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Reclaiming multi-contaminated soil: melatonin alleviates cadmium and microplastic toxicity to restore rice growth and yield

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The increasing presence of cadmium (Cd) and microplastics (MPs) in agricultural soils is a serious challenge to crop productivity and human health. Melatonin (MT) is a vital protectant that mitigates abiotic stresses, and it has shown promising results in mitigating Cd toxicity. Its specific role in mitigating the combined stress of Cd and MPs remains unexplored, which distinguishes our study from recent studies on single stressors. This study investigated the roles of MT in modulating plant physiological function, cadmium uptake and soil properties to increase rice resilience to combined Cd and MP stress. The study included different treatments: control (soil without Cd and MPs), Cd-polluted soil (30 mg kg⁻¹), MP-contaminated soil (1%), Cd + MPs, control (soil without Cd and MPs) + MT, Cd + MT, MPs + MT, and Cd + MPs + MT. The results demonstrated that Cd + MP toxicity reduced rice yield and aboveground biomass production by 25.39% and 64.74%, respectively. The presence of MPs exacerbated Cd uptake and led to a significant increase in oxidative stress, reduced chlorophyll synthesis, osmolyte and hormone synthesis, and nutrient uptake, leading to poor yield. Melatonin treatment increased rice yield and aboveground biomass by increasing antioxidant activity, chlorophyll synthesis, water uptake, gibberellic acid, indole-3-acetic acid synthesis, and the expression of genes associated with antioxidants. A key mechanism is that MT downregulates the expression of Cd transport genes (OsNRAMP1 and OsHMA3), leading to a decrease in Cd accumulation in roots and shoots (24.93% and 41%, respectively). Furthermore, MT improved plant nutritional status by increasing nitrogen (N: 71%), phosphorus (P: 34.17%), potassium (K: 38.76%), calcium (K: 82.12%) and magnesium (Mg: 27.83%) accumulation in plant tissues. These findings suggest that applying MT alleviates Cd and MP toxicity, making it a promising strategy for reclaiming multicontaminated soils.

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Introduction

Plastics are extensively used in daily life for packaging, textiles, and electronics.¹ Global production has reached 413.8 million tons,² which is leading to the accumulation of plastics in soil and water. Microplastics (MPs) are particles with a diameter >5 mm that easily accumulate in soil, damaging plant health and human health.³ Microplastics enter soil through different sources, including atmospheric deposition, plastic mulch, contaminated water, coated fertilizers, sewage sludge, compost, and street runoff.^{4,5} They change soil properties and negatively affect plant growth by altering plant function and soil properties.^{6–9} Microplastics cause membrane damage, decrease

chlorophyll synthesis and nutrient uptake, and affect the fates of toxic metals in soil.^{7,10,11} They have a large surface area, which allows the absorption of toxic metals, increasing their availability in soil and plants.^{12,13} They also trigger reactive oxygen species, which damage cellular structures and the photosynthetic apparatus and cause yield losses.^{14,15} Microplastics also affect soil properties, nutrient and water availability, and the soil carbon pool, and decrease soil fertility.^{16,17} They are absorbed by plants and enter humans *via* contaminated foods, which has health implications.¹⁸ These findings indicate that measures to restrict MP entry into soil and plants are needed.

Heavy metal and pesticide pollution is also a serious challenge for crops and humans.¹⁹ Cadmium is a naturally occurring heavy metal, and its concentration in soils has increased over time because of atmospheric deposition, agricultural chemicals, and widespread industrial use.²⁰ It is a very toxic metal for plants, as it disturbs cell division, chlorophyll synthesis, and photosynthetic efficiency and increases oxidative damage.²¹ Cadmium toxicity damages membrane integrity,

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disrupts electron transport, and inhibits carbon fixation.²¹ Furthermore, it also decreases lateral root growth, stomatal density, and stomatal conductance, triggers osmotic stress and diminishes water and nutrient uptake, consequently causing substantial yield losses.^{22–25} Recent findings indicate that the presence of MPs in growing media alters Cd availability by changing the soil pH and the adsorption and desorption processes.²⁶ Microplastics adsorb Cd ions, which are released into the soil medium, thereby increasing Cd uptake by plants.^{19,27} Microplastics also affect the desorption of Cd from soil particles²⁸ and soil microbial and enzyme activities, which indirectly affect Cd solubility.²⁶ The interaction of MPs and Cd facilitates the rapid spread of Cd within plant tissues, and it has more negative impacts on plants than do individual Cd and MPs.²⁹ Recent studies have shown that combining Cd and MPs reduces growth and biomass by increasing Cd availability.^{30,31} Similarly, other studies conducted on wheat crops have shown that combined exposure to Cd and MPs markedly decreases biomass yield and photosynthesis efficiency due to increased Cd uptake and availability, thereby increasing the degree of toxicity.^{32,33} Conversely, some authors reported that the presence of MPs in growing media had no effect on Cd accretion in plants^{34,35} and even reduced their toxic effects by reducing their availability.³⁶ This finding indicates that coexposure to Cd and MPs has no accepted conclusion; they can have synergistic and antagonistic effects. These interactions are affected by soil properties, microbial degradation, and physical and chemical weathering.³⁷ Consequently, it is important to understand the impacts of MPs and HMs on plants and develop measures to mitigate their toxic impacts.

Globally, biochar, hormones, and organic amendments are widely used to mitigate heavy metal toxicity. Among these methods, phytohormone application is considered an effective measure for counteracting HM toxicity. Melatonin (MT) plays a crucial role in mitigating Cd toxicity by increasing antioxidant activity and osmolyte production, maintaining hormonal balance, and regulating Cd to counteract Cd toxicity.³² Melatonin also protects the cellular apparatus from oxidative damage by increasing antioxidant activities, leading to increased growth under Cd stress.³⁹ It also favors transcriptional and posttranscriptional mechanisms, which help detoxify Cd and maintain cellular homeostasis.⁴⁰ Exogenous MT supplementation also promotes root growth, ensuring better water uptake, thereby increasing overall plant performance.⁴¹ Additionally, MT also protects the stomata and photosynthetic apparatus and upregulates the expression of photosynthetic and antioxidant genes, leading to better growth under Cd.⁴² In the case of MPs, exogenous MT application decreases MP uptake and transport by regulating genes associated with aquaporins and activates antioxidant defense to maintain better redox homeostasis.⁴³ Previous studies have explored the role of MT in mitigating Cd stress. Nevertheless, the entry of MPs into paddy soil has significantly increased, which is reported to increase Cd availability. Studies have reported that coexposure to Cd and MPs negatively affects rice plants. Melatonin has shown remarkable results in mitigating Cd toxicity; considering its efficiency, this study investigated how MT

mitigates the cototoxicity of Cd and MPs. Thus, we hypothesized that coexposure to Cd and MPs synergistically inhibits rice growth by increasing oxidative damage, disturbing nutrient homeostasis and increasing oxidative damage. We also hypothesized that MT application would ameliorate combined Cd and MP toxicity by increasing antioxidant activity, modulating the hormonal balance, downregulating the expression of Cd transport genes and increasing the soil–plant nutrient balance. This study was performed to explore the mechanism by which MT counteracts the coexposure toxicity of Cd and MPs. The objectives of this study were as follows: (i) to understand the role of MT in regulating plant physiological and biochemical processes in response to Cd and MP toxicity; (ii) to explore how MT affects osmolyte synthesis, hormonal balance and gene expression to counteract the toxic impacts of Cd and MPs; and (iii) to investigate the role of MT in improving rice productivity and regulating Cd uptake and nutrient accumulation in response to Cd and MP pollution. This study provides insights into novel cross-talk mechanisms at the gene–nutrient–hormone interface, offering an eco-friendly measure to increase crop productivity in multipollutant environments.

