tolerant crops and promoting sustainable agriculture in metal-contaminated soils.

## 1. INTRODUCTION

Environmental stress is a major constraint on agricultural productivity, significantly affecting plant growth, metabolism and yield. Among various environmental challenges, heavy metal contamination has become a pressing issue due to rapid industrialization, mining, and excessive use of chemical fertilizers (Sodango et al. 2018). Heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb), and arsenic (As) accumulate in agricultural soils, leading to toxic effects on plants and reducing overall food security (Sana et al. 2025). Cadmium, a highly toxic and non-essential metal, is particularly concerning due to its strong bioaccumulation and non-degradable nature. It disrupts critical physiological and biochemical processes in plants, including nutrient uptake, photosynthesis, and oxidative balance, ultimately impairing plant growth and development (Zhang et al. 2024).

Cadmium toxicity induces oxidative stress by triggering the overproduction of reactive oxygen species (ROS), leading to cellular damage through lipid peroxidation, protein oxidation, and DNA fragmentation (Zhang et al. 2020). Additionally, Cd exposure inhibits photosynthesis by degrading chlorophyll, reducing stomatal

conductance, and impairing the photosynthetic electron transport chain (Hasanuzzaman et al. 2019). Studies have also reported Cd-induced mitochondrial dysfunction, which disturbs ATP production and accelerates programmed cell death (Asgher et al. 2017). Prolonged Cd stress manifests as leaf chlorosis, root browning, reduced biomass accumulation, and overall metabolic disruption, severely affecting plant viability and productivity (Hosseini et al. 2025).

To counteract Cd toxicity, plants activate defense mechanisms such as the antioxidant enzyme system and stress-responsive signaling pathways. Recent studies suggest that gasotransmitters, including nitric oxide (NO) and hydrogen sulfide ($H_2S$), play crucial roles in enhancing plant resilience against abiotic stress (Shivaraj et al. 2020). NO is a key signaling molecule involved in various physiological processes, including antioxidant regulation, nutrient homeostasis and stress gene activation (Jiading et al. 2005). Research indicates that NO mitigates Cd-induced oxidative stress by scavenging ROS, enhancing antioxidant enzyme activities, and regulating metal ion transport (Leng et al. 2024). For instance, the exogenous application of sodium nitroprusside (SNP), a NO donor, has been shown to enhance Cd tolerance in plants by reducing lipid peroxidation and maintaining redox homeostasis (Bera et al. 2023).

Similarly, $H_2S$ has emerged as a crucial endogenous gasotransmitter that plays a significant role in plant growth, stomatal regulation, and stress adaptation. Studies indicate that $H_2S$ enhances plant defense against Cd toxicity by modulating antioxidant enzyme activities, reducing ROS accumulation, and improving photosynthetic efficiency (Li et al. 2022). Additionally, $H_2S$ interacts with NO and other signaling molecules, such as hydrogen peroxide ($H_2O_2$), to regulate stress responses and mitigate cellular damage (Zhou et al. 2025). Recent findings suggest that NO and $H_2S$ exhibit a combined role in preventing programmed cell death (PCD) under heavy metal stress, primarily through ROS inhibition and enhanced antioxidant defense (Xu et al. 2024).

Tomato (*Solanum lycopersicum*) serves as an ideal model for studying heavy metal toxicity and plant stress responses due to its high sensitivity to environmental stressors. Additionally, it is an economically significant crop, valued for its nutritional properties, including high levels of vitamins A, C, and E, along with essential minerals such as calcium, phosphorus, and iron (Sumalan et al. 2020). Moreover, tomato is a rich source of lycopene, a potent antioxidant known for its anti-carcinogenic properties and potential role in mitigating oxidative damage (Raja et al. 2023).

Therefore, this study aims to investigate the protective effects of exogenous NO and $H_2S$ application in alleviating Cd-induced stress in tomato plants. The research evaluates the physiological and biochemical impacts of Cd toxicity and explores the combined interactions between NO and $H_2S$ in enhancing plant stress tolerance. Understanding the molecular mechanisms underlying gasotransmitter-mediated stress responses will provide valuable insights into improving crop resilience in Cd-contaminated environments, thereby contributing to sustainable agricultural practices.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material and Growth Conditions

Tomato (*Solanum lycopersicum*) plants were selected for this study due to their high sensitivity to environmental stress and nutritional significance. Seeds were procured from a commercial source. 2% (v/v) sodium hypochlorite (NaOCl) solution was used to surface-sterilize the seeds for five minutes prior to sowing, after which they were thoroughly rinsed with sterile distilled water. The sterilized seeds were then germinated in a growth chamber under controlled conditions: temperature, 25 ± 2°C; relative humidity, 60–70%; photoperiod, 16-h light/8-h dark cycle; light intensity, 250 μmol $m^{-2}.s^{-1}$; growing medium, washed sand in plastic pots; and regular watering at fixed intervals. After two weeks of growth, uniform seedlings were selected for further experimentation.

For all experiments, $n = 3$ refers to three independent biological replicates, with each biological replicate consisting of three plants grown under identical conditions. For biochemical assays, three fully expanded leaves were collected from the second or third node of each plant within each biological replicate. Samples from different biological replicates were processed independently and were not pooled. Each biochemical assay was performed with three technical replicate readings from each extract.

