Global NEST Journal, **28** (3), 08052
Available online: 07/05/2026

Melatonin and Gamma-aminobutyric Acid Mitigates Cadmium Toxicity in Rice by Regulating Antioxidant Activities, Osmolytes Synthesis, and Reducing Oxidative Damages and Cadmium Accumulation

Zhan-Wu Gao[1*†], Hong-zhu Yu[2†], Yong Shi[1], Xin-Yu Hu[1], Yi-Xi Deng[1], Jameel M. Al-Khayri[3], Bader Alsubaie[3], Othman Al-dossary[3], Muneera Q. Al-mssallem[4] and Mustafa I. Almaghasla[5]*

[1]Jilin Provincial Key Laboratory of Western Jilin's Clean Energy, Baicheng Normal University, Baicheng 137000, China
[2]Jilin Academy of Agricultural Sciences (Northeast Agricultural Research Center of China), Changchun 136100, China; y22080@163.com
[3]Department of Agricultural Biotechnology, College of Agriculture and Food Sciences, King Faisal University, Saudi Arabia
[4]Department of Food Science and Nutrition, College of Agriculture and Food Sciences, King Faisal University, Saudi Arabia
[5]Plant Pests and Diseases Unit, College of Agriculture and Food Sciences, King Faisal University, Saudi Arabia

*Correspondence e-mail: gaozw261@nenu.edu.cn and malmghaslah@kfu.edu.sa

Abstract

Soil cadmium (Cd) contamination poses a serious challenge to crops. The exogenic application of growth hormones is an important strategy in addressing the challenge of Cd pollution. The study explores the impact of combined melatonin (MT) and gamma-aminobutyric acid (GABA) in mitigating Cd toxicity with these treatments; control, Cd stress (250 µg kg^{-1}), Cd stress + melatonin (MT: 100 µM), Cd stress + GABA (1 mM) and Cd+ MT + GABA. Cadmium toxicity significantly decreases rice growth and yield productivity by increasing oxidative markers, Cd accumulation, and decreasing photosynthetic pigments, osmolyte productions and nutrients availability. Melatonin and GABA significantly decreased the adversities of Cd and increased rice productivity. Co-applying MT and GABA decreased hydrogen peroxide (H_2O_2), malondialdehyde (MDA), electrolyte leakage (EL) by 34.59%, 44.74% and 105.40%, soil Cd availability, and increased chlorophyll synthesis (36.61- 54.67%) and antioxidant activities (48.38-121.34%), therefore lead to an increase in growth and yield. Further, MT and GABA also reduced Cd accretion in rice roots and shoots and increased the nutrients availability favoring the growth of rice plants in Cd stress conditions. These results underpin the potential of combined MT + GABA application in improving crop productivity and remediating Cd polluted soils.

Keywords: antioxidants, cadmium, GABA, hydrogen peroxide, yield.

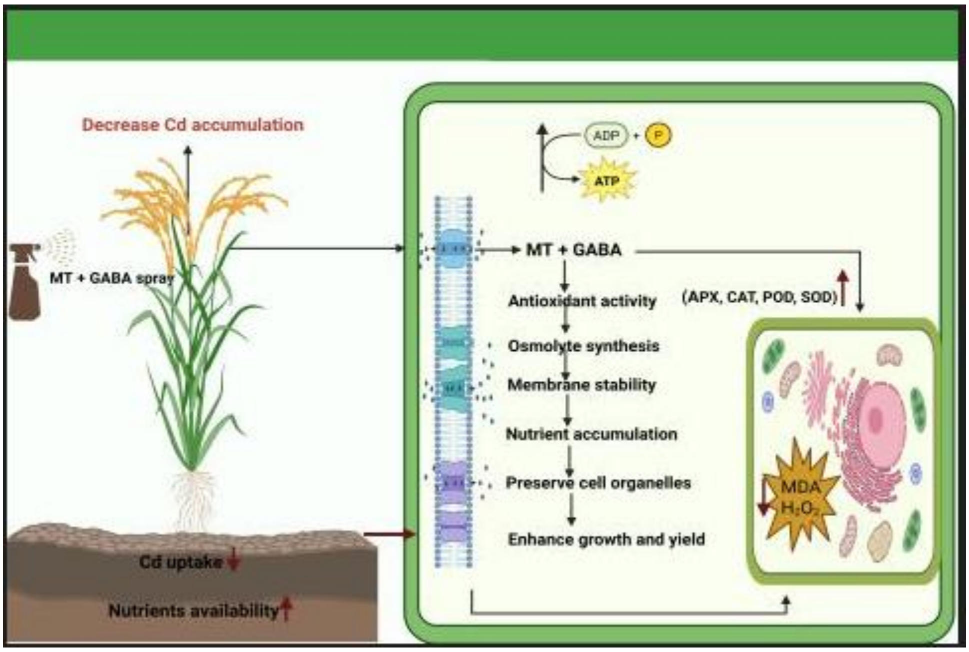
OPEN ACCESS

Received: 28/09/2025,

Accepted: 26/04/2026,

Available online: 07/05/2026

Graphical abstract



1. Introduction

Soil heavy metal (HM) pollution and air pollution are serious challenges to environmental quality and ecosystem sustainability (Mitra et al. 2022: Mohandas *et al.,* 2025a,b,c). Heavy metals are continuously soaring up in soils and water, thus it is essential to address this problem for safer food production (Rahimzadeh *et al.* 2017; Sivasubramanian et al., 2025). Among different HM, cadmium (Cd) is a seriously toxic metal posing serious threat to crop production, human health, and ecosystem sustainability (Peng and Shahidi 2021). It is a non-essential metal; however, its concentration is rapidly soaring due to mining, smelting, synthetic fertilizers, traffic, waste incinerators, and industrial effluents (Genchi *et al,* 2020; Wang and Yang 2021). Apart from these anthropogenic sources, Cd also enters the environment through weathering, forest fires, wind dust, and eruptions from volcanoes (Liu *et al.* 2013). The foods grown on Cd-contaminated soils lead to its entry into humans, which in turn causes kidney and liver damage, emphysema, and heart diseases (Li *et al.* 2022).

Cadmium is highly toxic to plants; however, plants quickly absorb Cd through roots, which negatively affects growth, cell division, chlorophyll synthesis, photosynthetic efficiency, and assimilate production (Moravčíková and Žiarovská 2023). The excessive concentration of Cd also causes oxidative damage and disturbs membranes, DNA, electron transport, and photosynthetic apparatus (Marron 2015). Cadmium toxicity induce stomatal closing, inhibits water uptake and carbon fixation (Huybrechts *et al.* 2020) thereby causes significant growth losses (Huybrechts *et al.* 2020). Additionally, Cd toxicity also disturbs nutrient uptake, decreases water uptake, damages the photosynthetic apparatus, and reduces antioxidant activities (Hassan *et al.* 2024). Cadmium pollution is a serious challenge to achieve agricultural goals, (Chen *et al.* 2018), therefore, it is mandatory to manage the Cd-polluted soils for sustainable and safer crop productivity.