Materials and methods

This study explored the role of MT in mitigating Cd and MP toxicity. The study was performed at Taohe Experimental Station, Baicheng city, Jilin Province, in 2023. Further details are presented in the following sections.

Experimental details

The study used soil taken from a research field (0–20 cm), which had an alkaline pH of 6.98. The soil nutrient analysis revealed available phosphorus (AP) and available potassium (AK) levels of 21.32 and 161.12 mg kg⁻¹, respectively, and a total nitrogen (TN) content of 1.82 mg kg⁻¹. The collected soil was thoroughly mixed, and debris was removed before the pots were filled. The pots were filled with 10 kg of dry soil, and CdCl₂ was used to attain the desired Cd concentration. The pots were subsequently placed in the dark under 70% light for two months. Then, the soil from the pots was removed, and the MPs were mixed with the Cd-contaminated soil and other pots according to the treatment methods and again placed in the dark for 7 days. Thereafter, the pots were filled with water, and five 25-day-old seedlings from the rice nursery were planted in each pot. A water level of 2–3 cm was maintained in the pots during the growing season to achieve good stand establishment. The different chemicals used in the study were provided by Sigma-Aldrich and were used for different analyses. The sterilized materials and standard procedures were used to measure different traits, and all the analyses were performed in triplicate to obtain reliable results.

Experimental treatments

A completely randomized design (CRD) was employed for the study, and each treatment was replicated three times. The study included different treatments: control (soil without Cd and



MPs), Cd-polluted soil (30 mg kg⁻¹), MP-polluted soil (1%), Cd + MPs, control (soil without Cd and MPs) + MT, Cd + MT, MPs + MT, and Cd + MPs + MT. foliar spraying of MT was performed at the jointing and tillering stages for two consecutive days. The pots were covered with a plastic sheet during spraying to avoid the direct entry of MT into the pots. The rates of MPs were selected from previous studies on the basis of field surveys and literature reviews.^{14,44} The rate of Cd accumulation was chosen from earlier studies reporting that 20–30 mg per kg Cd stress negatively affects plant growth and development.^{45,46} Melatonin was used at a rate of 100 μmol L⁻¹ as a foliar spray at different growth stages, and this concentration effectively mitigated Cd toxicity.^{47,48} Polyvinyl chloride MPs 50 μm in size and a density of 1.40 g cm⁻³ were used in the study. Moreover, further information about MP scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy is given in the supplementary section.

Measurement of physiological traits

Fresh leaves were collected and homogenized in 90% acetone solution. Thereafter, the absorbance was measured at different wavelengths, such as 665, 649, and 470 nm, to determine the chlorophyll concentration.⁴⁹ To determine the relative water content, the weights of the fresh leaves were measured (FW), after which the same leaves were soaked in water for 1 day. Then, they were removed from the water and weighed again (TW). These leaves were subsequently dried and weighed again (DW), after which the RWC was measured as (FW – DW)/(TW – DW) × 100. To measure electrolyte leakage (EL), fresh leaves were collected from plants, washed and boiled in water for 30 minutes to measure electrical conductivity (EC1). Thereafter, the leaves were again boiled in a water bath for 24 hours, the EC (EC2) was again measured, and the EL was determined as EL = EL1/EC2 × 100.

Measurement of biochemical traits

To measure the concentrations of malondialdehyde (MDA) and hydrogen peroxide (H₂O₂), the collected leaves were homogenized in 5 mL of trichloroacetic acid (TCA) solution. Later, to measure the MDA concentration, the enzyme extract was collected and mixed with 0.1% thiobarbituric acid (5 mL). The mixture was subsequently boiled (100 °C) for half an hour, after which the absorbance was measured at 520 °C to estimate the MDA concentration.⁵⁰ The hydrogen peroxide (H₂O₂) concentration was determined spectrophotometrically. The leaf extract was combined with 100 μL of potassium phosphate buffer (PBB) and 1 mL of potassium iodide, and the absorbance of the resulting solution was measured at 600 nm.⁵¹

To measure the antioxidant activity, leaf samples were collected and homogenized in 50 mL of PPB with a pH of 7.8. Then, the mixture was centrifuged (12 000 rpm) at 4 °C, and the supernatant was collected to measure the antioxidant activity. To measure ascorbate peroxidase (APX) activity, 100 μL of each enzyme extract, H₂O₂, and ascorbate mixture was mixed, and the absorbance was measured at 290 nm.⁵² In the case of catalase (CAT), we prepared a reaction mixture consisting of the

enzyme extract (2 mL), PPB (50 mL), and H₂O₂ (10 mM), and the absorbance was measured at 240 nm.⁵³ For peroxidase (POD), a reaction mixture containing PPB (50 mL), H₂O₂ (300 mM), and an enzyme extract (100 μL) was prepared, and the absorbance was measured at 470 nm.⁵³ Finally, for superoxide dismutase (SOD) activity, we took 25 mL of PPB, 400 μL of H₂O₂ and the enzyme extract, and the absorbance was measured at 560 nm to estimate the SOD activity.⁵⁴

The freshly collected leaves were ground and homogenized for 15 minutes at 15 000 rpm. Then, 3 mL of Bradford reagent was added to 1 of the enzyme extracts, and the absorbance was measured at 595 nm to determine the soluble protein (SP) content. To measure free amino acids (FAA), the mixture contained 1 mL of ninhydrin and pyridine, and 1 mL of the enzyme extract was prepared. The mixture was subsequently boiled (90 °C) for 30 minutes and subsequently allowed to cool, after which the absorbance was recorded at 570 °C to measure the FAA content. To measure proline, the leaves were ground with 3% sulfosalicylic acid. The extract was collected, and a mixture containing the enzyme extract, glacial acetic acid, and ninhydrin was prepared. The mixture was subsequently heated (90 °C) for 30 minutes, after which the absorbance was measured at 532 nm.⁵⁵

To measure the concentrations of abscisic acid (ABA) and indole acetic acid (IAA), freshly collected leaves (0.5 g) were ground in 2 mL of methanol (80%) and butylated hydroxytoluene (40 mg). The mixture was subsequently heated (4 °C) for 48 hours and subsequently centrifuged (1900×g) for 15 minutes. The supernatant was subsequently passed through C18 Sep-Pak cartridges using 10 mL of pure ethanol. This extract was subsequently collected and dissolved in 0.1% gelatin containing 2 mL of PPB and 0.1% Tween-20 with 2 mL of phosphate-buffered saline, and the ABA and IAA concentrations were subsequently measured according to the procedures of Weiler *et al.*⁵⁶ To measure the gibberellic acid (GA) concentration, 0.1 g of each leaf sample was homogenized in 3 mL of ethanol (96%), and the absorbance was measured at 254 nm to measure the GA concentration.⁵⁷ To quantify the hormones, calibration was performed with standards, which resulted in *r*² values exceeding 0.98. We also incorporated procedural blanks and triplicate samples to ensure overall analytical reliability.