The experiment was conducted in two phases. In Phase I, two-week-old seedlings were subjected to Cd stress for seven days, followed by foliar application of gasotransmitters for an additional seven days. In Phase II, after completion of the treatments, the plants were maintained for 80 additional days to evaluate early biochemical and physiological responses.

### 2.2. Cadmium Stress Exposure

To induce cadmium toxicity, tomato seedlings were exposed to cadmium chloride ($CdCl_2$) at a concentration of 20 μM. The $CdCl_2$ solution was administered through root irrigation, and the seedlings were maintained under these conditions for seven days. Visual stress symptoms, including leaf chlorosis, reduced growth, and root damage, were recorded.

### 2.3. Gasotransmitter Treatments

To assess the ameliorative effects of nitric oxide (NO) and hydrogen sulfide ($H_2S$), two exogenous treatments were applied following seven days of Cd stress. For the NO treatment, sodium nitroprusside (SNP, 100 µM) was used as an NO donor, whereas sodium hydrosulfide (NaHS, 100 µM) was used as an $H_2S$ donor for the $H_2S$ treatment. Moreover, for the combined treatment (NO + $H_2S$), a mixture of SNP (100 µM) and NaHS (100 µM) was applied to evaluate their combined effects. Each treatment was administered via foliar spray once daily for seven days, and the plants were maintained under the same growth conditions.

Plants grown under normal conditions without Cd exposure served as the control group. Plants exposed to $CdCl_2$ without any protective treatment served as the Cd-stressed control group. After seven days of treatment, all plants were transplanted into 3 L plastic pots (diameter: 15 cm, height: 20 cm) filled with a sand:soil:compost mixture (1:1:1). The plants were maintained for 80 days in a controlled greenhouse under the following conditions: temperature, 25 ± 3°C; relative humidity, 60–70%; and natural photoperiod. Growth parameters, including shoot length, root length, fresh biomass, and dry biomass, were recorded at the final harvest (80 days).

### 2.4. Physiological and Biochemical Assessments

To evaluate the effectiveness of NO and $H_2S$ in mitigating cadmium stress, several physiological and biochemical parameters were analyzed.

#### *2.4.1. Morphological and Growth Analysis*

Root length (RL) and shoot length (SL) were measured using a meter scale. The root length-to-shoot length ratio was also calculated. Biomass accumulation (g) patterns were assessed using the formulae modified by Hunt (1982).

Eighty days after the commencement of the experiment, plants were harvested and washed with sterile distilled water. The fresh mass (FM) of shoots and roots was recorded using a digital balance after blotting excess moisture. Dry mass (DM) was determined by drying the samples in a hot-air oven at 80˚C for 48 h, followed by weighing using a digital balance.

#### *2.4.2. Photosynthetic Pigment Analysis*

For pigment extraction, 20 mg of fresh leaf tissue from both treated and control plants was cut into small pieces and homogenized in 5 mL of 80% (v/v) acetone. The homogenate was centrifuged at 12,000 × g for 10 min, after which the pellet was resuspended in 80% acetone until it became colorless. The absorbance of the supernatant was measured using a Shimadzu UV-1780 UV–Vis spectrophotometer at 663.2, 646.5, and 470 nm. The concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids (Car) were determined using the following formulae (1, 2, and 3) described by Lichtenthaler (1987):

$$Chl\ a\ (\mu g.mL^{-1}) = 12.25\ A_{663.2} - 2.79\ A_{646.5} \quad \ldots(1)$$

$$Chl\ b\ (\mu g.mL^{-1}) = 21.50\ A_{646.5} - 5.10\ A_{663.2} \quad \ldots(2)$$

$$Car\ (\mu g.mL^{-1}) = [1000\ A_{470} - 1.82\ (Chla) - 85.02\ (Chlb)]/198 \quad \ldots(3)$$

Pigment concentrations calculated in $\mu g.mL^{-1}$ were converted to $mg.g^{-1}$ using:

Pigment ($mg.g^{-1}$) = [Pigment ($\mu g.mL^{-1}$) × extraction volume (mL)]/ [1000× tissue fresh weight(g)]

### 2.5. Oxidative Stress Markers

#### *2.5.1. Lipid Peroxidation Assay (MDA Content Measurement)*

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) content using the thiobarbituric acid (TBA) reaction method (Heath & Packer, 1968). Fresh leaf tissue (0.5 g) was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 15 min at 4°C. The supernatant was mixed with 0.5% TBA prepared in 20% TCA and incubated for 20 min at 95°C, followed by rapid cooling on ice. Absorbance was measured at 532 nm and corrected at 600 nm. MDA content was expressed as nmol MDA $g^{-1}$ fresh weight (FW), calculated using an extinction coefficient of 155 $mM^{-1}.cm^{-1}$.

