

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

4 Zhan-Wu Gao^{1**}, Hong-zhu Yu¹⁺, Yong Shi¹, Xin-Yu Hu¹, Yi-Xi Deng¹, Jameel M. AL-Khayri², Bader
5 Alsubaie², Othman Al-dossary², Muneera Q. Al-mssallem³, and Mustafa I. Almaghasla^{4*}

6 ¹Jilin Provincial Key Laboratory of Western Jilin's Clean Energy, Baicheng Normal University,
7 Baicheng 137000, China

8 ²Department of Agricultural Biotechnology, College of Agriculture and Food Sciences, King Faisal
9 University, Saudi Arabia

10 ³Department of Food Science and Nutrition, College of Agriculture and Food Sciences, King Faisal
11 University, Saudi Arabia

12 ⁴Plant Pests and Diseases Unit, College of Agriculture and Food Sciences, King Faisal University, Saudi
13 Arabia

14 Corresponding authors: gaozw261@nenu.edu.cn and malmghaslah@kfu.edu.sa

15 **Abstract**

16 Soil cadmium (Cd) contamination poses a serious challenge to crops. The exogenic application of growth
17 hormones is an important strategy in addressing the challenge of Cd pollution. The study explores the
18 impact of combined melatonin (MT) and gamma-aminobutyric acid (GABA) in mitigating Cd toxicity
19 with these treatments; control, Cd stress (250 $\mu\text{g kg}^{-1}$), Cd stress + melatonin (MT: 100 μM), Cd stress +
20 GABA (1 mM) and Cd+ MT + GABA. Cadmium toxicity significantly decreases rice growth and yield
21 productivity by increasing oxidative markers, Cd accumulation, and decreasing photosynthetic pigments,
22 osmolyte productions and nutrients availability. Melatonin and GABA significantly decreased the
23 adversities of Cd and increased rice productivity. Co-applying MT and GABA decreased hydrogen
24 peroxide (H_2O_2), malondialdehyde (MDA), electrolyte leakage (EL) by 34.59%, 44.74% and 105.40%,

25 soil Cd availability, and increased chlorophyll synthesis (36.61- 54.67%) and antioxidant activities
26 (48.38-121.34%), therefore lead to an increase in growth and yield. Further, MT and GABA also reduced
27 Cd accretion in rice roots and shoots and increased the nutrients availability favoring the growth of rice
28 plants in Cd stress conditions. These results underpin the potential combined MT + GABA application
29 in improving crop productivity and remediating Cd polluted soils.

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

31 **1. Introduction**

32 Soil heavy metal (HM) pollution and air pollution are serious challenges to environmental quality and
33 ecosystem sustainability (Mitra et al. 2022; Mohandas *et al.*, 2025a,b,c). Heavy metals are continuously
34 soaring up in soils and water, thus it is essential to address this problem for safer food production
35 (Rahimzadeh *et al.* 2017; Sivasubramanian et al., 2025). Among different HM, cadmium (Cd) is a
36 seriously toxic metal posing serious threat to crop production, human health, and ecosystem sustainability
37 (Peng and Shahidi 2021). It is a non-essential metal; however, its concentration is rapidly soaring due to
38 mining, smelting, synthetic fertilizers, traffic, waste incinerators, and industrial effluents (Genchi *et al.*,
39 2020; Wang and Yang 2021). Apart from these anthropogenic sources, Cd also enters the environment
40 through weathering, forest fires, wind dust, and eruptions from volcanoes (Liu *et al.* 2013). The foods
41 grown on Cd-contaminated soils lead to its entry into humans, which in turn causes kidney and liver
42 damage, emphysema, and heart diseases (Li *et al.* 2022).

43 Cadmium is highly toxic to plants; however, plants quickly absorb Cd through roots, which negatively
44 affects growth, cell division, chlorophyll synthesis, photosynthetic efficiency, and assimilate production
45 (Moravčíková and Žiarovská 2023). The excessive concentration of Cd also causes oxidative damage
46 and disturbs membranes, DNA, electron transport, and photosynthetic apparatus (Marron 2015).
47 Cadmium toxicity induce stomata closing, inhibits water uptake and carbon fixation (Huybrechts *et al.*
48 2020) thereby causes significant growth losses (Huybrechts *et al.* 2020). Additionally, Cd toxicity also
49 disturbs nutrient uptake, decreases water uptake, damages the photosynthetic apparatus, and reduces

50 antioxidant activities (Hassan *et al.* 2024). Cadmium pollution is a serious challenge to achieve
51 agricultural goals, (Chen *et al.* 2018), therefore, it is mandatory to manage the Cd-polluted soils for
52 sustainable and safer crop productivity.

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

63 Gamma-aminobutyric acid (GABA) also showed appreciable results to counteract abiotic stresses. It
64 improves stomata opening, regulates the osmotic pressure (Xu *et al.* 2021), improves carbon assimilation,
65 and antioxidant activity, thus regulating plant growth in stress conditions. Under Cd stress, GABA uptake
66 reduces Cd uptake and improves plant performance by increasing endogenous MT synthesis (Li *et al.*
67 2022). The role of single MT and GABA application in mitigating the toxic impacts of HMs. However,
68 the interactive effect of MT and GABA in mitigating the adverse impacts of Cd is poorly understood.
69 Therefore, we hypothesized that the interactive effects of MT and GABA can substantially reduce the
70 toxic impacts of Cd on rice as compared to their application alone. Therefore, this study aimed to explore
71 the interactive impacts of GABA on growth, yield, plant functioning, Cd uptake, accumulation, and soil
72 properties.