Measurement of tissue Cd and nutrient concentrations and soil properties

The root and shoot samples were collected and digested in a mixture of acids (HNO₃:HClO₄, 4:1). The samples were subsequently filtered, the volume was increased by adding water, and the Cd concentration was measured *via* atomic absorption spectrophotometry (AAS). The external calibration was performed using standard solutions, with calibration curves (*r*² > 0.995) verified every 10 samples. Moreover, the detection limits (DLs) and quantification limits (QLs) were measured three and ten times, and the standard deviation of the blanks was 0.05 μg L⁻¹ for Cd. Moreover, recovery rates were assessed through analysis of reference material and matrix-spiked samples, which resulted in recovery rates ranging from



Table 1 Effects of exogenous melatonin application on the growth and yield characteristics of rice plants growing in cadmium- and microplastic-contaminated soil^a

Treatments	RL (cm)	RFW (g)	RDW (g)	PH (cm)	TPP	PL	TGW (g)	GY/pot (g)	BY/yield (g)	HI (%)
Control	54.04 ^{ab} ± 1.36	18.16 ^a ± 0.53	7.26 ^b ± 0.13	109 ^a ± 2.94	12.33	19.07 ^b ± 0.20	26.58 ^a ± 0.22	183.00 ^{ab} ± 2.59	39.68 ^{ab} ± 2.05	21.67 ^a ± 0.81
Cd	37.07 ^f ± 1.28	8.65 ^d ± 0.24	5.05 ^e ± 0.12	81 ^d ± 2.87	10.33	14.59 ^f ± 0.31	17.05 ^e ± 0.24	132.30 ^e ± 3.05	19.84 ^{fg} ± 1.20	14.99 ^{cd} ± 0.67
MPs	39.41 ^{ef} ± 0.83	9.81 ^d ± 0.29	5.23 ^c ± 0.13	85 ^{cd} ± 2.88	10.33	15.52 ^e ± 0.15	19.22 ^f ± 0.82	147.27 ^d ± 3.60	22.60 ^{ef} ± 1.34	15.36 ^{cd} ± 0.92
Cd + MPs	35.68 ^e ± 2.60	8.32 ^d ± 0.09	4.47 ^f ± 0.12	77 ^d ± 3.86	10.33	13.61 ^g ± 0.24	14.75 ^f ± 0.53	122.87 ^e ± 4.64	16.45 ^g ± 0.68	13.41 ^d ± 0.81
MT	58.46 ^a ± 2.10	19.77 ^a ± 1.02	8.41 ^a ± 0.18	114 ^a ± 3.30	12.33	20.37 ^a ± 0.21	28.03 ^a ± 0.15	191.98 ^a ± 2.98	42.76 ^a ± 1.69	22.27 ^a ± 0.66
Cd + MT	47.07 ^{cd} ± 1.25	15.47 ^b ± 0.46	6.33 ^{cd} ± 0.18	104 ^{ab} ± 2.49	11.67	17.82 ^c ± 0.12	23.47 ^{bc} ± 0.37	166.67 ^c ± 4.15	29.95 ^{cd} ± 0.89	17.98 ^{bc} ± 0.71
MPs + MT	49.35 ^b ± 0.74	16.15 ^b ± 0.25	60.75 ^c ± 0.17	105 ^{ab} ± 2.45	11.67	18.58 ^b ± 0.13	24.71 ^b ± 0.58	176.93 ^{bc} ± 2.99	34.74 ^{bc} ± 1.09	19.65 ^{ab} ± 0.93
Cd + MPs + MT	43.40 ^{de} ± 1.63	12.49 ^c ± 0.58	6.06 ^d ± 0.09	96 ^{bc} ± 3.74	11.33	17.05 ^d ± 0.17	22.00 ^c ± 0.23	154.07 ^d ± 1.38	27.10 ^{de} ± 2.30	17.58 ^{bc} ± 1.37

^a RL: root length, RFW: root fresh weight, RDW: root dry weight, PH: plant height, TPP: tillers/plant, TGW: thousand grain weight, GY: grain yield, BY: biological yield, HI: harvest index. Cd: cadmium, MT: melatonin, MPs: microplastics. The presented data are the means ($n = 3$) ± SDs, and letters within columns indicate significant differences at $P \leq 0.05$.

91–104%. The concentrations of calcium, potassium, and magnesium in the plant tissues were measured *via* a flame photometer, the P concentration was measured *via* a spectrophotometer, and the N concentration was measured *via* the Kjeldahl method. A soil suspension was prepared at a 1 : 5 ratio (soil: deionized water), and the pH of the suspension was then measured with a pH meter. The soil AP and AK contents were determined *via* spectrophotometer and flame photometer techniques, whereas the TN concentration was measured *via* the Kjeldahl method. The soil samples were collected, and digestion was performed at 160 °C by adding HClO₄ and HNO₃. The samples were subsequently filtered, the volume was increased by adding water, and the Cd concentration was measured *via* atomic absorption spectrometry.

Measurement of growth and yield attributes

The roots were carefully separated, and their length was measured and weighed to measure the fresh weight. The roots were then oven-dried to measure the dry weight. The tillers were manually counted, and the plant height and panicle length were measured with a measuring tape. The pots were harvested and weighed to measure biomass and grain yield, while the harvest index was determined as the ratio of grain yield to biomass yield.

Measurement of gene expression

Total RNA was isolated from plant leaf samples *via* the TRIzol reagent for gene expression analysis.⁵⁸ Complementary DNA (cDNA) was then synthesized from 1 µg of RNA with the PrimeScript™ RT Reagent Kit (with gDNA Eraser). The cDNA was subsequently diluted to 80 µl and used for qRT-PCR, which was performed *via* a TB Green™ Premix Ex Taq™ II kit (TaKaRa). The qRT-PCR was performed in a reaction volume containing 1 µL of cDNA, 1 µM concentrations of both the forward and reverse primers, and 10 µL of a commercial PCR master mix. Three biological replicates were used to ensure the robustness and reliability of the results, and the expression of different genes was measured *via* the methods of Livak and Schmittgen.⁵⁹ A comprehensive list of the different primers used for measuring gene expression is provided in Table S2.