#### *2.5.2. Hydrogen Peroxide ($H_2O_2$) Quantification*

Hydrogen peroxide ($H_2O_2$) accumulation was determined following the method of Velikova et al. (2000). Leaf tissue (0.5 g) was homogenized in 5 mL of 0.1% (w/v) TCA and centrifuged at 12,000 × g for 15 min at 4°C. The supernatant was reacted with 1 M KI prepared in a 10 mM potassium phosphate buffer (pH 7.0). Absorbance was measured at 390 nm, and $H_2O_2$ content was quantified using a standard curve and expressed as µmol $H_2O_2$ $g^{-1}$ FW.

### 2.6. Antioxidant Enzyme Activity Assays

All enzyme activities were measured at 25°C using a UV–visible spectrophotometer.

#### *2.6.1. Superoxide Dismutase (SOD) Activity*

SOD activity was determined based on its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) (Beauchamp & Fridovich, 1971). Enzyme extracts were prepared by homogenizing 0.5 g of leaf tissue in 3 mL of ice-cold 50 mM phosphate buffer (pH 7.8) containing 1% PVPP, followed by centrifugation at 12,000 × g for 20 min at 4°C. The

reaction mixture contained 50 mM phosphate buffer, 13 mM methionine, 75 µM NBT, 10 µM EDTA, 2 µM riboflavin, and enzyme extract in a total volume of 1 mL. The reaction was carried out at 25°C, and absorbance was measured at 560 nm. SOD activity was expressed as U $mg^{-1}$ protein.

#### *2.6.2. Catalase (CAT) Activity*

Catalase (CAT) activity was determined by monitoring the decomposition of $H_2O_2$ at 240 nm according to Aebi (1984). The reaction mixture (1 mL) contained 50 mM phosphate buffer (pH 7.0) and 10 mM $H_2O_2$. CAT activity was calculated using an extinction coefficient of 39.4 $M^{-1}$ $cm^{-1}$ and expressed as µmol $H_2O_2$ decomposed $min^{-1}$ $mg^{-1}$ protein.

#### *2.6.3. Ascorbate Peroxidase (APX) Activity*

APX activity was determined by measuring the oxidation of ascorbate at 290 nm following Nakano and Asada (1981). The reaction mixture (1 mL) contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, and 0.1 mM $H_2O_2$. Absorbance was recorded at 290 nm, and APX activity was expressed as µmol ascorbate oxidized $min^{-1}$ $mg^{-1}$ protein, using an extinction coefficient of 2.8 $mM^{-1}$ $cm^{-1}$.

#### *2.6.4. Peroxidase (POD) Activity*

Peroxidase (POD) activity was determined using guaiacol as a substrate following the method of Chance and Maehly (1955). The reaction mixture (1 mL) contained 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, and 10 mM $H_2O_2$. The increase in absorbance at 470 nm was recorded, and POD activity was expressed as $\Delta$A470 $min^{-1}$ $mg^{-1}$ protein using an extinction coefficient of 26.6 $mM^{-1}$ $cm^{-1}$.

### 2.7. Protein Estimation

Total protein content in enzyme extracts was determined using the Bradford method (Bradford, 1976), with bovine serum albumin (BSA) as the standard.

### 2.8. Proline Estimation

Proline content was estimated according to Bates et al. (1973). Leaf tissue (0.5 g) was homogenized in 10 mL of 3% (w/v) sulfosalicylic acid and centrifuged. Then, 2 mL of the supernatant was reacted with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid, followed by incubation at 100°C for 1 h. The reaction was terminated on ice. The chromophore was extracted using 4 mL of toluene, and absorbance was measured at 520 nm using toluene as a blank. Proline concentration was calculated from a standard curve and expressed as µmoL.$g^{-1}$ FW.

### 2.9. Statistical Analysis

All experiments were conducted with at least three independent biological replicates (n = 3). Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test ($p < 0.05$) to determine significant differences among treatments. Results are presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software, and graphs were generated using GraphPad Prism 8. A summary of treatment means and representative statistical indicators is provided in Supplementary Table S1.

## 3. RESULTS

The present study investigates the role of NO and $H_2S$ in mitigating Cd-induced stress in *Solanum lycopersicum* plants. The results are analyzed in terms of growth parameters, oxidative stress markers, antioxidant enzyme activity, and physiological responses to evaluate the protective effects of gasotransmitters against Cd toxicity.

### 3.1. Effect of Cd and Gasotransmitters on Plant Growth and Biomass

#### *3.1.1. Morphological Observations*

Cd exposure caused significant growth inhibition in tomato seedlings (Fig. 1a). Plants exhibited leaf chlorosis, stunted growth, root browning, and reduced shoot elongation after seven days of Cd treatment (Fig. 1b). In contrast, plants treated with NO and $H_2S$ individually, as well as in combination (NO + $H_2S$), showed notable improvements in growth parameters compared to Cd-stressed plants (Fig. 1c–e). NO and $H_2S$ treatments increased shoot length by 11.48 cm and 10.66 cm, respectively. In contrast, the combined NO + $H_2S$ treatment enhanced shoot length by 13.12 cm (Fig. 2a). Similarly, NO and $H_2S$ treatments increased root length by 5.74 cm and 5.33 cm, respectively. In comparison, the combined treatment increased root length by 7.0 cm (Fig. 2b). Overall, the NO + $H_2S$ treatment resulted in approximately 60% improvement in both shoot and root length compared with Cd-stressed plants. Fig.s 2c and 2d show higher fresh and dry biomass, respectively, indicating improved water retention and metabolic activity. Moreover, reduced leaf chlorosis suggests enhanced chlorophyll stability. These findings indicate that exogenous application of NO and $H_2S$ enhances plant tolerance to Cd stress, potentially by improving nutrient uptake and reducing oxidative stress.