Different practices such as micro-nutrients, hormones, biochar, organic amendments, and controlled irrigation are using in mitigating Cd toxicity (Wang *et al.* 2022). Recently, role of plant hormones is well acknowledged in mitigating toxic impacts of abiotic stress. Melatonin (MT) is an imperative hormone with tremendous potential in mitigating toxicity of heavy metals (El-Yazied *et al.* 2022) and improving plant performance (Moustaka *et al.* 2024). Melatonin-mediated improvement in growth under HM pollution is linked with improved osmolyte synthesis, leaf photosynthesis, and antioxidants activities (Malik *et al.* 2022). In the tomato crop, exogenously applied MT increased Cd toxicity via boosting the antioxidant activity and synthesis of phytochelatin (PC) (Hasan *et al.* 2015). Melatonin increases chlorophyll synthesis, water uptake, osmolyte synthesis, and decreases the oxidative damage and Cd accumulation (Cai *et al.* 2017).

Gamma-aminobutyric acid (GABA) also showed appreciable results to counteract abiotic stresses. It improves stomatal opening, regulates the osmotic pressure (Xu *et al.* 2021), improves carbon assimilation, and antioxidant activity, thus regulating plant growth in stress conditions. Under Cd stress, GABA uptake reduces Cd uptake and improves plant performance by increasing endogenous MT synthesis (Li *et al.* 2022). The role of single MT and GABA application in mitigating the toxic impacts of HMs. However, the interactive effect of MT and GABA in mitigating the adverse impacts of Cd is poorly understood. Therefore, we hypothesized that the interactive effects of MT and GABA can substantially reduce the toxic impacts of

Cd on rice as compared to their application alone. Therefore, this study aimed to explore the interactive impacts of GABA on growth, yield, plant functioning, Cd uptake, accumulation, and soil properties.

2. Materials and methods

2.1. *Growth conditions and plant materials*

The study was performed at Baicheng Normal University and soil was taken from experimental soil (0-20 cm) and sieved to fill the pots with 10 kg capacity after removing debris. The soil was silt-loam with acid pH (5.97), total nitrogen (TN: 1.66 g kg^{-1}), and available phosphorus (AP) and available potassium (AK) concentrations of 38.42 and 117.19 mg kg^{-1}. The pots were filled, and $CdCl_2$ was used to induce Cd toxicity. The soil was equilibrated for two months, and during this period, a field capacity level of 70% was maintained.

2.2. *Experimental treatments*

The current study was designed in a complete randomized design comprising of three replications along with five treatments: control, Cd stress (250 µg kg^{-1}), Cd stress + melatonin (MT: 100 µM), Cd stress + GABA (1 mM), and Cd+ MT + GABA. The pots were carefully handled, and recommended practices were followed to ensure good stand establishment. Melatonin and GABA were applied as a foliar spray at the tillering stage. The application of these rates of MT and GABA proved beneficial in mitigating cadmium stress in rice (Nayyar *et al.* 2014; Ashraf *et al.* 2022; Jiang *et al.* 2022). The certified references materials, along with sterilized instruments and analytical grade chemicals, were used to get reliable results. All biochemical assay were performed in three replicates with established protocols to get reliable results.

2.3. *Measurement of growth and photosynthetic traits*

Fresh rice leaves were collected, and chlorophyll (Chl) and carotenoid (Cart.) concentrations were determined using the methods of Arnon (1949). The freshly collected leaves (0.5 g) were homogenized in 80% methanol and extract was collected, and absorbance was recorded at 645, 480, and 663 for determining Chl-a and Chl-b and Cart concentrations. The fresh leaves were randomly collected and weighed (FW), and then they were soaked in water and after 24 hours they were weighed (TW). Later, these leaves were removed from water and oven dried (65°C) until constant weight (DW). Finally, leaf relative water contents (RWC) were calculated with following formula: RWC (%) = FW − DR/TW − DR × 100.

2.4. *Measurement of biochemical attributes*

Fresh rice leaves (0.5 g) were collected and dipped in water for 30 minutes, and the electrical conductivity (EC_1) was measured. Thereafter, the same leaves were taken and boiled in water (90 °C) for 1 hour. After that, the leaves were removed from the water, and the second EC (EC_2) was measured. Finally, EL was calculated using the following formula: EL = $EC_1/EC_2 \times 100$. The freshly collected leaves (0.5 g) were homogenized using the potassium phosphate buffer (PPB) and centrifuged (14000 rpm) for 15 minutes. The obtained extract was treated with Bradford reagent (2 mL), and absorbance was measured at 595 nm (Bradford, 1976). For free amino acids (FAA), leaves were ground using PPB, and then the extract was mixed with ninhydrin (1 mL) and pyridine (1 mL). Later, the mixture was boiled for 30 min, and the absorbance was read at 570 nm (Hamilton and Van-Slyke 1943). To quantify hydrogen peroxide (H_2O_2), we homogenized 0.5 g of fresh leaf tissue in 5 mL of trichloroacetic acid (TCA). The resulting homogenate was centrifuged, and we then mixed 1 mL of the supernatant with a reaction solution containing potassium phosphate buffer (PBB) and 1 M potassium iodide (KI). The absorbance of this mixture was measured at 390 nm. For malondialdehyde (MDA), leaves were ground in trichloroacetic acid (TCA) solution and centrifuged to obtain the extract, and then 5 mL of thiobarbituric acid (TBA) was added and boiled (100 °C) for 30 minutes, and later the absorbance (532 nm) was taken. For ascorbate peroxidase (APX) activity leaves (0.5 g) were homogenized in PPB buffer and centrifuged (10000 rpm) for 15 min, and absorbance noted at 290 nm (Nakano and Asada, 1987). For the catalase (CAT) activity, 0.5 g freshly collected leaves were blended by using PPB and centrifuged (10000 rpm), and absorbance was noted at 240 nm (Aebi 1984). For determining superoxide dismutase (SOD) activity, we prepared the mixture containing 400 µL H_2O_2, 25 mL buffer, 100 µL Triton, 50 µL extract was made and reading done at 560 nm (Mukherjee and Choudhuri 1983). In case of peroxidase (POD), freshly collected leaves were homogenized in PPB, and later, the absorbance was noted at 470 nm, and POD activity was measured by the methods of Zhang (1992). For ascorbic acid (AsA), 0.5 g of leaves was homogenized in TCA solution (5 mL) and centrifuged (10000 rpm) for 15 minutes, and later AsA contents were determined with the methods of Zhang (1992).

2.5. *Measurement of growth traits, Cd concentration in plant tissues and soil properties*

The height of each plant was measured, and tillers were counted and average was done. Five panicles from each replication were selected to determine their length and grains per panicle. The plants were hand harvested for assessing the grain and biomass yield. The rice samples were collected, dried (65°C), and powdered to determine Cd concentration. The samples were digested (180°C) by using two acids (HCl and $HClO_4$, 4:1), then this mixture was filtered and diluted to 50 mL, and Cd in the samples was measured by atomic absorption spectrophotometry. The soil samples were collected, and pH meter was used for measuring pH in a soil and water solution (3:1). Nitrogen in soil samples was assessed with Kjeldahl apparatus, while AP and AK were assessed with the Olsen and ammonium acetate extraction flame photometry method. The translocation factors and biological accumulation coefficient were calculated with the procedures of Malik *et al.* (2010). The soil samples were digested in HNO_3:$HClO_4$ (4:1). Thereafte,r they were fileted and diluted with water and Cd concentration was estimated using atomic absorption spectrometry.