Statistical analysis

The collected data were analyzed *via* one-way analysis of variance *via* Statistix 8.1, and the significance among means was separated *via* the honestly significant difference test at 5% probability. Furthermore, the homogeneity of variances was controlled by Bartlett's test, and normality was studied by the Kolmogorov–Smirnov test. The figures used in the experiment were generated *via* Sigmpot-10 software.

Results

Growth and yield parameters

The results revealed that Cd, MPs and their combination significantly ($p < 0.05$) reduced the growth and yield traits of the rice plants (Table 1). The minimum root length (RL: 35.68 cm),

root fresh weight (RFW: 8.32 g), root dry weight (RDW: 4.47 g), and plant height (PH: 77.33 cm) were measured in response to combined Cd + MP exposure (Table 1). Exogenous MT spray mitigated Cd- and MP-induced toxicity and substantially ($p < 0.05$) increased the RL, RFW, RDW, and PH (Table 1). Overall, the longest roots (58.46 cm), with maximum fresh (19.77 g) and dry biomass (8.41 g), and PH (114 cm) were found in the plants that received MT in the Cd- and MP-free soils (Table 1). Furthermore, MT increased the RL, RFW, RDW, and PH by 21.63%, 50.12%, 35.57% and 24.14%, respectively, in the Cd + MP-polluted soil (Table 1). The results revealed that the different treatments had a nonsignificant ($p < 0.05$) effect on tiller production during the growing period (Table 1). Different treatment combinations significantly affected the panicle length (PL), 1000 GW, biomass yield (BY), grain yield (GY) and harvest index (HI) (Table 1). Cadmium and MPs significantly ($p < 0.05$) decreased the aforementioned traits; nevertheless, coexposure to Cd + MPs caused a greater reduction in these traits (Table 1). For example, compared with the control, combined Cd + MPs resulted in reductions of 40.11%, 80.20%, 48.93%, 141.21% and 61.59% in PL, 1000-GW, BY, GY, and HI, respectively (Table 1). Exogenously applied MT mitigated Cd + MP toxicity and enhanced the PL, 1000-GW, BY, GY, and HI by 28.58%, 49.15%, 25.39%, 64.74% and 31.09%, respectively, in Cd + MP-polluted soil (Table 1).

Physiological traits and oxidative markers

The results demonstrated that compared to control treatments, Cd, MP, and specifically, their co-exposure significantly ($p < 0.05$) decreased chlorophyll (Chl) concentration and enhanced the production of oxidative markers (Table 2). Notably, Cd + MPs decreased the Chl *a*, Chl *b*, and carotenoid contents and the RWC by 53.44%, 83.82%, 40.49% and 56.86%, respectively, while they significantly increased EL, MDA, and H₂O₂ production (Table 2). Nevertheless, exogenic MT increased photosynthetic pigments and mitigated the production of oxidative markers. Under Cd + MP stress, MT increased the contents of Chl *a*, Chl *b*, and carotenoids and the RWC by 20.68%, 48.59%, 46.59% and 28.09%, respectively, compared with those in the

Cd + MP treatment (Table 2). Moreover, MT also decreased EL, MDA, and H₂O₂ production by 28.53%, 39.05% and 52.12%, respectively, under Cd + MP stress (Table 2).

Antioxidant activities and osmolyte and hormone synthesis

Different treatment combinations significantly ($p < 0.05$) affected the antioxidant activity (Table 1). Overall, the lowest activities of all the antioxidants were observed in the Si group, indicating that the plants faced no stress conditions and that they had the lowest antioxidant activity. Melatonin treatment significantly increased the APX, CAT, POD, and SOD activities in the presence of Cd or MPs alone or in combination with each other. Notably, compared with the Cd + MP treatment, the Cd + MP treatment increased APX, CAT, POD, and SOD activities by 48.38%, 35.30%, 29.33%, and 86.74%, respectively (Fig. 1). The synthesis of TSPs, FAAs, and proline was significantly affected by different treatment combinations. We observed that Cd and MP stress, particularly coexposure, decreased TSP and FAA contents by 85.58% and 97.93%, respectively, whereas they increased proline synthesis by 85.71% (Fig. 1 and 2A). Melatonin treatment increased ($p < 0.05$) osmolyte synthesis in the presence of Cd, MP, or their combination (Fig. 1). Notably, MT increased TSP, FAA, and proline synthesis by 20.18%, 37.59%, and 42.51%, respectively, under Cd + MP stress compared with those in the Cd + MP group without MT application (Fig. 1 and 2A). The synthesis of hormones was significantly affected by Cd and MP stress (Fig. 1). We observed that Cd and MPs reduced GA and IAA while increasing ABA synthesis (Fig. 2). Specifically, the combination of Cd + MPs decreased GA and IAA synthesis but increased ABA synthesis (Fig. 2). Melatonin spray increased ($p < 0.05$) the GA and IAA concentrations by 56.58% and 50.69%, respectively, in response to Cd + MPs. However, it decreased ABA synthesis by 55.32% under Cd + MP stress, which helped counteract Cd + MP toxicity (Fig. 2).

Antioxidants and cadmium uptake gene expression

The effects of single Cd and MPs and their combined treatments ($p < 0.05$) are presented in Fig. 3. Cadmium, MPs, and Cd

Table 2 Effects of exogenous melatonin application on photosynthetic pigments, leaf water contents and oxidative stress markers in rice plants growing in cadmium- and microplastic-contaminated soil^a

Treatments	Chl- <i>a</i> (mg per g FW)	Chl- <i>b</i> (mg per g FW)	Cart. (mg per g FW)	RWC (%)	EL (%)	MDA (μ mol per g FW)	H ₂ O ₂ (μ mol per g FW)
Control	1.78 ^a ± 0.053	1.25 ^{ab} ± 0.036	3.21 ^{ab} ± 0.066	80 ^{ab} ± 2.16	10.46 ^f ± 0.53	5.42 ^c ± 0.21	4.26 ^c ± 0.14
Cd	1.21 ^c ± 0.059	0.74 ^{ef} ± 0.030	2.14 ^{de} ± 0.069	54 ^c ± 1.25	50.92 ^b ± 1.02	12.82 ^b ± 0.28	9.69 ^b ± 0.38
MPs	1.29 ^{de} ± 0.029	0.84 ^e ± 0.017	2.39 ^c ± 0.13	61 ^d ± 1.41	47.00 ^{bc} ± 1.33	11.07 ^c ± 0.72	8.22 ^c ± 0.15
Cd + MPs	1.16 ^e ± 0.033	0.68 ^f ± 0.028	1.91 ^e ± 0.055	51 ^e ± 0.82	56.17 ^a ± 1.70	14.67 ^a ± 0.42	11.12 ^a ± 0.82
MT	1.86 ^a ± 0.058	1.33 ^a ± 0.048	3.48 ^a ± 0.0152	84 ^a ± 1.24	8.71 ^f ± 1.37	5.06 ^e ± 0.14	3.54 ^f ± 0.12
Cd + MT	1.50 ^{bc} ± 0.017	1.09 ^{cd} ± 0.029	2.96 ^{bc} ± 0.061	70 ^c ± 2.05	40.30 ^{de} ± 0.70	9.85 ^{cd} ± 0.27	6.72 ^d ± 0.14
MPs + MT	1.61 ^b ± 0.026	1.16 ^{bc} ± 0.028	3.08 ^{bc} ± 0.037	76 ^b ± 2.09	36.04 ^c ± 1.69	8.66 ^d ± 0.39	6.23 ^d ± 0.19
Cd + MPs + MT	1.40 ^{cd} ± 0.029	1.01 ^d ± 0.053	2.80 ^c ± 0.062	65 ^{cd} ± 0.47	43.70 ^{cd} ± 1.82	10.55 ^c ± 0.47	7.31 ^{cd} ± 0.18

^a Cd: cadmium, MT: melatonin, MPs: microplastics. Chl: chlorophyll, Cart. carotenoid, RWC: relative water content, EL: electrolyte leakage, MDA: malondialdehyde, H₂O₂: hydrogen peroxide. The presented data are the means ($n = 3$) ± SDs, and letters within columns indicate significant differences at $P \leq 0.05$.