73 **2. Materials and methods**

74 *2.1. Growth conditions and plant materials*

75 The study was performed at Baicheng Normal University and soil was taken from experimental soil (0-
76 20 cm) and sieved to fill the pots with 10 kg capacity after removing debris. The soil was silt-loam with
77 acid pH (5.97), total nitrogen (TN: 1.66 g kg⁻¹), and available phosphorus (AP) and available potassium
78 (AK) concentrations of 38.42 and 117.19 mg kg⁻¹. The pots were filled, and CdCl₂ was to impost Cd
79 toxicity. The soil was equilibrated for two months, and during this period, a field capacity level of 70%
80 was maintained.

81 2.2. Experimental treatments

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

91 2.3. Measurement of growth and photosynthetic traits

92 The fresh leaves of rice were taken and chlorophyll (Chl) and carotenoid (Cart.) concentrations by the
93 methods of Arnon (1949). The freshly collected leaves (0.5 g) were homogenized in 80% methanol and
94 extract was collected, and absorbance was recorded at 645, 480, and 663 for determining Chl-a and Chl-
95 b and Cart concentrations. The fresh leaves were randomly collected and weighed (FW), and then they
96 were soaked in water and after 24 hours they were weighed (TW). Later, these leaves were removed from
97 water and oven dried (65°C) until constant weight (DW). Finally, leaf relative water contents (RWC)
98 were calculated with following formula: $RWC (\%) = \frac{FW - DR}{TW - DR} \times 100$.

100 *2.4. Measurement of biochemical attributes*

101 Fresh rice leaves (0.5 g) were collected and dipped in water for 30 minutes, and the electrical conductivity
102 (EC_1) was measured. Thereafter, the same leaves were taken and boiled in water (90 °C) for 1 hour. After
103 that, the leaves were removed from the water, and the second EC (EC_2) was measured. Finally, EL was
104 calculated using the following formula: $EL = EC_1/EC_2 \times 100$. The freshly collected leaves (0.5 g) were
105 homogenized using the potassium phosphate buffer (PPB) and centrifuged (14000 rpm) for 15 minutes.
106 The obtained extract was treated with Bradford reagent (2 mL), and absorbance was measured at 595 nm
107 (Bradford, 1976). For free amino acids (FAA), leaves were ground using PPB, and then the extract was
108 mixed with ninhydrin (1 mL) and pyridine (1 mL). Later, the mixture was boiled for 30 min, and the
109 absorbance was read at 570 nm (Hamilton and Van-Slyke 1943). To quantify hydrogen peroxide (H_2O_2),
110 we homogenized 0.5 g of fresh leaf tissue in 5 mL of trichloroacetic acid (TCA). The resulting
111 homogenate was centrifuged, and we then mixed 1 mL of the supernatant with a reaction solution
112 containing potassium phosphate buffer (PBB) and 1 M potassium iodide (KI). The absorbance of this
113 mixture was measured at 390 nm. For malondialdehyde (MDA), leaves were ground in trichloroacetic
114 acid (TCA) solution and centrifuged to obtain the extract, and then 5 mL of thiobarbituric acid (TBA)
115 was added and boiled (100 °C) for 30 minutes, and later the absorbance (532 nm) was taken. For
116 ascorbate peroxidase (APX) activity leaves (0.5 g) were homogenized in PPB buffer and centrifuged
117 (10000 rpm) for 15 min, and absorbance noted at 290 nm (Nakano and Asada, 1987). For the catalase
118 (CAT) activity, 0.5 g freshly collected leaves were blended by using PPB and centrifuged (10000 rpm),
119 and absorbance was noted at 240 nm (Aebi 1984). For determining superoxide dismutase (SOD) activity,
120 we prepared the mixture containing 400 μ L H_2O_2 , 25 mL buffer, 100 μ L Triton, 50 μ L extract was made
121 and reading done at 560 nm (Mukherjee and Choudhuri 1983). In case of peroxidase (POD), freshly
122 collected leaves were homogenized in PPB, and later, the absorbance noted at 470 nm, and POD activity
123 was measured by the methods of Zhang (1992). For ascorbic acid (AsA), 0.5 g of leaves was

124 homogenized in TCA solution (5 mL) and centrifuged (10000 rpm) for 15 minutes, and later AsA
125 contents were determined with the methods of Zhang (1992).

126 *2.5. Measurement of growth traits, Cd concentration in plant tissues and soil properties*

127 The height of each plant was measured, and tillers were counted and average was done. Five panicles
128 from each replication were selected to determine their length and grains per panicle. The plants were
129 hand harvested for assessing the grain and biomass yield. The rice samples were collected, dried (65°C),
130 and powdered to determine Cd concentration. The samples were digested (180°C) by using two acids
131 (HCl and HClO₄, 4:1), then this mixture was filtered and diluted to 50 mL, and Cd in the samples was
132 measured by atomic absorption spectrophotometry. The soil samples were collected, and pH meter was
133 used for measuring pH in a soil and water solution (3:1). Nitrogen in soil samples was assessed with
134 Kjeldahl apparatus, while AP and AK were assessed with the Olsen and ammonium acetate extraction
135 flame photometry method. The translocation factors and biological accumulation coefficient were
136 calculated with the procedures of Malik *et al.* (2010). The soil samples were digested in HNO₃:HClO₄
137 (4:1), thereafter they were filtered and diluted by adding water and Cd concentration was estimated with
138 atomic absorption spectrometry.