2.6. *Data Analysis*

A one-way ANOVA was conducted to assess significance among treatments, and Tukey's honestly significant difference (HSD) was employed for separating means. Moreover, Sigma-plot 10 was used for making figures, and PCA and correlation matrix were prepared by R-studio.

3. Results

3.1. *Effect of MT and GABA on photosynthetic pigments*

The chl-a, chl-b, and carotenoid were decreased by 56.69%, 70% and 42.14% respectively, under Cd stress (**Table 1**). Melatonin and GABA mitigated this reduction and resulted in a remarkable increase in photosynthetic traits. The combined MT and GABA remained the top-performing and resulted in an increase of 36.61%, 54.67, and 32.20% in chl-a, chl-b, and Cart synthesis in Cd-polluted soil (**Table 1**).

3.2. *Effect of MT and GABA on oxidative markers, osmolytes and antioxidants activities*

Cadmium toxicity caused a marked decrease in RWC of rice plants. Cd toxicity reduced leaf RWC by 43.25% respectively (**Table 1**) while MT and GABA considerably increased leaf RWC (**Table 1**). The production of all the oxidative markers substantially increased in Cd stress. The results depicted that Cd toxicity increases EL, MDA, and H_2O_2 synthesis by 299.29%, 298.45% and 323.19% as the control (**Table 1**). Co-applying MT and GABA causes a marked decrease in EL, MDA, and H_2O_2 production (**Table 1**). Cadmium toxicity increased the proline synthesis, while it decreased the synthesis of total soluble protein (TSP) and FAA (**Figure 1**). Both MT and GABA increased the synthesis of all the osmolytes under Cd stress conditions. For instance, co-applying MT and GABA enhanced the proline, TSP, and FAA by 45.67%, 75.68% and 102.73% respectively, under Cd stress conditions (**Figure 1**). Melatonin and GABA boosted the antioxidant activity. Co-applying MT and GABA enhanced APX, CAT, POD, SOD, and AsA activities by 62.46%, 89.40%, 48.38%, 66.19% and 121.34% respectively than control (**Figure 1**).

3.3. *Effect of MT and GABA on growth and yield traits*

Cadmium toxicity decreased the RL, their fresh and dry matter production by 49.49%, 79.84% and 84.65% respectively (**Table 2**). Interestingly, exogenous treatments with MT and GABA significantly increased root length (RL) and their fresh and dry matter production by 38.93%, 53.91% and 68.58% respectively in Cd-polluted soil (**Table 2**). Cadmium stress considerably decreased yield and yield traits.

The exogenous applied MT and GABA significantly increased the plant height (PH), tillers per plant (TPP), hundred kernel weight (HKW), grain yield (GY), biological yield (BY), and harvest index (HI) of rice plants 22.47%, 16.08%, 58.30%, 62.28%, 30.28% and 24.31% respectively (**Table 2**).

3.4. *Effect of MT and GABA on root and shoot Cd concentration on TF, BAC and BAF*

The results showed that Cd stress results in a significant increase in root and shoot Cd concentration (**Figure 2**). MT and GABA application significantly diminished Cd accretion in the plant, and a high reduction was noted with co-applying MT and GABA (**Figure 2**). Furthermore, these findings indicated that translocation factor (TF), bio-accumulation concentration (BAC), and bio-concentration factor (BCF) of Cd were significantly diminished with the supplementation of MT and GABA. The highest reduction in TF, BAC, and BCF was obtained after co-applying MT and GABA than their single application (**Figure 2**).

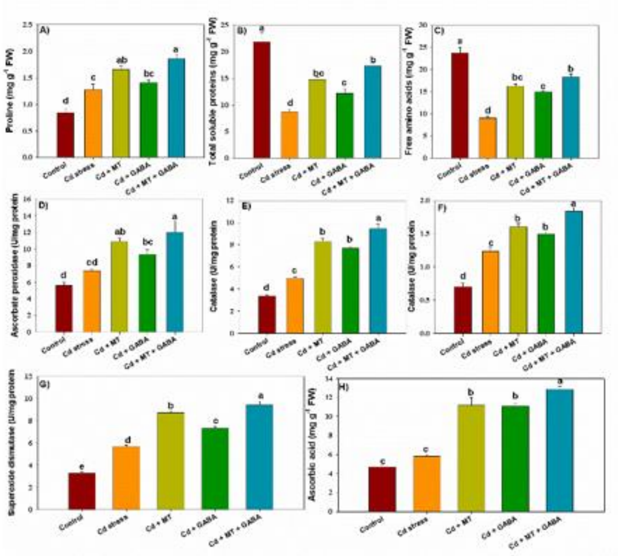


Figure 1. Impact of exogenously applied MT and GABA on the osmolyte synthesis and antioxidant activities of rice planted in Cd polluted soil. The presented data is mean of three replications with ± SD and different letters depicts the significance at *P ≤ 0.05*.

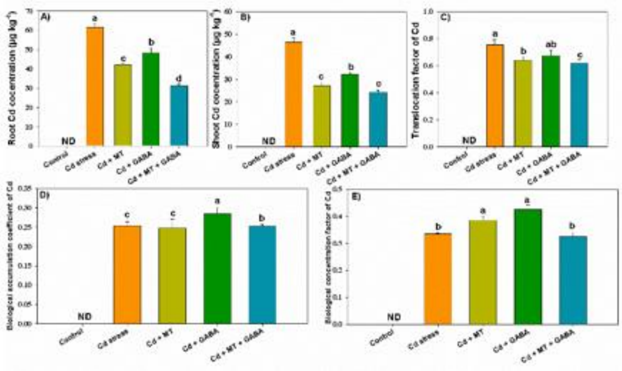


Figure 2. Impact of exogenously applied MT and GABA on root and shoot Cd concentration, TF, BAC and BCF of rice planted in Cd polluted soil. The presented data is mean of three replication with ± SD and different letters depicts the significance at *P ≤ 0.05*.

3.5. *Effects of MT and GABA on soil properties*

The results depicted that MT and GABA supplementation significantly decreased the soil Cd concentration after harvesting rice plants (**Figure 3**). Co-applying MT+GABA decreased soil Cd by 90.61%, while MT and GABA decreased soil Cd concentrations by 66.65% and 61.83% respectively (**Figure 3**). Further, the results also indicated that Cd stress and exogenously applied GABA and MT had non-significant impacts on soil pH (**Figure 3**). Cd reduced AP, AK, and TN concentration; conversely, MT and GABA caused a marked increase in the availability of AP, AK, and TN (**Figure 3**).