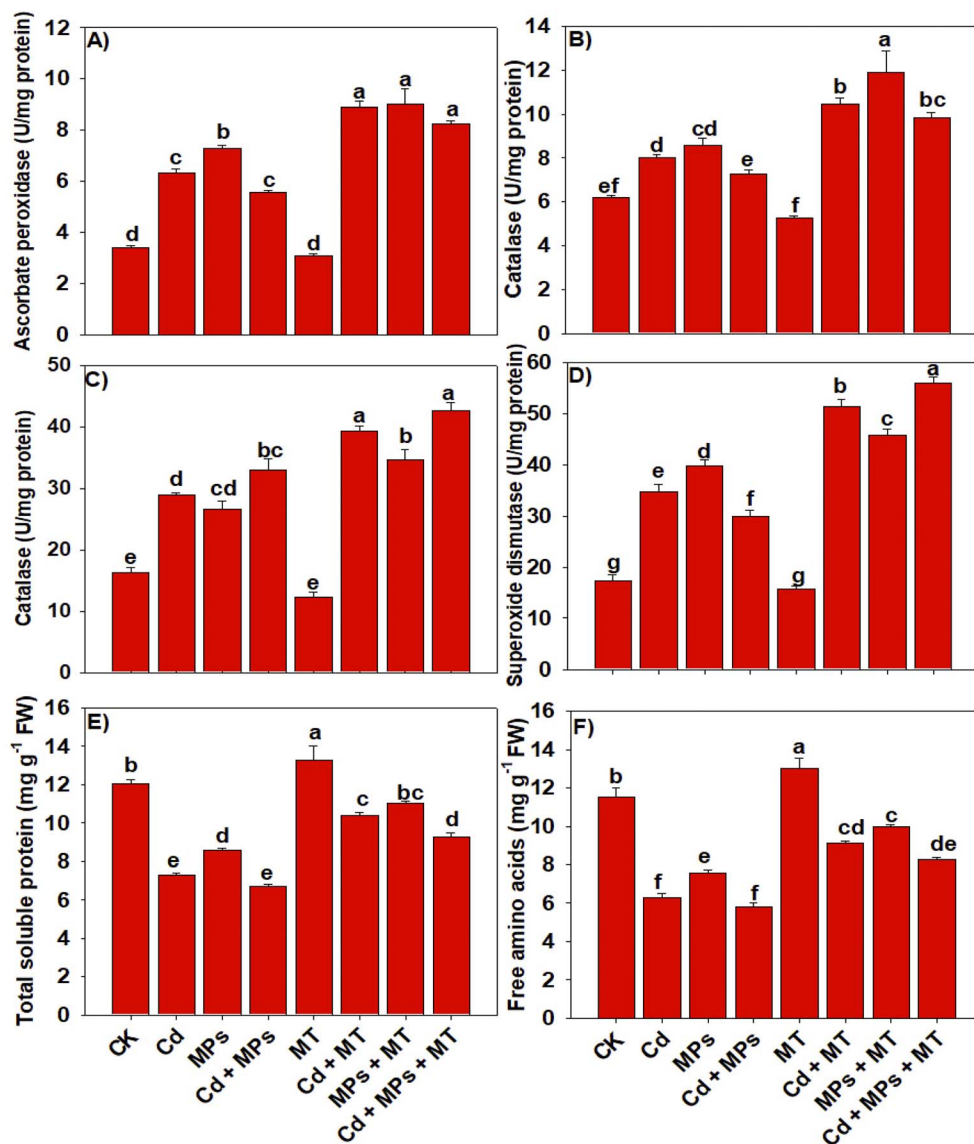


Fig. 1 Effects of exogenous melatonin application on antioxidants activities (A–D), total soluble proteins (E) and free amino acids (F) synthesis of rice plants grown in cadmium and microplastics contaminated soil. The presented data is mean ($n = 3$) with \pm SD and letters within bars indicating the significant differences at $P \leq 0.05$.

+ MPs enhanced the expression of genes involved in antioxidant activity (Fig. 3). Furthermore, MT treatment also caused a significant ($p < 0.05$) increase in the expression of genes related to antioxidant activity. We observed that, compared with the control treatment, Cd + MPs significantly increased *OsApx6*, *OsCAT*, *OsPOD*, and *OsSOD* expression (Fig. 3). In the Cd + MPs group, exogenously applied MT increased *OsApx6*, *OsCAT*, *OsPOD*, and *OsSOD* expression by 29.49%, 36.36%, 19.09% and 48.26%, respectively, compared with that in the Cd + MPs group without MT (Fig. 3). The expression of Cd uptake genes was also significantly ($p < 0.05$) affected by the different treatments. For example, Cd and MPs increased the expression of *OsNRAMP1* and *OsHMA3*, which increased Cd uptake, whereas MT decreased the expression of these genes, thereby leading to a decrease in Cd uptake (Fig. 3). For example, MT spray

decreased the expression of *OsNRAMP1* and *OsHMA3* by 47.65% and 37.82%, respectively, in the Cd + MPs group without MT (Fig. 3).