139 *2.5. Data Analysis*

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

143 **3. Results**

144 *3.1. Effect of MT and GABA on photosynthetic pigments*

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

149 *3.2. Effect of MT and GABA on oxidative markers, osmolytes and antioxidants activities*

150 Cadmium toxicity caused a marked decrease in RWC of rice plants. Cd toxicity reduced leaf RWC by
151 43.25% respectively (Table 1) while MT and GABA considerably increased leaf RWC (Table 1). The
152 production of all the oxidative markers substantially increased in Cd stress. The results depicted that Cd
153 toxicity increases EL, MDA, and H₂O₂ synthesis by 299.29%, 298.45% and 323.19% as the control
154 (Table 1). Co-applying MT and GABA causes a marked decrease in EL, MDA, and H₂O₂ production
155 (Table 1). Cadmium toxicity increased the proline synthesis, while it decreased the synthesis of total
156 soluble protein (TSP) and FAA (Figure 1). Both MT and GABA increased the synthesis of all the
157 osmolytes under Cd stress conditions. For instance, co-applying MT and GABA enhanced the proline,
158 TSP, and FAA by 45.67%, 75.68% and 102.73% respectively, under Cd stress conditions (Figure 1).
159 Melatonin and GABA boosted the antioxidant activity. Co-applying MT and GABA enhanced APX,
160 CAT, POD, SOD, and AsA activities by 62.46%, 89.40%, 48.38%, 66.19% and 121.34% respectively
161 than control (Figure 1).

162 *3.3. Effect of MT and GABA on growth and yield traits*

163 Cadmium toxicity decreased the RL, their fresh and dry matter production by 49.49%, 79.84% and
164 84.65% respectively (Table 2). Interestingly, exogenous treatments with MT and GABA significantly
165 increased root length (RL) and their fresh and dry matter production by 38.93%, 53.91% and 68.58%
166 respectively in Cd-polluted soil (Table 2). Cadmium stress considerably decreased yield and yield traits.
167 The exogenous applied MT and GABA significantly increased the plant height (PH), tillers per plant
168 (TPP), hundred kernel weight (HKW), grain yield (GY), biological yield (BY), and harvest index (HI)
169 of rice plants 22.47%, 16.08%, 58.30%, 62.28%, 30.28% and 24.31% respectively (Table 2).

170 *3.4. Effect of MT and GABA on root and shoot Cd concentration on TF, BAC and BAF*

171 The results showed that Cd stress results in a significant increase in root and shoot Cd concentration
172 (Figure 2). MT and GABA application significantly diminished Cd accretion in the plant, and a high
173 reduction was noted with co-applying MT and GABA (Figure 2). Furthermore, these findings indicated

174 that translocation factor (TF), bio-accumulation concentration (BAC), and bio-concentration factor
175 (BCF) of Cd were significantly diminished with the supplementation of MT and GABA. The highest
176 reduction in TF, BAC, and BCF was obtained after co-applying MT and GABA than their single
177 application (Figure 2).

178 *3.5. Effects of MT and GABA on soil properties*

179 The results depicted that MT and GABA supplementation significantly decreased the soil Cd
180 concentration after harvesting rice plants (Figure 3). Co-applying MT+GABA decreased soil Cd by
181 90.61%, while MT and GABA decreased soil Cd concentrations by 66.65% and 61.83% respectively
182 (Figure 3). Further, the results also indicated that Cd stress and exogenously applied GABA and MT had
183 non-significant impacts on soil pH (Figure 3). Cd reduced AP, AK, and TN concentration; conversely,
184 MT and GABA caused a marked increase in the availability of AP, AK, and TN (Figure 3).

185 *3.6. Principal component and correlation analysis*

186 The first two principal components, Dim1 (95.3%) and Dim2 (3.3%), explain a cumulative 98.6% of the
187 variance, indicating that most of the variation in growth traits is captured by these two dimensions (Figure
188 4 a). The results displayed that Cd stress significantly alters plant growth traits. The Cd stress group is
189 clustered far from other groups, suggesting a strong impact of Cd on growth. Among the treatments, the
190 Cd + MT and Cd + GABA groups are positioned closer to the center, implying a partial recovery of
191 growth traits. The Cd + MT + GABA (light blue crosses) group is located near the control, indicating a
192 strong mitigation effect on Cd stress. The vector directions suggest that traits such as GY, BY, PH, RDW,
193 RFW and HI are strongly influenced by Cd stress and recovery treatments. The correlation matrix shows
194 strong positive correlations (red circles) between various growth parameters. Traits such as GY, BY, PH,
195 RDW, RFW, TPP, and HI exhibit high correlation coefficients, suggesting that these traits are highly
196 interdependent under Cd stress and recovery treatments. The treatments such as MT, GABA, and their
197 combination effectively restore growth performance, leading to improved biomass and grain yield
198 (Figure 4 b).

199 The PCA components explained 63.3% and 35.5% of the total variance, respectively. Vectors
200 representing antioxidant enzyme activities, oxidative stress markers (H₂O₂, MDA, EL), and TSP and
201 FAA displayed distinct clustering patterns. Control samples were separated from Cd-treated groups,
202 while combined treatments (Cd + MT and Cd + GABA) showed unique clustering, indicating their
203 potential in mitigating Cd-induced stress (Figure 4 c). Notably, oxidative stress markers (H₂O₂, MDA,
204 EL) exhibited a positive correlation with cadmium stress, while antioxidant responses clustered
205 oppositely, suggesting their protective functions. Antioxidant enzymes displayed positive linking with
206 AsA, Pro, TSP, indicating their collaborative role in mitigating oxidative stress. Conversely, markers of
207 oxidative damage negatively correlated with chlorophyll content and carotenoids, suggesting that higher
208 oxidative stress levels lead to reduced photosynthetic efficiency. Additionally, RWC was negatively
209 associated with MDA and EL, highlighting the impact of cadmium stress on cellular water retention. The
210 inclusion of MT and GABA influenced these interactions, underscoring their potential in alleviating
211 cadmium-induced toxicity (Figure 4d).