### 3.2. Effect of NO and $H_2S$ on Photosynthetic Pigments

#### *3.2.1. Chlorophyll and Carotenoid Content*

Cd-stressed plants exhibited a significant reduction ($p < 0.05$) in total chlorophyll (Chl a and Chl b) and carotenoid content compared with control plants. Cd toxicity is known to disrupt chlorophyll biosynthesis and accelerate pigment degradation, leading to impaired photosynthesis.

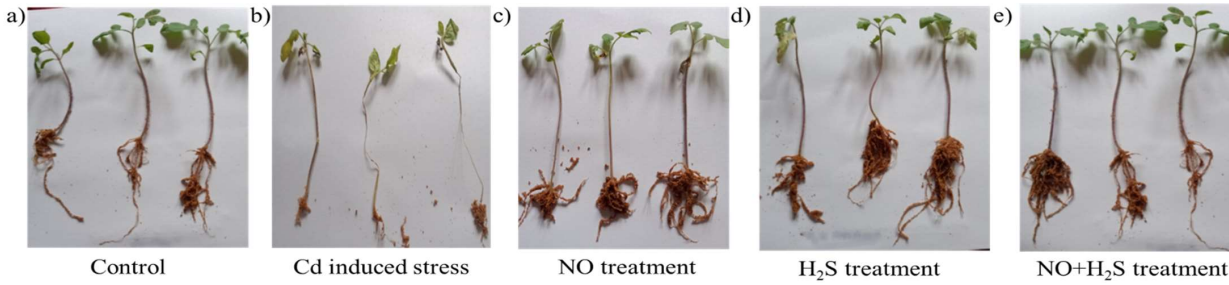


Fig. 1: Morphological parameters (a) Control (b) Cd induced stress (c) NO treatment (d) H2S treatment (e) NO+H2S treatment.

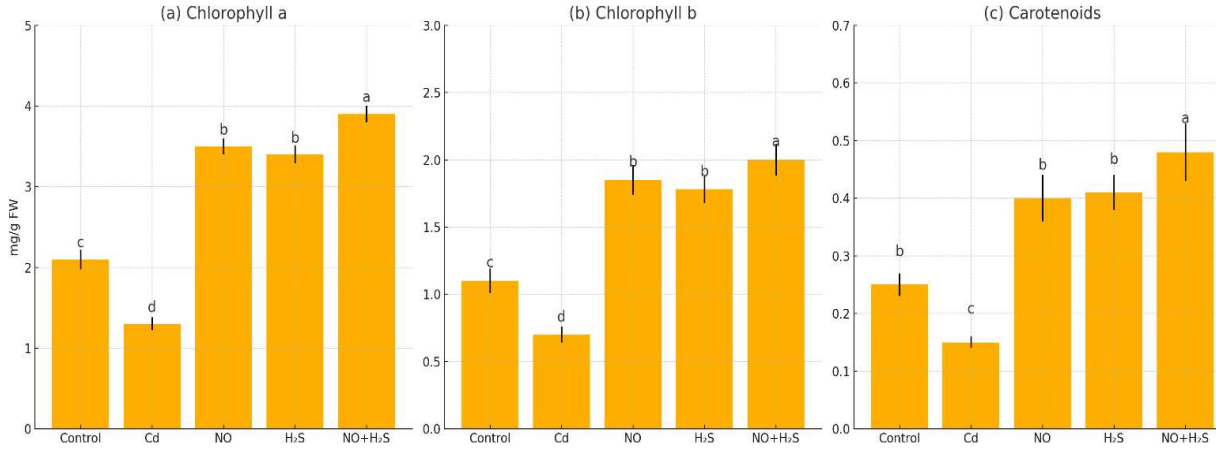


Fig. 2: Effect of NO, H2S and NO+H2S on (a) shoot length (b) root length.

Fig. 3a shows that Cd-stressed plants exhibited 40% decrease in Chl a content (1.26 mg.g$^{-1}$) compared with control plants (2.1 mg.g$^{-1}$). However, NO and $H_2S$ treatments alleviated Chl a loss, with the NO + $H_2S$ combination showing the highest restoration (3.927 mg.g$^{-1}$), corresponding to approximately 87% of control levels. Individual NO and $H_2S$ treatments also enhanced Chl a content to 3.465 mg.g$^{-1}$ and 3.36 mg.g$^{-1}$, respectively (Fig. 3a). Similarly, Fig. 3b showed 34% decrease in Chl b content (0.726 mg.g$^{-1}$) under Cd stress compared with control plants (1.1 mg.g$^{-1}$). NO and $H_2S$ treatments improved Chl b content, with NO + $H_2S$ combination showing the highest restoration (1.98 mg.g$^{-1}$), corresponding to approximately 80% of control levels. Individual NO and $H_2S$ treatments increased Chl b content to 1.82 mg.g$^{-1}$ and 1.76 mg.g$^{-1}$, respectively (Fig. 3b)./ Fig. 3c shows that carotenoid content increased in NO- and $H_2S$-treated plants (0.41 mg.g$^{-1}$ and 0.40 mg.g$^{-1}$, respectively), whereas the NO + $H_2S$ combination showed the highest restoration (0.46 mg.g$^{-1}$), corresponding to approximately 87% of control levels. This suggests a role for carotenoids in ROS detoxification and photoprotection. Overall, these results indicate that NO and $H_2S$ mitigate Cd-induced chlorophyll degradation, likely by protecting chloroplast membranes and enhancing antioxidant defense mechanisms.