Cadmium and nutrient accumulation in plant tissues

The cadmium concentration in plant tissues increased in the presence of MPs, indicating that MPs act as vectors for Cd, thereby increasing Cd availability and accumulation in plant tissues (Fig. 4). Compared with the absence of MT, melatonin treatment significantly ($p < 0.05$) decreased Cd accumulation in plant tissues (Fig. 4). Melatonin treatment decreased ($p < 0.05$) the accumulation of Cd in roots and shoots by 60.91% and 33.40%, respectively, compared with that in the absence of MT application in the Cd + MP-contaminated soil (Fig. 4). The coexposure of plants to cadmium, MPs, and particularly



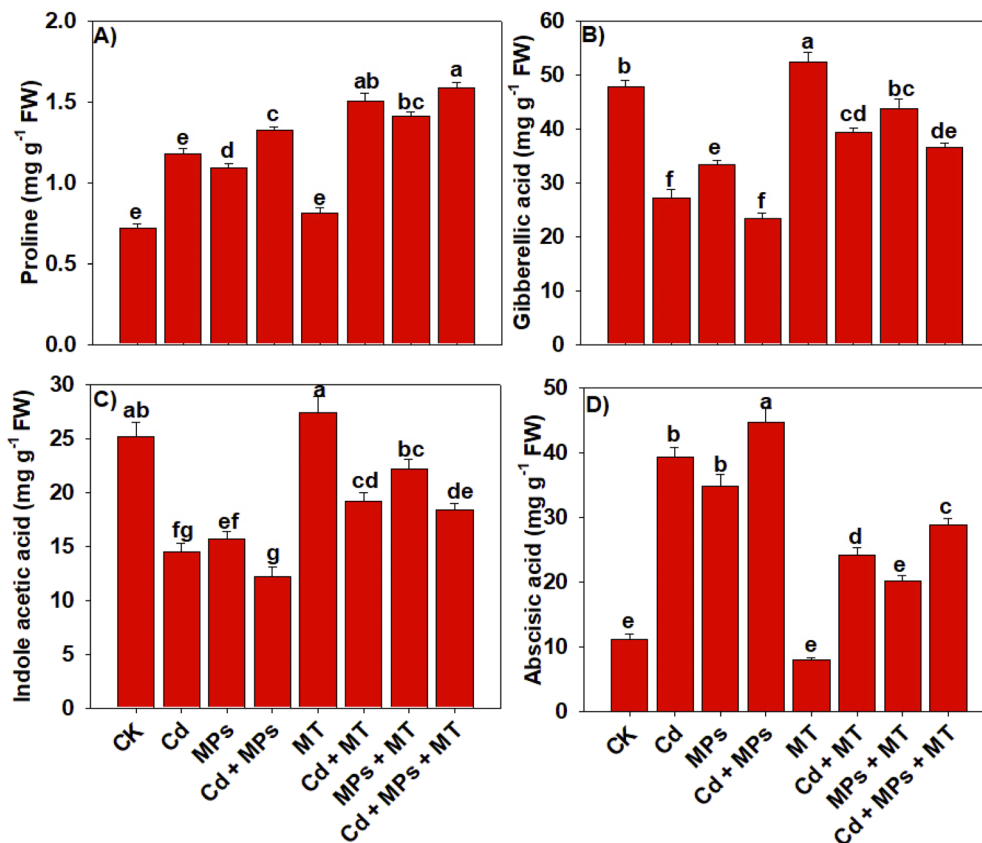


Fig. 2 Effects of exogenous melatonin application on proline (A), gibberellin acid (B), indole acetic acid (C) and abscisic acid (D) concentration of rice plants grown in cadmium and microplastics contaminated soil. The presented data is mean ($n = 3$) with \pm SD and letters within bars indicating the significant differences at $P \leq 0.05$.

cadmium, significantly ($p < 0.05$) inhibited nutrient accumulation in plant tissues. We observed that Cd + MPs decreased the nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) concentrations in the seedlings by 18.35%, 11.78%, 5.59%, 35.21% and 10.43%, respectively, compared with those in the Cd stress treatment (Table 3). Nevertheless, MT substantially ($p < 0.05$) increased the nutrient concentration in the rice seedlings. In the Cd + MPs group, MT treatment significantly increased the N, P, K, Ca, and Mg concentrations in the seedlings by 71%, 34.17%, 38.76%, 82.12% and 27.83%, respectively, over those in the Cd + MPs group without MT (Table 3).

Soil cadmium and nutrient availability after rice plants were harvested

The present results revealed that the presence of MPs in growing media significantly ($p < 0.05$) increased Cd availability (Fig. 5). However, the MT supply decreased the soil Cd availability by 29.87% and 23.53% in the Cd- and Cd + MP-contaminated soils, respectively (Fig. 5). Different treatments also had a minor effect on the soil pH; for example, the combined effects of Cd and MP caused a slight decrease in the soil pH, whereas MT increased the soil pH under all the stress conditions (Fig. 5). The availability of soil TN, AP, and AK

significantly ($p < 0.05$) decreased under Cd and MP stress; however, a greater reduction was observed under combined Cd and MP stress. Notably, the TN, AP, and AK availability decreased by 1.97%, 8.61% and 9.67%, respectively, in the Cd + MP-contaminated soil compared with that in the Cd-contaminated soil alone (Fig. 5). Melatonin significantly ($p < 0.05$) increased the soil TN, AP, and AK availability under Cd and MP stress. We observed that under Cd + MP stress, MT enhanced soil TN, AP, and AK by 62.56%, 27.68% and 24.06%, respectively, in the Cd + MP group without MT (Fig. 5).

Discussion

Effects of melatonin on growth and yield under cadmium and microplastic stress

Microplastics are a serious threat to humans, animals, and plants. They also work as vectors for toxic metals, increase their availability and pose more deleterious impacts.^{9,13} The study findings revealed that Cd + MPs cause a substantial reduction in above- and belowground biomass. This decrease in growth was linked to poor root growth, chlorophyll content, and disturbed hormonal balance resulting in increased oxidative damage. Roots directly face MP + Cd pollution, which decreases their ability to take up water and nutrients and causes growth losses.^{37,60} Previously, Liu *et al.* reported that Cd and MPs