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

222

223

224 4. Discussion

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

241 Cadmium negatively effects the photosynthetic efficiency of plants. The higher accumulation of Cd
242 constrains assimilate supply, which in turn impairs the leaf functioning. In the current study, Cd toxicity
243 significantly decreased the chlorophyll synthesis, which was linked with Cd-induced increase in
244 oxidative stress (Table 1), and damaged the light-harvesting system and disrupted the structure of the
245 chloroplast (Song *et al.* 2024; Vazquez-Marquez *et al.* 2024). Opposite to this, MT and GABA mitigated
246 the adversities of Cd and ensured better chlorophyll synthesis. This was linked with a reduction in
247 oxidative stress (Table 1) and the ability of MT and GABA to maintain the integrity of D1 protein and
248 nutrient uptake (Figure 3), and reduces Cd uptake, which enhances antioxidant activities and supports

249 photosynthesis under stress conditions (Yang *et al.* 2023). Melatonin improves the activity of the
250 photosynthetic apparatus by reducing the stress-induced damage to the thylakoid membrane (Mushtaq *et*
251 *al.* 2022). MT also increases the photosynthetic-related gene expressions (CB1₂ and CAB₇), and an
252 increase in expression of these significantly increases chlorophyll synthesis and leads to better
253 photosynthetic efficiency (Altaf *et al.* 2024).

254 Cadmium stress significantly decreased RWC of rice plants (Table 1), which was linked with Cd-induced
255 damage to membranes, leading to loss of water and resulting in less RWC (Imran *et al.*, 2021). However,
256 MT and GABA maintained the better RWC by improving root growth (Table 1). Cadmium toxicity also
257 facilitated the increase in oxidative damage which was evidenced in the form of enhanced H₂O₂ and
258 MDA production (Table 1). The foliar applied MT and GABA reduced EL, H₂O₂, and MDA synthesis
259 by boosting antioxidant activities (Malik *et al.*, 2022). This suggests that co-applying MT and GABA
260 decreased Cd toxicity by decreasing membrane damage and enhancing antioxidant activities (Buttar *et*
261 *al.* 2020; Lv *et al.* 2023). Likewise, Kumar *et al.* (2019) also witnessed that GABA application reduced
262 the H₂O₂ production under As stress. Notably, co-applying MT and GABA significantly enhanced
263 antioxidant activity and reduced MDA and H₂O₂ production as compared to their sole application. A
264 possible reason for this increase could be that GABA increased the MT synthesis, thus leading to an
265 increase in antioxidant activities (Aghdam and Fard 2017).

266 Cadmium toxicity increased the antioxidant activities; however, this increase was not enough to decrease
267 the toxic impacts of Cd. This indicates the ability of rice plants to increase antioxidant activities to
268 counteract Cd toxicity (Chattha *et al.* 2021). Nevertheless, GABA and MT significantly enhanced all the
269 antioxidant activities, which mitigates the toxicity of Cd (Hasanuzzaman *et al.* 2017). Amino acids and
270 proteins are vital for stress tolerance; however, Cd toxicity decreased the synthesis of TSP and FAA.
271 Cadmium toxicity decreased the N uptake (Figure 3), which is a building block of protein and amino acid
272 synthesis. Further, Cd might also disturb the metabolism of protein and amino acids synthesis (Zemanová
273 *et al.* 2014), leading to a reduction in the accumulation of TSP and FAA. The exogenously applied MT

274 and GABA increased the FAA and TSP, which were linked with enhanced N uptake (Figure 3), and
275 antioxidant activities (Figure 1). These results align with previous reports indicating GABA and MT
276 provide tolerance to plants through increasing the synthesis of osmo-regulatory substances (Wang *et al.*
277 2026).

278 Maximum Cd contents was noted in roots as compared to shoots plant. This increased accumulation in
279 roots was linked with the fact that the root comes in contact with Cd first, or it is also linked with
280 compartmentalization of Cd in root vacuoles (Aamer *et al.* 2018). The lower Cd concentration was
281 reported in shoots, indicating that less Cd was transported to aerial plant parts. Though MT and GABA
282 significantly decreased Cd accretion in rice. GABA application reduces uptake of Cd by decreasing
283 Cd²⁺ flux and declining gene expression linked with Cd uptake and transportation (Li *et al.* 2022). MT
284 application also decreases Cd accumulation by reducing its uptake. Co-applying GABA and MT
285 significantly decreased Cd uptake, which indicates the interaction between GABA and MT in inhibiting
286 Cd absorption (Lv *et al.* 2023). MT and GABA diminished soil Cd availability and increased the
287 availability of NPK (Figure 3). MT application improves microbial growth, which degrades the Cd and
288 fixes it in the soil, thereby reducing its availability (Liang *et al.* 2017). Applying MT increases root
289 exudate (malate and citrate), which increases bacterial and fungal growth and soil enzyme activities,
290 leading to better nutrient availability (Yang *et al.* 2020).

291 **5. Conclusion**

292 Cadmium toxicity significantly inhibited rice growth, via increasing oxidative damages, causing, Cd
293 accumulation and decreasing chlorophyll synthesis. Nevertheless, co-applying MT and GABA
294 significantly decreased the toxic impacts of Cd. Combined MT and GABA showed a positive impact in
295 remediating Cd-polluted soils and improving rice productivity by increasing soil health, antioxidant
296 activities, osmolyte synthesis, and plant resilience to oxidative damage. Co-applying MT and GABA can
297 serve as a promising approach to mitigate the adversities of Cd. This approach can decrease soil Cd
298 availability, prevents ground-water contamination, and promote safer rice production. Thus,

299 implementing this approach can enhance food safety and sustainable crop productivity. However, the
300 efficiency of MT and GABA can be enhanced by using more sensitive analytical instruments such as
301 ICP-MS for trace metal quantification. Moreover, validating the gene expression data with qRT-PCR
302 alongside enzyme activity assays is also needed to understand its role in mitigating Cd toxicity.
303 Additionally, conducting field trials across diverse climate and soil conditions can also enhance its
304 efficiency in mitigating Cd toxicity.