Table 1: Impact of exogenously applied MT and GABA on the photosynthetic pigments, oxidative markers and osmolyte synthesis rice planted in Cd polluted soil

Treatments	Chlorophyll-a (mg g-1 FW)	Chlorophyll-b (mg g-1 FW)	Carotenoid (mg g-1 FW)	Relative water contents (%)	Electrolyte leakage (%)	Malondialdehyde (µ mol g-1 FW)	Hydrogen peroxide (µ mol g-1 FW)
Control	4.45a±0.135	2.55a±0.091	7.15a±0.026	92.93a±1.79	17.13d±1.11	2.59d±0.13	1.94e±0.11
Cd stress	2.84d±0.059	1.50c±0.041	5.03c±0.102	64.87d±1.93	68.40a±1.47	10.32a±0.33	8.21a±0.18
Cd + MT	3.52c±0.029	2.06b±0.048	6.05b±0.057	80.20b±1.63	35.79c±2.49	8.10b±80.09	7.06c±0.04
Cd + GABA	3.28c±0.042	1.87b±0.075	5.83b±0.061	73.00c±1.88	42.10b±1.74	8.37b±0.181	7.58b±0.24
Cd + MT + GABA	3.88b±0.073	2.32a±0.082	6.65a±0.0239	85.33b±2.41	33.30c±0.82	7.13c±0.074	6.10d±0.09

The presented data is mean of three replication with ± SD and different letters depicts the significance among means at P ≤ 0.05. Cd: cadmium, MT: melatonin, GABA: γ-aminobutyric acid.

Table 2: Impact of exogenously applied MT and GABA on the agronomic traits of rice planted in Cd polluted soil

Treatments	RL (cm)	RFW (g)	RDW (g)	PH (cm)	TPP	HKW	GY/pot (g)	BY/yield (g)	HI (%)
Control	53.37a±2.33	14.01a±0.42	7.70a±0.99	121a±2.94	11.00a±0.82	5.11a±0.10	62.97a±1.84	212.48a±7.20	30.85a±1.13
Cd stress	35.70c±2.07	7.79d±0.56	4.17c±0.06	89c±3.22	8.33b±0.47	3.07c±0.13	36.80c±3.21	148.39d±3.33	24.84b±2.39
Cd + MT	46.08b±1.55	10.29bc±0.68	6.64ab±0.10	103b±2.16	10.00ab±1.41	4.40b±0.07	56.35ab±0.72	188.00bc±2.45	29.98a±.066
Cd + GABA	44.12b±2.02	9.22cd±0.54	6.09b±0.09	102b±2.06	9.00ab±0.44	4.16b±0.04	50.33b±0.78	172.71c±4.97	29.19ab±1.2
Cd + MT + GABA	49.60ab±2.57	11.99b±0.64	7.03ab±0.15	109b±2.63	9.67ab±0.47	4.89a±0.06	59.72a±2.51	193.33b±4.19	30.88a±0.76

RL: root length, RFW and RDW indicates fresh and dry weights of roots, PH and TPP are plant height and tillers/plant, HKW: 100 kernel weight, GY and BY are grain and biomass yield and HI is harvest index. Cd: cadmium, MT: melatonin, GABA: γ-aminobutyric acid. The presented data is mean of three replication with ± SD and different letters depicts the significance among means at P ≤ 0.05.

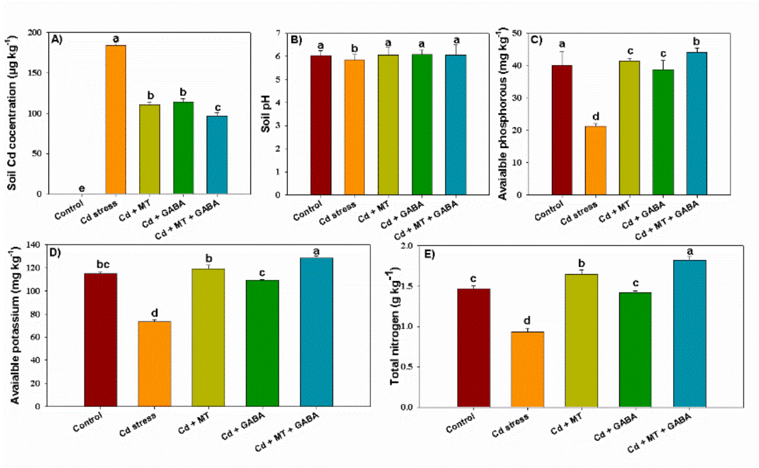


Figure 3: Impact of exogenously applied MT and GABA on soil Cd concentration, soil pH and soil N, P, and K concentration in Cd polluted soil. The presented data is mean of three replications with ± SD and different letters depicts the significance at P ≤ 0.05.

3.6. *Principal component and correlation analysis*

The first two principal components, Dim1 (95.3%) and Dim2 (3.3%), explain a cumulative 98.6% of the variance, indicating that most of the variation in growth traits is captured by these two dimensions (**Figure 4a**). The results displayed that Cd stress significantly alters plant growth traits. The Cd stress group is clustered far from other groups, suggesting a strong impact of Cd on growth. Among the treatments, the Cd + MT and Cd + GABA groups are positioned closer to the center, implying a partial recovery of growth traits. The Cd + MT + GABA (light blue crosses) group is located near the control, indicating a strong mitigation effect on Cd stress. The vector directions suggest that traits such as GY, BY, PH, RDW, RFW and HI are strongly influenced by Cd stress and recovery treatments. The correlation matrix shows strong positive correlations (red circles) between various growth parameters. Traits such as GY, BY, PH, RDW, RFW, TPP, and HI exhibit high correlation coefficients, suggesting that these traits are highly interdependent under Cd stress and recovery treatments. The treatments such as MT, GABA, and their combination effectively restore growth performance, leading to improved biomass and grain yield (**Figure 4b**).