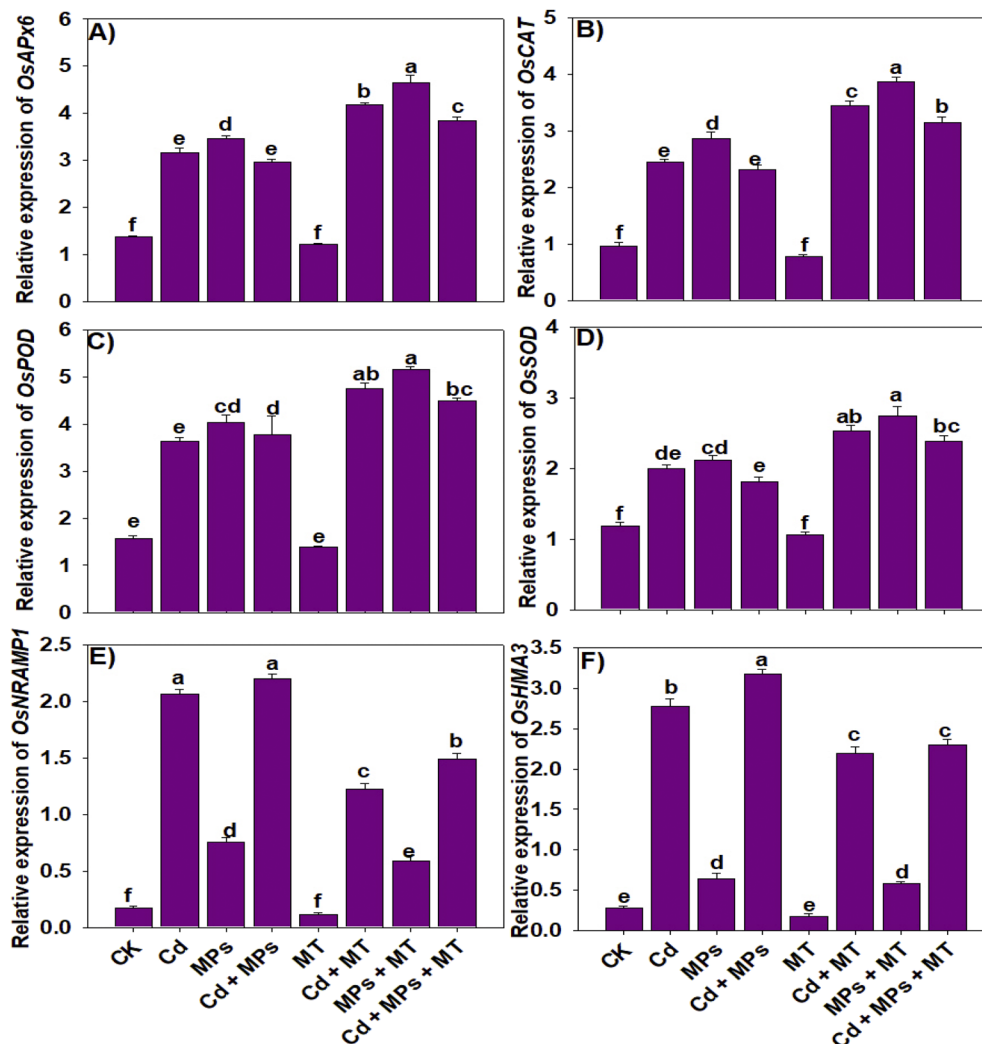


Fig. 3 Effects of exogenous melatonin application on expression of antioxidant genes (A–D) and cadmium uptake genes (E and F) of rice plants grown in cadmium and microplastics contaminated soil. The presented data is mean ($n = 3$) with \pm SD and letters within bars indicating the significant differences at $P \leq 0.05$.

decreased root growth and biomass production due to their synergistic toxic effects.⁶¹ Cadmium and MPs cause oxidative damage by increasing the level of ROS (Table 2) by

compromising antioxidant activity and hormone synthesis. Cd and MPs also indirectly decreased water and nutrient uptake, which altered soil functioning, thereby reducing rice growth

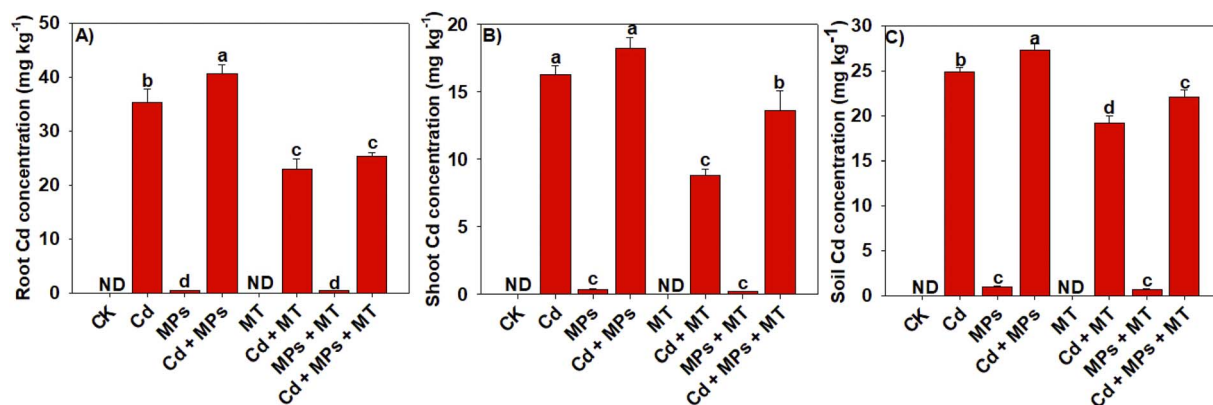


Fig. 4 Effects of exogenous melatonin application on root Cd (A), shoot Cd (B) and soil Cd (C) concentrations. The presented data is mean ($n = 3$) with \pm SD and letters within bars indicating the significant differences at $P \leq 0.05$.



Table 3 Effects of exogenous melatonin application on nitrogen, phosphorus, potassium, calcium and magnesium concentrations in rice plants growing in cadmium- and microplastic-contaminated soil^a

Treatments	Nitrogen (mg per kg DW)	Phosphorous (mg per kg DW)	Potassium (mg per kg DW)	Calcium (mg per kg DW)	Magnesium (mg per kg DW)
Control	32.91 ^a ± 1.78	38.92 ^{ab} ± 0.99	54.11 ^{ab} ± 1.32	73.67 ^a ± 1.37	64.43 ^b ± 0.98
Cd	15.15 ^{ef} ± 0.46	24.37 ^f ± 0.94	35.50 ^{de} ± 1.79	40.93 ^e ± 1.21	38.21 ^{ef} ± 1.98
MPs	18.36 ^{de} ± 0.83	33.33 ^{cd} ± 0.86	39.34 ^d ± 0.84	48.50 ^d ± 2.16	40.73 ^{de} ± 1.26
Cd + MPs	12.80 ^f ± 0.44	21.80 ^f ± 0.43	32.94 ^e ± 1.67	30.27 ^f ± 0.077	34.60 ^f ± 1.74
MT	37.08 ^a ± 1.68	42.01 ^a ± 1.71	59.07 ^a ± 2.75	77.64 ^a ± 1.95	69.77 ^a ± 1.85
Cd + MT	24.64 ^{bc} ± 1.75	32.57 ^{de} ± 0.52	50.98 ^{bc} ± 1.09	59.75 ^c ± 1.09	50.15 ^c ± 1.30
MPs + MT	28.53 ^b ± 0.75	36.47 ^{bc} ± 0.74	53.18 ^{ab} ± 0.93	65.91 ^b ± 1.34	54.88 ^c ± 1.31
Cd + MPs + MT	21.88 ^{cd} ± 1.24	29.25 ^e ± 1.66	45.71 ^c ± 2.94	55.13 ^c ± 2.09	44.08 ^d ± 0.48

^a Cd: cadmium, MT: melatonin, MPs: microplastics. The presented data are the means ($n = 3$) ± SDs, and letters within columns indicate significant differences at $P \leq 0.05$.

and yield.⁶² The presence of MPs in the growth media also decreased the TN, AP, and AK contents of the plants, further inhibiting their growth and yield. Microplastics also increase the availability and accumulation of Cd, which causes oxidative stress, damages membrane integrity and chlorophyll synthesis, and contributes to a reduction in growth and yield.⁸ Microplastics absorbed by roots block some channels linked with ion exchange, leading to a reduction in yield.^{63,64} Melatonin

counteracts abiotic stress and improves plant performance.⁶⁵ We observed that MT enhanced rice biomass and grain yield through different mechanisms. Melatonin increases chlorophyll synthesis by decreasing oxidative damage, which in turn enhances assimilate production and leads to better yields.^{38,66,67} The melatonin supplies increased root growth, which increased nutrient and water absorption, resulting in better growth.⁶⁸ We also observed that MT enhanced antioxidant activities and