### 3.3. Oxidative Stress Markers: Lipid Peroxidation and Hydrogen Peroxide Levels

#### *3.3.1. Lipid Peroxidation (MDA Content)*

Cadmium stress significantly increased malondialdehyde (MDA) content, an indicator of lipid peroxidation and membrane damage. Cd-treated plants exhibited a 2.5-fold increase in MDA content compared with control plants (Fig. 4a). NO and $H_2S$ treatments reduced MDA accumulation by approximately 40%, indicating reduced membrane damage. The NO + $H_2S$ combination showed the strongest protective effect, with MDA levels nearly restored to control conditions.

#### *3.3.2. Hydrogen Peroxide ($H_2O_2$) Accumulation*

Cd stress also led to excessive $H_2O_2$ production, further exacerbating oxidative damage. Fig. 4b shows that $H_2O_2$ levels were significantly higher in Cd-stressed plants, with an approximately three-fold increase compared with the control. Treatment with NO and $H_2S$ significantly reduced $H_2O_2$ accumulation, with the combined NO + $H_2S$ treatment showing the maximum reduction (~55%) (Fig. 4b).

Overall, the reduction in MDA and $H_2O_2$ levels suggests that NO and $H_2S$ enhance the plant's ability to

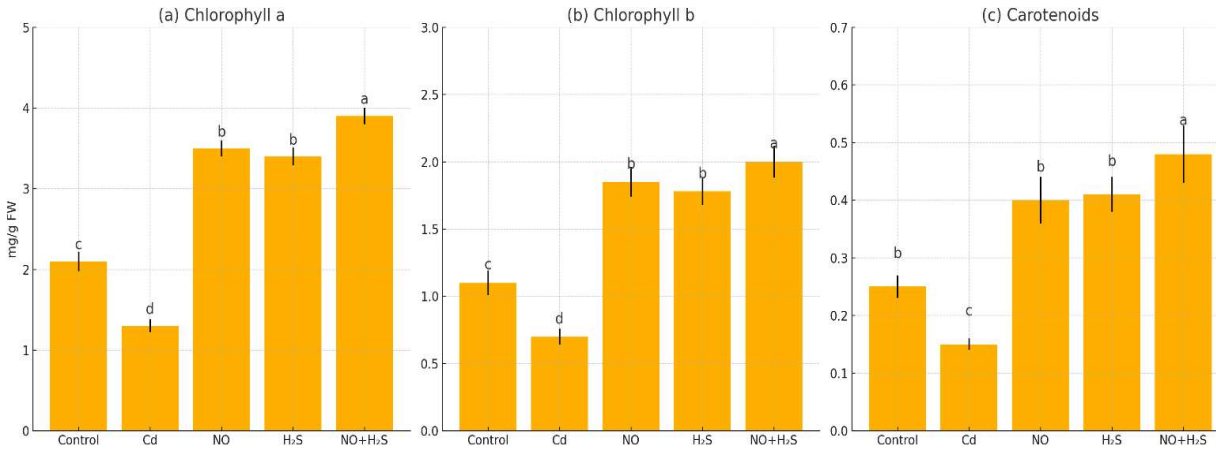


Fig. 3: Effect of NO, $H_2S$ and NO+$H_2S$ on (a) Chl a, (b) Chl b and (c) carotenoid.

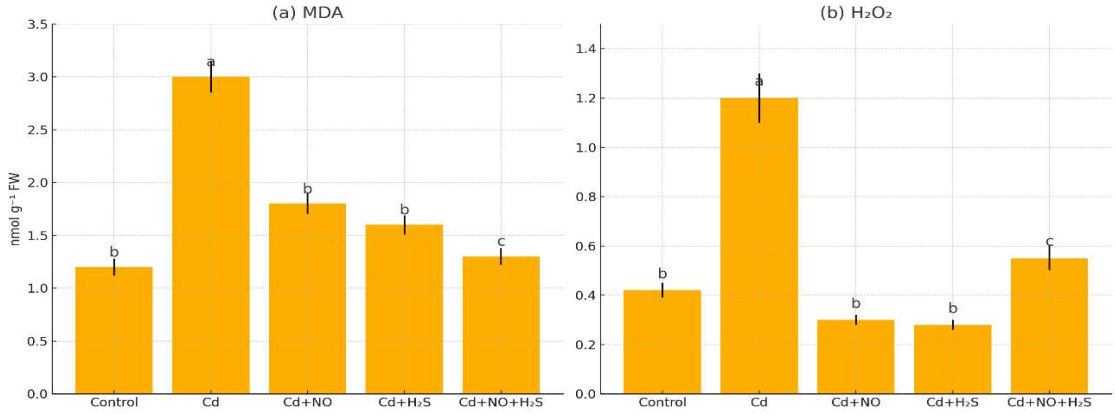


Fig. 4: Effect of NO, $H_2S$ and NO+$H_2S$ on (a) MDA content, (b) $H_2O_2$ content.

detoxify reactive oxygen species (ROS), thereby improving membrane stability and stress tolerance.