305 **Authors' Contributions**

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

310 **Data availability**

311 Data will be made available on request.

312 **Acknowledgements**

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

316 **Funding**

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

319 **Conflict of Interests**

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

321 **References**

322 Aamer, M., Muhammad, U.H., Li, Z., Abid, A., Su, Q., & Liu, Y. (2018). Foliar application of
323 glycinebetaine (GB) alleviates the cadmium (Cd) toxicity in spinach through reducing Cd uptake

324 and improving the activity of anti-oxidant system. Applied Ecology and Environmental
325 Research, 16(6), [doi:10.15666/aeer/1606_75757583](https://doi.org/10.15666/aeer/1606_75757583).

326 Aebi, H. (1984). Catalase in vitro. Methods in Enzymology, 105, 121-126. [doi:10.1016/S0076-
327 6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3).

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

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

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

337 Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant
338 Physiology, 24, 1. [doi:10.1104/pp.24.1.1](https://doi.org/10.1104/pp.24.1.1).

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

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

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

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

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

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

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

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

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

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

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

- 374 Hasan, M.K., Ahammed, G.J., Yin, L., Shi, K., Xia, X., & Zhou, Y. (2015). Melatonin mitigates cadmium
375 phytotoxicity through modulation of phytochelatin biosynthesis, vacuolar sequestration, and
376 antioxidant potential in *Solanum lycopersicum* L. *Frontiers in Plant Science*, 6, 601.
377 [doi:10.3389/fpls.2015.00601](https://doi.org/10.3389/fpls.2015.00601).
- 378 Hasanuzzaman, M., Nahar, K., Gill, S.S., Alharby, H.F., Razafindrabe, B.H., & Fujita, M. (2017).
379 Hydrogen peroxide pretreatment mitigates cadmium-induced oxidative stress in *Brassica napus*
380 L.: an intrinsic study on antioxidant defense and glyoxalase systems. *Frontiers in Plant Science*,
381 8, 115. [doi:10.3389/fpls.2017.00115](https://doi.org/10.3389/fpls.2017.00115).
- 382 Hassan, M.U., Huang, G., Haider, F.U., Khan, T.A., Noor, M.A., Luo, F., Zhou, Q., Yang, B, Ul Haq,
383 M.I., & Iqbal, M.M. (2024). Application of zinc oxide nanoparticles to mitigate cadmium
384 toxicity: Mechanisms and future prospects. *Plants*, 13, 1706.
385 <https://doi.org/10.3390/plants13121706>
- 386 Huybrechts, M., Hendrix, S., Bertels, J., Beemster, G.T., Vandamme, D., & Cuypers, A. (2020). Spatial
387 analysis of the rice leaf growth zone under controlled and cadmium-exposed
388 conditions. *Environmental and Experimental Botany*, 177, 104120.
389 [doi:10.1016/j.envexpbot.2020.104120](https://doi.org/10.1016/j.envexpbot.2020.104120).
- 390 Imran, K., Seleiman, M.F., Chattha, M.U., Jalal, R.S., Mahmood, F., & Hassan, F.A. (2021). Enhancing
391 antioxidant defense system of mung bean with a salicylic acid exogenous application to mitigate
392 cadmium toxicity. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 49, 12303-12303.
393 <https://doi.org/10.15835/nbha49212303>
- 394 Jiang, Y., Huang, S., Ma, L., Kong, L., Pan, S., Tang, X., Tian, H., Duan, M., & Mo, Z. (2022). Effect
395 of exogenous melatonin application on the grain yield and antioxidant capacity in aromatic rice
396 under combined lead–cadmium stress. *Antioxidants*, 11, 776. [doi: 10.3390/antiox11040776](https://doi.org/10.3390/antiox11040776)
- 397 Kumar, N., Gautam, A., Dubey, A. K., Ranjan, R., Pandey, A., Kumari, B., Gayatri, S., Sachin, M.,
398 Puneet, S.C., Saripella, S., Venekatest, D., & Mallick, S. (2019). GABA mediated reduction of

399 arsenite toxicity in rice seedling through modulation of fatty acids, stress responsive amino acids
400 and polyamines biosynthesis. *Ecotoxicology and Environmental Safety*, 173, 15-27.
401 <https://doi.org/10.1016/j.ecoenv.2019.02.017>

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

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

408 Liu, Y., Xiao, T., Ning, Z., Li, H., Tang, J., & Zhou, G. (2013). High cadmium concentration in soil in
409 the Three Gorges region: Geogenic source and potential bioavailability. *Applied geochemistry*,
410 37, 149-156. [10.1016/j.apgeochem.2013.07.022](https://doi.org/10.1016/j.apgeochem.2013.07.022).

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

414 Malik, R.N., Husain, S.Z., & Nazir, I. (2010). Heavy metal contamination and accumulation in soil and
415 wild plant species from industrial area of Islamabad, Pakistan. *Pakistan Journal of Botany*, 42,
416 291–301.

417 Malik, Z., Afzal, S., Dawood, M., Abbasi, G.H., Khan, M.I., & Kamran, M. (2022). Exogenous melatonin
418 mitigates chromium toxicity in maize seedlings by modulating antioxidant system and suppresses
419 chromium uptake and oxidative stress. *Environmental Geochemistry and Health*, 44(5), 1451-
420 1469. [doi:10.1007/s10653-021-00908-z](https://doi.org/10.1007/s10653-021-00908-z).