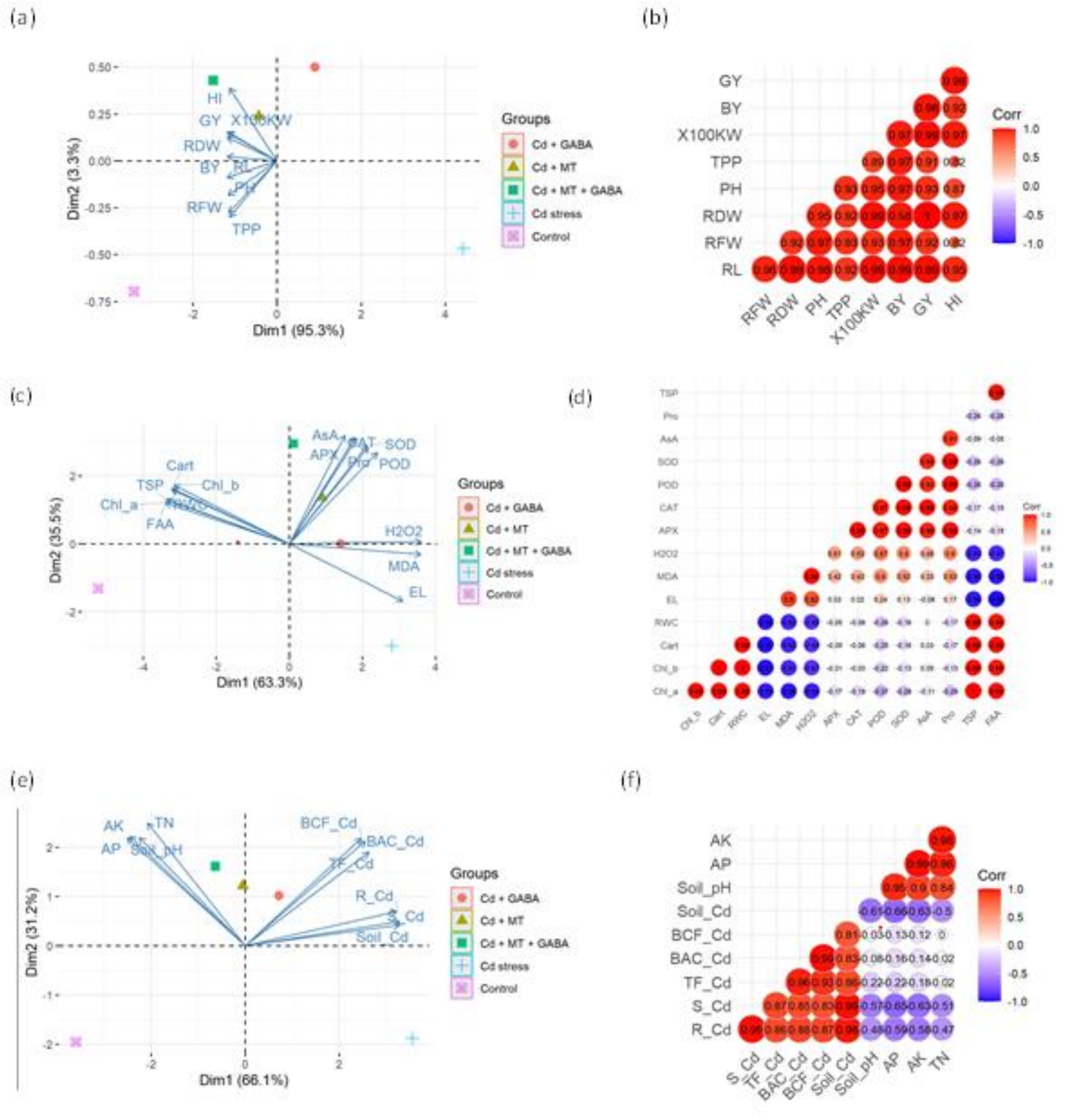


Figure 4: The principal component and correlation analysis for the impact of exogenously applied MT and GABA on growth, morpo-physiological functioning, soil properties and lead accumulation. GY: grain yield, BY: biomass yield, KW: kernel weight, TPP: tillers/plant, PH: plant height, RDW: root dry weight, RFW: root fresh weight, TSP: total soluble protein, Pro: proline, AsA: ascorbic acid, Chl: chlorophyll, RWC: relative water contents, EL: electrolyte leakage, MDA: malondialdehyde, H_2O_2: hydrogen peroxide, TSP: total soluble pro-tein, APX: ascorbate peroxidase, CAT: catalase, POD: peroxidase, SOD: superoxide dismutase. AK: available potassium, AP: available phosphorus, BAC: bio-accumulation factor, BCF: bio-concentration factor.

The PCA components explained 63.3% and 35.5% of the total variance, respectively. Vectors representing antioxidant enzyme activities, oxidative stress markers (H_2O_2, MDA, EL), and TSP and FAA displayed distinct clustering patterns. Control samples were separated from Cd-treated groups, while combined treatments (Cd + MT and Cd + GABA) showed unique clustering, indicating their potential in mitigating Cd-induced stress (**Figure 4c**). Notably, oxidative stress markers (H_2O_2, MDA, EL) exhibited a positive correlation with cadmium stress, while antioxidant responses clustered oppositely, suggesting their protective functions. Antioxidant enzymes displayed positive linking with AsA, Pro, TSP, indicating their collaborative role in mitigating oxidative stress. Conversely, markers of oxidative damage negatively correlated with chlorophyll content and carotenoids, suggesting that higher oxidative stress levels lead to reduced photosynthetic efficiency. Additionally, RWC was negatively associated with MDA and EL, highlighting the impact of cadmium stress on cellular water retention. The inclusion of MT and GABA influenced these interactions, underscoring their potential in alleviating cadmium-induced toxicity (**Figure 4d**).

PCA was used to analyze the distribution of different experimental groups and the relationships among soil and plant Cd accumulation parameters under various treatments. The biplot showed distinct clustering patterns for Cd content in plant tissues and soil Cd levels, with control samples positioned differently from Cd-treated groups. Combined treatments (Cd + MT and Cd + GABA) exhibited unique clustering patterns, indicating their role in modulating Cd accumulation and soil parameters (Figure 4e). The correlation matrix highlighted significant relationships between soil properties and Cd accumulation in plant tissues, with strong positive correlations among Cd accumulation factors and negative correlations between soil parameters and plant Cd accumulation. The introduction of MT and GABA modulated these interactions, suggesting their potential in mitigating cadmium accumulation in plants (**Figure 4f**).

4. Discussion

The detrimental impacts of Cd contamination on ecosystem health and organisms presents a significant challenge. Therefore, remediation of Cd-polluted soils is mandatory to safeguard living organisms and the ecosystem (Yang *et al.* 2023). Phyto-hormones got a considerable role for remediating Cd-polluted soils. Melatonin and GABA are important hormones with tremendous potential to improve plant growth under abiotic stresses (Yang *et al.* 2023). Therefore, this study assessed the impacts of MT and GABA in mitigating Cd toxicity in rice. Cadmium toxicity considerably decreased rice growth and yield (**Table 2**). Further, Cd toxicity also inhibits cell expansion and reduces root growth, causing reduction in growth (Alam *et al.* 2020). Cadmium decreased rice growth by increasing in oxidative damage, reducing nutrient uptake (Figure 3), photosynthetic pigments (**Table 1**), antioxidant activities (Figure 2), and protein degradation (Alam *et al.* 2020). Nevertheless, MT and GABA significantly increased the rice growth and grain productivity, aligning with previous studies (Guo *et al.* 2022). The exogenous applied GABA improves amylase activities, starch metabolism, osmolyte synthesis, and antioxidant activity; therefore, it reduces the adversities of stress (Cheng *et al.* 2018). Melatonin also maintains better homeostasis, antioxidant, and nutrient uptake, therefore, ensures better growth (Altaf *et al.* 2024). Co-applying MT and GABA enhanced rice growth via increase antioxidant activity, soil properties, and decreasing Cd accumulation (Song *et al.* 2024).