### 3.4. Antioxidant Enzyme Activity

Heavy metal stress induces oxidative stress, leading to the activation of the plant's antioxidant defense system. In this study, key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD), were analyzed to evaluate stress responses.

#### *3.4.1. Superoxide Dismutase (SOD) Activity*

SOD represents the first line of defense against oxidative stress by converting superoxide radicals ($O_2^{\bullet-}$) into $H_2O_2$. Fig. 5a shows that Cd stress increased SOD activity (~1.8-fold) compared with the control, indicating an initial stress response. NO and $H_2S$ treatments further enhanced SOD activity, suggesting a strengthened antioxidant response. The NO + $H_2S$ combination resulted in the highest SOD activity (~2.2-fold increase) (Fig. 5a), thereby improving the plant's capacity to neutralize superoxide radicals.

#### *3.4.2. Catalase (CAT) Activity*

Hydrogen peroxide ($H_2O_2$) is detoxified by CAT into water and oxygen, thereby reducing oxidative stress. Fig. 5b shows that Cd-treated plants exhibited a decline in CAT activity, indicating Cd-induced enzymatic inhibition. NO and $H_2S$ treatments restored CAT activity, with the NO + $H_2S$ combination showing a 55% increase compared with Cd-stressed plants (Fig. 5b).

#### *3.4.3. Ascorbate Peroxidase (APX) and Peroxidase (POD) Activities*

APX and POD play crucial roles in scavenging $H_2O_2$ via the ascorbate–glutathione cycle and related detoxification pathways. Cd stress significantly reduced APX and POD activities. In contrast, NO and $H_2S$ treatments enhanced both APX and POD activities, thereby improving ROS detoxification capacity. The NO + $H_2S$ combination showed the highest activation of APX and POD (Figs. 5c and 5d), indicating a synergistic role in stress mitigation.

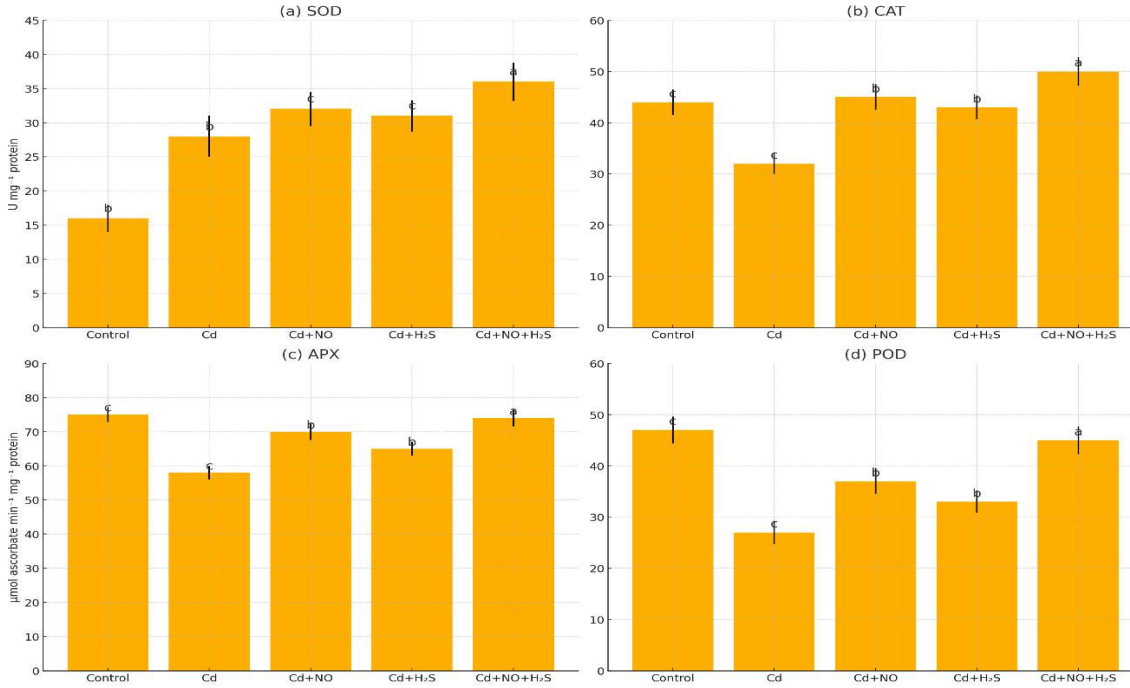


Fig. 5: Effect of NO, $H_2S$ and NO+$H_2S$ on (a) SOD activity, (b) CAT activity, (c) APX activity and (d) POD activity.