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

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

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

432 Marron, N. (2015). Agronomic and environmental effects of land application of residues in short-rotation
433 tree plantations: A literature review. *Biomass and Bioenergy*, 81, 378-400. doi:
434 [10.1016/j.biombioe.2015.07.025](https://doi.org/10.1016/j.biombioe.2015.07.025).

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

438 Moravčiková, D., Žiarovská, J. (2023). The effect of cadmium on plants in terms of the response of gene
439 expression level and activity. *Plants* 12:1848. [doi:10.3390/plants12091848](https://doi.org/10.3390/plants12091848).

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

443 Mukherjee, S.P., & Choudhuri, M.A. (1983). Implications of water stress-induced changes in the levels
444 of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiologia Plantarum*
445 58(2), 166-170. [doi:10.1111/j.1399-3054.1983.tb04162.x](https://doi.org/10.1111/j.1399-3054.1983.tb04162.x).

446 Mushtaq, N., Iqbal, S., Hayat, F., Raziq, A., Ayaz, A., & Zaman, W. (2022). Melatonin in micro-tom
447 tomato: Improved drought tolerance via the regulation of the photosynthetic apparatus, membrane

448 stability, osmoprotectants, and root system. *Life*, 12(11), 1922.
449 <https://doi.org/10.3390/life12111922>

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

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

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

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

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

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

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

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

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

480 Xu, B., Long, Y., Feng, X., Zhu, X., Sai, N., & Chirkova, L. (2021). GABA signalling modulates stomatal
481 opening to enhance plant water use efficiency and drought resilience. *Nature Communications*,
482 12, 1952. [doi:10.1038/s41467-021-21694-3](https://doi.org/10.1038/s41467-021-21694-3).

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

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

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

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

495 **Table 1:** Impact of exogenous 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 (%)	Malondialdehyd e (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

496 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-
497 aminobutyric acid.

498

499 **Table 2:** Impact of exogenous 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/yard (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

500 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
501 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
502 letters depicts the significance among means at P ≤ 0.05.

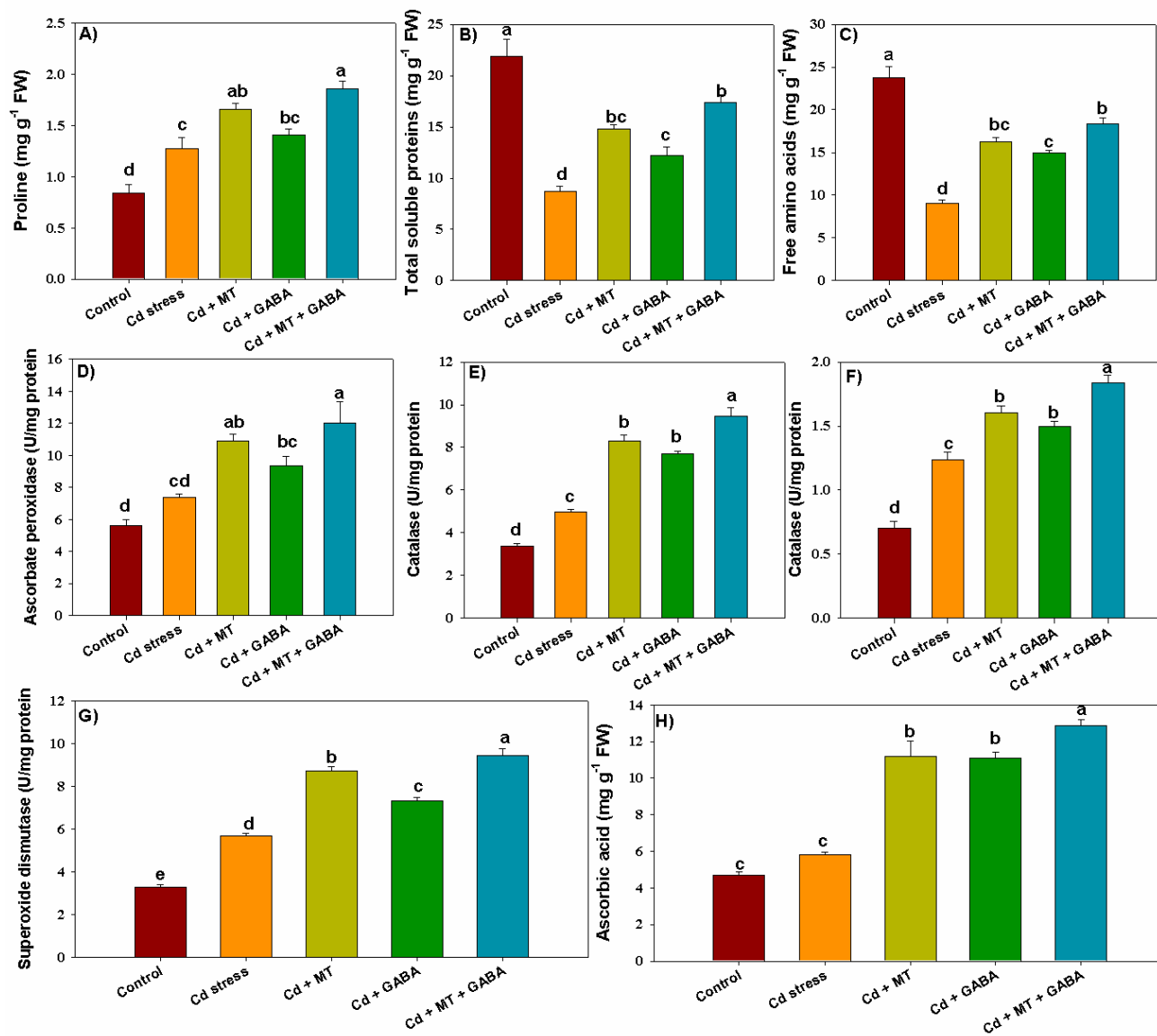


Figure 1: Impact of exogenous applied MT and GABA on the osmolyte synthesis and antioxidant activities of rice planted in Cd polluted soil. The presented data is mean of three replication with \pm SD and different letters depicts the significance at $P \leq 0.05$.