Cadmium negatively affects the photosynthetic efficiency of plants. The higher accumulation of Cd constrains assimilate supply, which in turn impairs the leaf functioning. In the current study, Cd toxicity significantly decreased the chlorophyll synthesis, which was linked with Cd-induced increase in oxidative stress (**Table 1**), and damaged the light-harvesting system and disrupted the structure of the chloroplast (Song *et al.* 2024; Vazquez-Marquez *et al.* 2024). Opposite to this, MT and GABA mitigated the adversities of Cd and ensured better chlorophyll synthesis. This was linked with a reduction in oxidative stress (**Table 1**) and the ability of MT and GABA to maintain the integrity of D1 protein and nutrient uptake (Figure 3), and reduces Cd uptake, which enhances antioxidant activities and supports photosynthesis under stress conditions (Yang *et al.* 2023). Melatonin improves the activity of the photosynthetic apparatus by reducing the stress-induced damage to the thylakoid membrane (Mushtaq *et al.* 2022). MT also increases the photosynthetic-related gene expressions ($CB1_2$ and CAB_7), and an increase in expression of these significantly increases chlorophyll synthesis and leads to better photosynthetic efficiency (Altaf *et al.* 2024).

Cadmium stress significantly decreased RWC of rice plants (**Table 1**), which was linked with Cd-induced damage to membranes, leading to loss of water and resulting in less RWC (Imran *et al.,* 2021). However, MT and GABA maintained the better RWC by improving root growth (**Table 1**). Cadmium toxicity also facilitated the increase in oxidative damage which was evidenced in the form of enhanced H_2O_2 and MDA production (**Table 1**). The foliar applied MT and GABA reduced EL, H_2O_2, and MDA synthesis by boosting antioxidant activities (Malik et al., 2022). This suggests that co-applying MT and GABA decreased Cd toxicity by decreasing membrane damage and enhancing antioxidant activities (Buttar *et al.* 2020; Lv *et al.* 2023). Likewise, Kumar *et al.* (2019) also witnessed that GABA application reduced the H_2O_2 production under As stress. Notably, co-applying MT and GABA significantly enhanced antioxidant activity and reduced MDA and H_2O_2 production as compared to their sole application. A possible reason for this increase could be that GABA increased the MT synthesis, thus leading to an increase in antioxidant activities (Aghdam and Fard 2017).

Cadmium toxicity increased the antioxidant activities; however, this increase was not enough to decrease the toxic impacts of Cd. This indicates the ability of rice plants to increase antioxidant activities to counteract Cd toxicity

(Chattha *et al.* 2021). Nevertheless, GABA and MT significantly enhanced all the antioxidant activities, which mitigates the toxicity of Cd (Hasanuzzaman *et al.* 2017). Amino acids and proteins are vital for stress tolerance; however, Cd toxicity decreased the synthesis of TSP and FAA. Cadmium toxicity decreased the N uptake (Figure 3), which is a building block of protein and amino acid synthesis. Further, Cd might also disturb the metabolism of protein and amino acids synthesis (Zemanová *et al.* 2014), leading to a reduction in the accumulation of TSP and FAA. The exogenously applied MT and GABA increased the FAA and TSP, which were linked with enhanced N uptake (Figure 3), and antioxidant activities (Figure 1). These results align with previous reports indicating that GABA and MT provide tolerance to plants through increasing the synthesis of osmo-regulatory substances (Wang *et al.* 2026).

Maximum Cd content was noted in roots as compared to shoots plant. This increased accumulation in roots was linked with the fact that the root comes in contact with Cd first, or it is also linked with compartmentalization of Cd in root vacuoles (Aamer *et al.* 2018). The lower Cd concentration was reported in shoots, indicating that less Cd was transported to aerial plant parts. Though MT and GABA significantly decreased Cd accretion in rice. GABA application reduces uptake of Cd by decreasing Cd^{2+} flux and declining gene expression linked with Cd uptake and transportation (Li *et al.* 2022). MT application also decreases Cd accumulation by reducing its uptake. Co-applying GABA and MT significantly decreased Cd uptake, which indicates the interaction between GABA and MT in inhibiting Cd absorption (Lv *et al.* 2023). MT and GABA diminished soil Cd availability and increased the availability of NPK (Figure 3). MT application improves microbial growth, which degrades the Cd and fixes it in the soil, thereby reducing its availability (Liang *et al.* 2017). Applying MT increases root exudate (malate and citrate), which increases bacterial and fungal growth and soil enzyme activities, leading to better nutrient availability (Yang *et al.* 2020).

5. Conclusion

Cadmium toxicity significantly inhibited rice growth, via increasing oxidative damages, causing, Cd accumulation and decreasing chlorophyll synthesis. Nevertheless, co-applying MT and GABA significantly decreased the toxic impacts of Cd. Combined MT and GABA showed a positive impact in remediating Cd-polluted soils and improving rice productivity by increasing soil health, antioxidant activities, osmolyte synthesis, and plant resilience to oxidative damage. Co-applying MT and GABA can serve as a promising approach to mitigate the adversities of Cd. This approach can decrease soil Cd availability, prevents ground-water contamination, and promote safer rice production. Thus, implementing this approach can enhance food safety and sustainable crop productivity. However, the efficiency of MT and GABA can be enhanced by using more sensitive analytical instruments such as ICP-MS for trace metal quantification. Moreover, validating the gene expression data with qRT-PCR alongside enzyme activity assays is also needed to understand its role in mitigating Cd toxicity. Additionally, conducting field trials across diverse climate and soil conditions can also enhance its efficiency in mitigating Cd toxicity.

Authors' Contributions

Conceptualization, ZG and HY, Writing – original draft: ZG, HY, YS, Data collection: YS, XH and YD, Investigation, YS, XH and YD, Writing, original draft: ZG and HY, Funding acquisition: ZG and HY, Writing – reviewing and editing: JMA, BA, OA, MA and MA. All authors have read and agreed to the published version of the manuscript."

Data availability

Data will be made available on request.

Acknowledgements

The authors extend their appreciation for the support of the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU262258].

Funding

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU262258].

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Aamer, M., Muhammad, U.H., Li, Z., Abid, A., Su, Q., & Liu, Y. (2018). Foliar application of glycinebetaine (GB) alleviates the cadmium (Cd) toxicity in spinach through reducing Cd uptake and improving the activity of anti-oxidant system. Applied Ecology and Environmental Research, 16(6), doi:10.15666/aeer/1606_75757583.

Aebi, H. (1984). Catalase in vitro. Methods in Enzymology, 105, 121-126. doi:10.1016/S0076-6879(84)05016-3.

Aghdam, M.S., & Fard, J.R. (2017). Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (Fragaria× anannasa cv. Selva) by enhancing GABA shunt activity. Food Chemistry, 22, 1650-1657. doi:10.1016/j.foodchem.2016.10.123.

Alam, M.Z., Carpenter-Boggs, L., Hoque, M.A., & Ahammed, G.J. (2020). Effect of soil amendments on antioxidant activity and photosynthetic pigments in pea crops grown in arsenic contaminated soil. Heliyon, 6(11), https://doi.org/10.1016/j.heliyon.2020.e05475

Altaf, M.M., Awan, Z.A., Ashraf, S., Altaf, M.A., Zhu, Z., & Alsahli, A.A. (2024). Melatonin induced reversibility of vanadium toxicity in muskmelon by regulating antioxidant defense and glyoxalase systems. Journal of Hazardous Materials, 473, 134452. doi:10.1016/j.jhazmat.2024.134452.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology, 24, 1. doi:10.1104/pp.24.1.1.