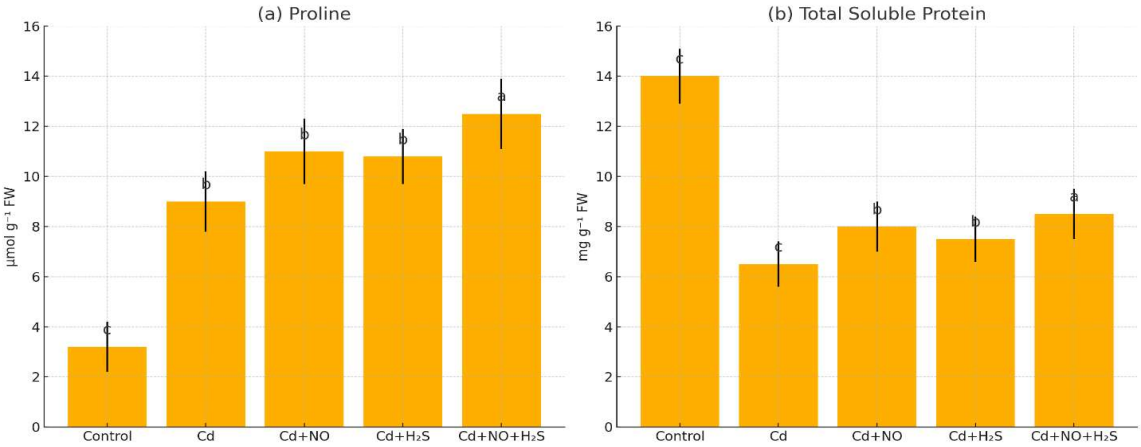


Fig. 6: Effect of NO, $H_2S$ and NO+$H_2S$ on (a) Proline content, (b) Total soluble protein content.

### 3.5. Proline Accumulation and Protein Content

#### *3.5.1. Proline Content*

Proline acts as an osmoprotectant and reactive oxygen species (ROS) scavenger, accumulating under stress conditions. Fig. 6a shows that Cd stress significantly increased proline levels (~3-fold compared with the control), indicating an adaptive stress response. Exogenous application of NO and $H_2S$ further enhanced proline accumulation, with the highest levels observed under the NO + $H_2S$ treatment (~4-fold increase) (Fig. 6a).

#### *3.5.2. Total Soluble Protein Content*

Total soluble protein content serves as an indicator of metabolic activity and stress adaptation. Cd stress led to a decline in protein content, suggesting enhanced protein degradation due to oxidative damage. Fig. 6b shows that NO and $H_2S$ treatments restored protein levels, with NO + $H_2S$-treated plants exhibiting a 30% increase compared with Cd-stressed plants.

## 4. DISCUSSION

The present study demonstrates that exogenous nitric oxide

(NO) and hydrogen sulfide ($H_2S$) effectively mitigate cadmium (Cd)-induced stress in *Solanum lycopersicum* by enhancing plant growth, reducing oxidative damage, and improving physiological responses. The combined NO + $H_2S$ treatment exhibited the strongest protective effects, suggesting a synergistic interaction between these two gasotransmitters in alleviating heavy metal toxicity.

### 4.1. Growth Restoration and Reduced Leaf Chlorosis

Cadmium stress significantly inhibited plant growth, as evidenced by reduced shoot and root length, biomass accumulation, and leaf chlorosis. These findings are consistent with previous reports showing that Cd toxicity suppresses cell elongation, disrupts water balance, and induces nutrient deficiency in plants (Hosseini et al. 2025). However, exogenous NO and $H_2S$ treatments reversed these effects, with the combined NO + $H_2S$ treatment showing the highest increase in shoot and root length (~60% improvement over Cd-stressed plants). This improvement may be attributed to enhanced nutrient uptake, reduced oxidative damage, and regulation of key signaling pathways associated with plant growth (Zhang et al. 2024).

### 4.2. Enhanced Photosynthetic Pigment Content and Chloroplast Protection

Cd stress caused a significant reduction in chlorophyll *a* and *b* content, as well as carotenoid levels. This aligns with previous studies indicating that Cd disrupts chlorophyll biosynthesis, damages chloroplast membranes, and accelerates pigment degradation through excessive reactive oxygen species (ROS) production (Hasanuzzaman et al. 2019). NO and $H_2S$ treatments restored chlorophyll and carotenoid levels, with the NO + $H_2S$ combination achieving near-control levels (~87% restoration). These findings suggest that gasotransmitters play a key role in stabilizing chlorophyll structure and enhancing photosynthetic efficiency, likely by reducing oxidative stress and maintaining chloroplast integrity (Shivaraj et al. 2020). Similar protective effects of NO and $H_2S$ on photosynthetic pigments under heavy metal stress have been reported in wheat (Zhang et al. 2022) and rice (Zhou et al. 2020).