503
504
505
506
507
508
509
510
511
512

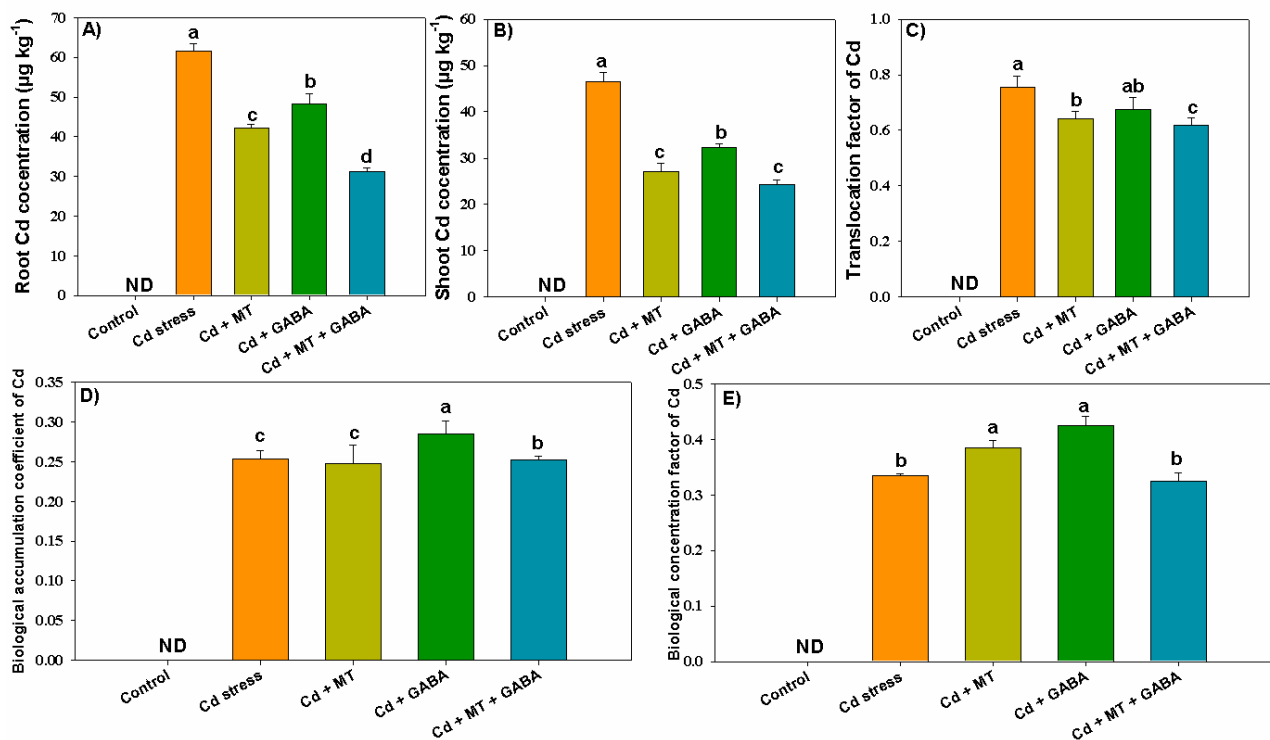


Figure 2: Impact of exogenous applied MT and GABA on root and shoot Cd concentration, TF, BAC and BCF of rice planted in Cd polluted soil. The presented data is mean of three replication with \pm SD and different letters depicts the significance at $P \leq 0.05$.