Ashraf, U., Mahmood, S., Anjum, S.A., Abbas, R.N., Rasul, F., Iqbal, J., Mo, Z., & Tang, X. (2022). Exogenous gamma-aminobutyric acid application induced modulations in the performance of aromatic rice under lead toxicity. Frontiers in Plant Science, 13, 933694. https://doi.org/10.3389/fpls.2022.933694

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annals of Biochemistry, 72, 248-254, https://doi.org/10.1016/0003-2697(76)90527-3.

Buttar, Z.A., Wu, S.N., Arnao, M.B., Wang, C., Ullah, I., & Wang, C. (2020). Melatonin suppressed the heat stress-induced damage in wheat seedlings by modulating the antioxidant machinery. Plants, 9, 809. doi:10.3390/plants9070809.

Cai, S.Y., Zhang, Y., Xu, Y.P., Qi, Z.Y., Li, M.Q., & Ahammed, G.J. (2017). HsfA1a upregulates melatonin biosynthesis to confer cadmium tolerance in tomato plants. Journal of Pineal Research, 62, 12387. doi:10.1111/jpi.12387.

Chattha, M., Arif, W., Khan, I., Soufan, W., Chattha, M.B., & Hassan, M.U. (2021). Mitigation of cadmium induced oxidative stress by using organic amendments to improve the growth and yield of mash beans [Vigna mungo (L.)]. Agronomy, 11, 2152. doi: 10.3390/agronomy11112152.

Chen, J., Dou, R., Yang, Z., You, T., Gao, X., & Wang, L. (2018). Phytotoxicity and bioaccumulation of zinc oxide nanoparticles in rice (Oryza sativa L.). Plant Physiology and Biochemistry, 130, 604-612. doi:10.3390/ijms19092520

Cheng, B., Li, Z., Liang, L., Cao, Y., Zeng, W., & Zhang, X. (2018). The γ-aminobutyric acid (GABA) alleviates salt stress damage during seeds germination of white clover associated with Na+/K+ transportation, dehydrins accumulation, and stress-related genes expression in white clover. International Journal of Molecular Sciences, 19, 2520. doi:10.3390/ijms19092520.

El-Yazied, A.A., Ibrahim, M.F., Ibrahim, MA., Nasef, I.N., Al-Qahtani, S.M., & Al-Harbi, N.A. (2022). Melatonin mitigates drought induced oxidative stress in potato plants through modulation of osmolytes, sugar metabolism, ABA homeostasis and antioxidant enzymes. Plants, 11, 1151. doi:10.3390/plants11091151.

Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A., & Catalano, A. (2020). The effects of cadmium toxicity. International Journal of Environmental Research and Public Health, 17, 3782. doi:10.3390/ijerph17113782.

Guo, Y., Li, D., Liu, L., Sun, H., Zhu, L., & Zhang, K. (2022). Seed priming with melatonin promotes seed germination and seedling growth of Triticale hexaploide L. under PEG-6000 induced drought stress. Frontiers in Plant Science, 13, 932912. doi:10.3389/fpls.2022.932912

Hamilton, P.B., Van Slyke, D.D. (1943). Amino acid determination with ninhydrin. Journal of Biological Chemistry, 150, 231-250.

Hasan, M.K., Ahammed, G.J., Yin, L., Shi, K., Xia, X., & Zhou, Y. (2015). Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in Solanum lycopersicum L. Frontiers in Plant Science, 6, 601. doi:10.3389/fpls.2015.00601.

Hasanuzzaman, M., Nahar, K., Gill, S.S., Alharby, H.F., Razafindrabe, B.H., & Fujita, M. (2017). Hydrogen peroxide pretreatment mitigates cadmium-induced oxidative stress in Brassica napus L.: an intrinsic study on antioxidant defense and glyoxalase systems. Frontiers in Plant Science, 8, 115. doi:10.3389/fpls.2017.00115.

Hassan, M.U., Huang, G., Haider, F.U., Khan, T.A., Noor, M.A., Luo, F., Zhou, Q, Yang, B, Ul Haq, M.I., & Iqbal, M.M. (2024). Application of zinc oxide nanoparticles to mitigate cadmium toxicity: Mechanisms and future prospects. Plants, 13, 1706. https://doi.org/10.3390/plants13121706

Huybrechts, M., Hendrix, S., Bertels, J., Beemster, G.T., Vandamme, D., & Cuypers, A. (2020). Spatial analysis of the rice leaf growth zone under controlled and cadmium-exposed conditions. Environmental and Experimental Botany, 177, 104120. doi:10.1016/j.envexpbot.2020.104120.

Imran, K., Seleiman, M.F., Chattha, M.U., Jalal, R.S., Mahmood, F., & Hassan, F.A. (2021). Enhancing antioxidant defense system of mung bean with a salicylic acid exogenous application to mitigate cadmium toxicity. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49, 12303-12303. https://doi.org/10.15835/nbha49212303

Jiang, Y., Huang, S., Ma, L., Kong, L., Pan, S., Tang, X., Tian, H., Duan, M., & Mo, Z. (2022). Effect of exogenous melatonin application on the grain yield and antioxidant capacity in aromatic rice under combined lead–cadmium stress. Antioxidants, 11, 776. doi: 10.3390/antiox11040776

Kumar, N., Gautam, A., Dubey, A. K., Ranjan, R., Pandey, A., Kumari, B., Gayatri, S., Sachin, M., Puneet, S.C., Saripella, S., Venekatest, D., & Mallick, S. (2019). GABA mediated reduction of arsenite toxicity in rice seedling through modulation of fatty acids, stress responsive amino acids and polyamines biosynthesis. Ecotoxicology and Environmental Safety, 173, 15-27. https://doi.org/10.1016/j.ecoenv. 2019.02.017

Li, G.Z., Wang, Y.Y., Liu, J., Liu, H.T., Liu, H.P., & Kang, G.Z. (2022). Exogenous melatonin mitigates cadmium toxicity through ascorbic acid and glutathione pathway in wheat. Ecotoxicology and Environmental Safety, 237, 113533. doi: 10.1016/j.ecoenv.2022.113533.

Liang, C., Li, A., Yu, H., Li, W., Liang, C., & Guo, S. (2017). Melatonin regulates root architecture by modulating auxin response in rice. Frontiers in Plant Science, 8, 134. doi:10.3389/fpls.2017.00134.

Liu, Y., Xiao, T., Ning, Z., Li, H., Tang, J., & Zhou, G. (2013). High cadmium concentration in soil in the Three Gorges region: Geogenic source and potential bioavailability. Applied geochemistry, 37, 149-156. 10.1016/j.apgeochem. 2013.07.022.