### 4.3. Reduction in Oxidative Stress Markers (MDA and $H_2O_2$ Levels)

Cd exposure significantly increased malondialdehyde (MDA) content and hydrogen peroxide ($H_2O_2$) levels, indicating lipid peroxidation and membrane damage. Cd-induced oxidative stress leads to ROS accumulation, which disrupts cellular structures and triggers programmed cell death (Zhang et al. 2020, Li et al. 2022). NO and $H_2S$ treatments effectively reduced MDA and $H_2O_2$ levels (~40% and ~55% reduction, respectively), with the combined treatment showing the strongest protective effect. These results align with studies suggesting that NO and $H_2S$ function as ROS modulators, enhancing membrane stability and stress tolerance (Zhou et al. 2025). The pronounced effect of the NO + $H_2S$ combination supports the hypothesis that these gasotransmitters act synergistically in regulating ROS detoxification pathways (Hasanuzzaman et al. 2019). Furthermore, these findings are consistent with reports showing that NO and $H_2S$ enhance the expression of stress-responsive genes and activate detoxification enzymes, thereby protecting cellular structures from oxidative damage (Zhang et al. 2022).

### 4.4. Enhanced Antioxidant Defense System

The antioxidant defense system, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD), plays a central role in mitigating oxidative stress in plants. In this study, Cd stress increased SOD activity (~1.8-fold), indicating an initial defense response, but inhibited CAT, APX, and POD activities. This agrees with previous findings showing that Cd disrupts enzymatic function by altering redox homeostasis and interfering with metal cofactors (Jiading et al. 2005).

NO and $H_2S$ treatments further enhanced SOD activity and restored CAT, APX, and POD activities, indicating an improved ROS detoxification system. The NO + $H_2S$ combination resulted in the highest enzymatic activation, reinforcing the hypothesis that gasotransmitters act synergistically to strengthen antioxidant defense mechanisms (Leng et al. 2024, Xu et al. 2024). The ability of NO and $H_2S$ to regulate these enzyme activities highlights their potential role in mitigating heavy metal-induced oxidative damage in plants. Similar responses have been reported in *Oryza sativa* (Zhou et al. 2020) and *Brassica napus* (Zhang et al. 2020), where NO and $H_2S$ enhanced antioxidant capacity under heavy metal stress.

### 4.5. Increased Proline Accumulation and Protein Stability

Proline accumulation acts as an osmoprotectant and ROS scavenger under abiotic stress conditions. Cd exposure significantly increased proline levels (~3-fold), indicating an adaptive stress response. However, NO and $H_2S$ treatments further enhanced proline accumulation, with the highest increase observed under the NO + $H_2S$ treatment (~4-fold), suggesting improved osmotic balance and reduced oxidative stress (Sumalan et al. 2020).

Additionally, Cd stress led to a decline in total protein content, likely due to protein oxidation and degradation

induced by ROS accumulation. NO and $H_2S$ treatments restored protein levels, with the NO + $H_2S$ combination showing a ~30% increase over Cd-stressed plants. This suggests that gasotransmitters promote protein stability and metabolic resilience under heavy metal stress. Similar NO–$H_2S$ cross-talk has been reported in *Arabidopsis* (Zhang et al. 2020) and *Triticum aestivum* (Zhou et al. 2025).

### 4.6. Potential Mechanisms of NO and $H_2S$ in Cd Stress Mitigation

The protective effects of NO and $H_2S$ can be attributed to several potential mechanisms:

a. **ROS Scavenging and Antioxidant Regulation:** NO and $H_2S$ enhance antioxidant enzyme activity, reducing ROS accumulation and oxidative damage (Shivaraj et al. 2020, Zhou et al. 2025).

b. **Chloroplast and Photosynthetic Protection:** NO and $H_2S$ improve chlorophyll stability, prevent pigment degradation, and enhance photosynthetic efficiency (Jiading et al. 2005).

c. **Membrane Stabilization:** Reduced MDA levels suggest that NO and $H_2S$ protect cellular membranes from lipid peroxidation (Kolupaev et al. 2023).

d. **Osmotic Adjustment:** Increased proline accumulation indicates that NO and $H_2S$ help maintain osmotic balance under stress (Sumalan et al. 2020).

e. **Combined Interaction:** The NO + $H_2S$ combination exhibits the strongest effects, suggesting a cooperative role in stress mitigation (Xu et al. 2024)

## 5. CONCLUSIONS

This study provides compelling evidence that NO and $H_2S$ play a crucial role in mitigating Cd-induced stress in tomato plants. The NO + $H_2S$ combination exhibited the strongest protective effects, emphasizing their combined interaction in regulating plant stress responses. These findings contribute to a deeper understanding of gasotransmitter-mediated stress mitigation strategies, which could be applied to develop Cd-tolerant crop varieties for sustainable agriculture.

Future research should focus on investigating gene expression changes related to NO and $H_2S$ signaling pathways under Cd stress. Moreover, assessing the practical use of NO and $H_2S$ in agricultural settings to improve crop resilience. Further, exploring interactions between NO, $H_2S$, and other phytohormones in heavy metal detoxification. By understanding and leveraging the protective roles of NO and $H_2S$, sustainable strategies can be developed to enhance crop productivity in Cd-contaminated environments.

## 6. ACKNOWLEDGMENTS

The authors wish to thank the Management of the Department of Botany, Rammanohar Lohia Avadh University, Ayodhya, K.S Saket Post-Graduate College, Ayodhya, and the Center for Biotechnology, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, for encouragement and motivation to carry out the research work.

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