513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534

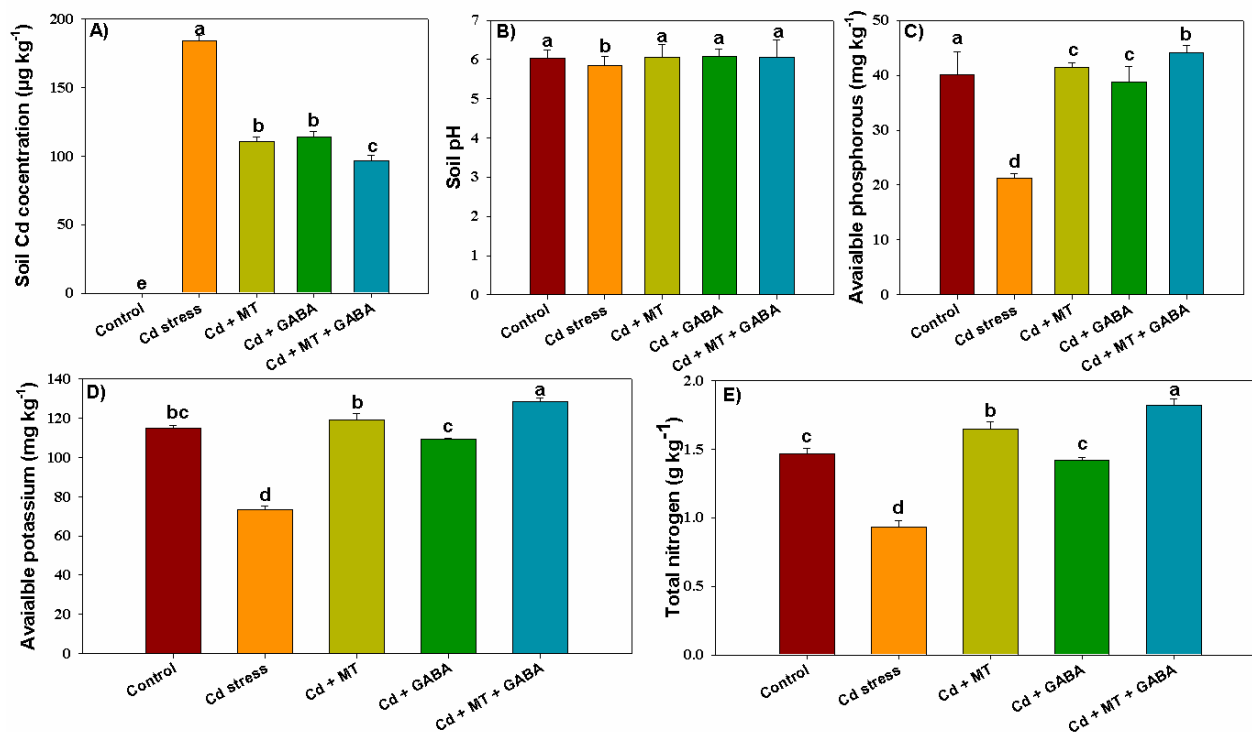


Figure 3: Impact of exogenous applied MT and GABA on soil Cd concentration, soil pH and soil N, P, and K concentration in Cd polluted soil. The presented data is mean of three replication with \pm SD and different letters depicts the significance at $P \leq 0.05$.

535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556

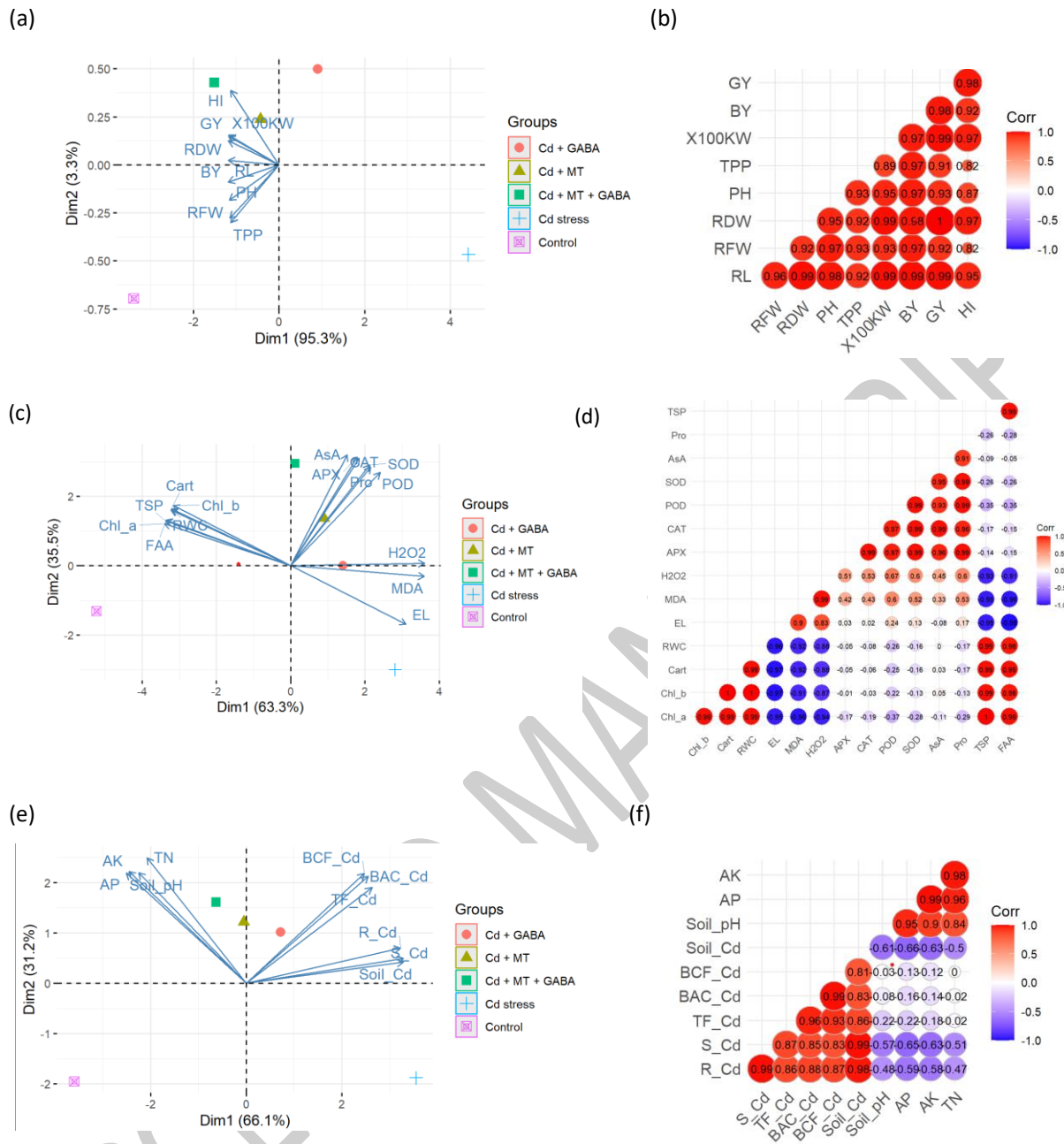


Figure 4: The principal component and correlation analysis for the impact of exogenous applied MT and GABA on growth, morpo-physiological functioning, soil properties and lead accumulation. GY: grain yield, BY: biomass yield, KW: kernel weight, TPP: tillers/plant, PH: plant height, RDW: root dry weight, RFW: root fresh weight, TSP: total soluble protein, Pro: proline, AsA: ascorbic acid, Chl: chlorophyll, RWC: relative water contents, EL: electrolyte leakage, MDA: malondialdehyde, H₂O₂: 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.