Lv. Y., Zhao, Y., He, Y., Wang, J., Zheng, Y., & Chen, X. (2023). Synergistic effects of gamma-aminobutyric acid and melatonin on seed germination and cadmium tolerance in tomato. Plant Signaling & Behavior, 18, 2216001. doi:10.1080/15592324.2023.2216001.

Malik, R.N., Husain, S.Z., & Nazir, I. (2010). Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. Pakistan Journal of Botany, 42, 291–301.

Malik, Z., Afzal, S., Dawood, M., Abbasi, G.H., Khan, M.I., & Kamran, M. (2022). Exogenous melatonin mitigates chromium toxicity in maize seedlings by modulating antioxidant system and suppresses chromium uptake and oxidative stress. Environmental Geochemistry and Health, 44(5), 1451-1469. doi:10.1007/s10653-021-00908-z.

Mohandas, P., Subramanian, P., & Surendran, R. (2025a). Deep feedforward air pollution classification empowered by Sophia-G optimization. In 4th international conference on innovative mechanisms for industry applications (ICIMIA). Pp, 1696-1702. doi:10.1109/ICIMIA67127.2025.11200570

Mohandas, P., Subramanian, P., & Surendran, R. (2025b) AI-driven ResNet50 with channel attention for Tamil Nadu air pollution level classification. In 4th international conference on innovative mechanisms for industry applications (ICIMIA). Pp, 1508-1513. doi:10.1109/ICIMIA67127.2025.11200911

Mohandas, P., Subramanian, P., & Surendran, R. (2025c). Optimizing air pollution prediction in urban environments using a hybrid rnn-pbo model with iot data. In 2025 4th international conference on innovative mechanisms for industry applications (ICIMIA). Pp. 480-487. doi: 10.1109/ICVADV63329.2025.10961386

Marron, N. (2015). Agronomic and environmental effects of land application of residues in short-rotation tree plantations: A literature review. Biomass and Bioenergy, 81, 378-400. doi: 10.1016/j.biombioe.2015.07.025.

Mitra, S., Chakraborty, A.J., Tareq, A.M., Emran, T.B., Nainu, F., & Khusro, A. (2022). Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University-Science, 34, 101865. doi:10.1016/j.jksus.2022.101865.

Moravčíková, D., Žiarovská, J. (2023). The effect of cadmium on plants in terms of the response of gene expression level and activity. Plants 12:1848. doi:10.3390/plants12091848.

Moustaka, J., Sperdouli, I., İşgören, S., Şaş, B., & Moustakas, M. (2024). Deciphering the mechanism of melatonin-induced enhancement of photosystem II function in moderate drought-stressed oregano plants. Plants, 13, 2590. doi:10.3390/plants13182590.

Mukherjee, S.P., & Choudhuri, M.A. (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiologia Plantarum 58(2), 166-170. doi:10.1111/j.1399-3054.1983.tb04162.x.

Mushtaq, N., Iqbal, S., Hayat, F., Raziq, A., Ayaz, A., & Zaman, W. (2022). Melatonin in micro-tom tomato: Improved drought tolerance via the regulation of the photosynthetic apparatus, membrane stability, osmoprotectants, and root system. Life, 12(11), 1922. https://doi.org/10.3390/life12111922

Nakano, Y., Asada, K. (1987). Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. Plant and Cell Physiology, 28, 131-140. doi:10.1093/oxfordjournals.pcp.a077268.

Nayyar, H., Kaur, R., Kaur, S., & Singh, R. (2014). γ-Aminobutyric acid (GABA) imparts partial protection from heat stress injury to rice seedlings by improving leaf turgor and upregulating osmoprotectants and antioxidants. Journal of Plant Growth Regulation, 33, 408-419. doi:10.1007/s00344-013-9389-6.

Peng, H., & Shahidi, F. (2021). Cannabis and cannabis edibles: a review. Journal of Agricultural and Food Chemistry, 69, 1751-1774.

Rahimzadeh, M.R., Rahimzadeh, M.R., Kazemi, S., & Moghadamnia, A.A. (2017). Cadmium toxicity and treatment: An update. Caspian Journal of Internal Medicine, 8, 135. doi: 10.22088/cjim.8.3.135.

Song, C., Manzoor, M.A., Mao, D., Ren, X., Zhang, W., & Zhang, Y. (2024). Photosynthetic machinery and antioxidant enzymes system regulation confers cadmium stress tolerance to tomato seedlings pretreated with melatonin. Scientia Horticulturae, 323, 112550. doi:10.1016/j.scienta.2023.112550.

Vazquez-Marquez, A.M., Bernabé-Antonio, A., Correa-Basurto, J., Burrola-Aguilar, C., Zepeda-Gómez C, & Cruz-Sosa, F. (2024). Changes in growth and heavy metal and phenolic compound accumulation in Buddleja cordata cell suspension culture under Cu, Fe, Mn, and Zn enrichment. Plants, 13, 1147. doi:10.3390/plants13081147.

Wang, J., & Yang, S. (2021). Dose-dependent responses of Arabidopsis thaliana to zinc are mediated by auxin homeostasis and transport. Environmental and Experimental Botany, 189, 104554. doi:10.1016/j.envexpbot.2021.104554.

Wang, L., Shen, X., Chen, X., Ouyang, Q., Tan, X., & Tao, N. (2022). Exogenous application of melatonin to green horn pepper fruit reduces chilling injury during postharvest cold storage by regulating enzymatic activities in the antioxidant system. Plants, 11, 2367. doi:10.3390/plants11182367.

Wang, X., Chen, C., Sun, X., Zaman, Q. U., Wang, C., & Tang, H. (2026). Synergistic role of gamma-aminobutyric acid and melatonin in mitigating lead stress in rice. BMC Plant Biology, 26(1), 19. https://doi.org/10.1186/s12870-025-07774-2

Xu, B., Long, Y., Feng, X., Zhu, X., Sai, N., & Chirkova, L. (2021). GABA signalling modulates stomatal opening to enhance plant water use efficiency and drought resilience. Nature Communications, 12, 1952. doi:10.1038/s41467-021-21694-3.

Yang, Y., Cao, Y., Li, Z., Zhukova, A., Yang, S., & Wang, J. (2020). Interactive effects of exogenous melatonin and Rhizophagus intraradices on saline-alkaline stress tolerance in Leymus chinensis. Mycorrhiza, 30, 357-371. doi:10.1007/s00572-020-00942-2

Yang, Z., He, Y., Ma, Q., Wang, H., & Zhang, Q. (2023). Alleviative effect of melatonin against the nephrotoxicity induced by cadmium exposure through regulating renal oxidative stress, inflammatory reaction, and fibrosis in a mouse model. Ecotoxicology and Environmental Safety, 265, 115536. doi:10.1016/j.ecoenv.2023.115536.

Zemanová, V., Pavlík, M., Pavlíková, D., & Tlustoš, P. (2014). The significance of methionine, histidine and tryptophan in plant responses and adaptation to cadmium stress. Plant, Soil and Environment, 60, 426–432.

Zhang, X.Z. (1992). The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. Research Methodology of Crop Physiology, 208-211.