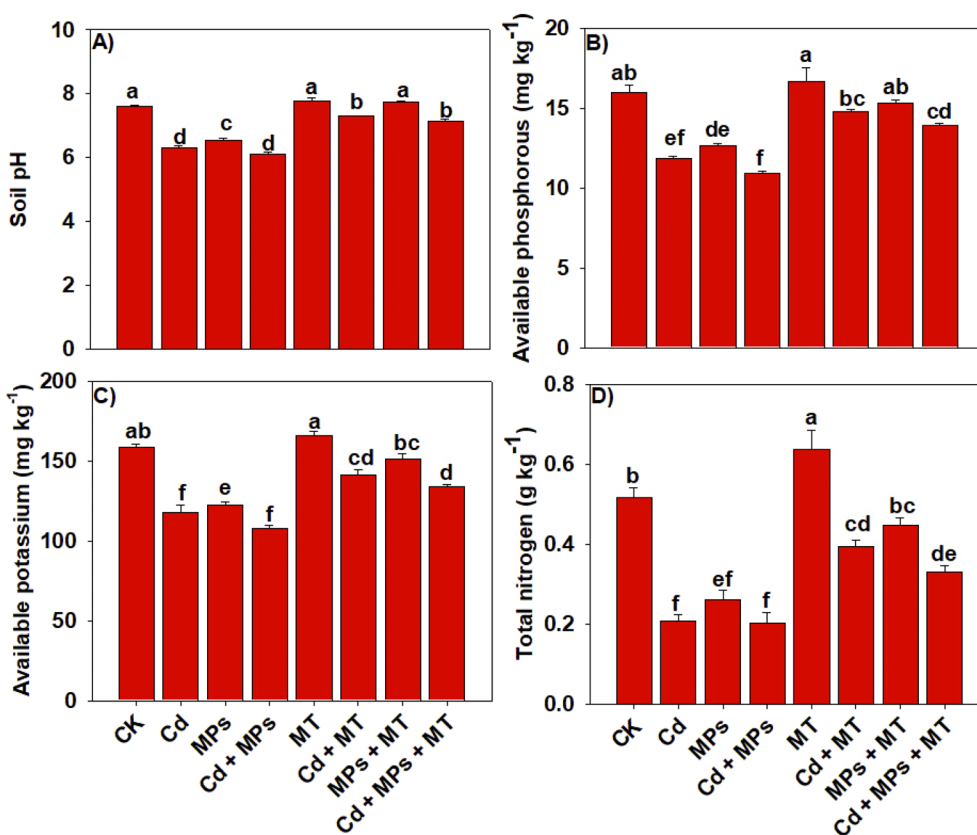


Fig. 5 Effects of exogenous melatonin application on soil pH (A), available phosphorus (B), available potassium (C) and total nitrogen (D) concentration after rice harvesting from cadmium and microplastics contaminated soil. The presented data is mean ($n = 3$) with ±SD and letters within bars indicating the significant differences at $P \leq 0.05$.



increased proline, GA, and IAA synthesis, which improved rice yield by decreasing oxidative damage. Melatonin significantly decreased Cd accumulation in plant tissues, which was a major reason for the increase in rice yield. The indoleamine structure of MT facilitates redox reactions, scavenges ROS, and modulates the cellular redox potential. Melatonin might also alter Cd speciation and bioavailability by increasing the soil pH (Fig. 5). This increase in soil pH reduces the mobility of toxic metals, thereby decreasing their uptake and accumulation in plants.⁶⁹ Microplastics serve as vectors for Cd and enhance its uptake *via* surface adsorption. However, melatonin interferes with this uptake, possibly by competing for sorption sites or forming ternary complexes. The downregulation of the Cd uptake gene after MT application reflects the response to these chemistry-driven changes in Cd bioavailability. Thus, the MT-mediated decrease in Cd uptake enhances rice yield and represents a promising chemico biological strategy for remediating multicontaminated soils.

Effects of melatonin on photosynthetic pigments, oxidative markers and antioxidants under cadmium and microplastic stress

Heavy metals decrease chlorophyll synthesis and the photosynthetic rate.⁷⁰ Cadmium and MPs decrease chlorophyll synthesis, which validates previous findings that chlorophyll synthesis decreases under stress conditions.⁷¹ However, these findings contradict those of previous studies by Li *et al.*⁷² and Wang *et al.*,³⁰ who reported nonsignificant and no impacts of Cd and MPs on chlorophyll synthesis. We observed that Cd and MPs enhanced Cd accumulation in plant tissues, which might cause chlorophyll degradation and lead to a reduction in its synthesis. Conversely, exogenous MT significantly enhanced chlorophyll synthesis by alleviating oxidative damage to chloroplasts, increasing magnesium uptake (Table 3), increasing the expression of chlorophyll biosynthesis genes, and interacting with hormones, which promoted chloroplast development and delayed senescence.^{73,74} Similarly, previous authors also reported that MT increases chlorophyll synthesis by increasing the expression of genes (POR, CAO, and CHL) involved in chlorophyll synthesis.⁷⁵

The results demonstrated that Cd and MPs significantly enhanced H₂O₂, MDA, and EL production. The increased Cd accumulation in roots and shoots is responsible for Cd- and MP-mediated ROS production.⁷⁶ This increase in ROS production might be associated with the downregulation of DEGs associated with these antioxidants. However, the MT supply significantly decreased the levels of oxidative markers under Cd and MP toxicity by increasing antioxidant activities. Generally, plants rely on antioxidant enzymes to counteract the toxic impacts of pollutants.⁷⁷ We observed that the antioxidants of rice plants increased under Cd and MP toxicity, which aligns with previous studies by Wang *et al.*⁷⁸ reporting the same findings. Previously, Nasser *et al.*⁷⁹ reported that MPs enhance their antioxidant activity to counter their toxicity, whereas Wang *et al.*⁷⁸ reported a nonsignificant impact of MPs on their antioxidant activity. Melatonin is a potent ROS scavenger that

reduces MDA and H₂O₂ production by increasing antioxidant activity (Table 2). The melatonin-mediated increase in antioxidant activity maintains redox homeostasis, thereby protecting plants from the oxidative damage caused by abiotic stress.^{80–82} Buttar *et al.*⁸³ also reported that MT decreased MDA production by increasing antioxidant activities. Furthermore, we observed that MT also significantly increased the expression of genes associated with antioxidants. These modifications in gene expression increase antioxidant activity and lead to ROS scavenging upon Cd and MP stress.³⁷

Effects of melatonin on osmolytes and hormones under cadmium and microplastic stress

Plants accumulate different osmolytes and hormones to counteract abiotic stresses. The maintenance of osmolytes and hormones plays a crucial role in stress tolerance.⁸⁴ We observed that proline synthesis increased in the presence of Cd and MPs, whereas TSP and FAA synthesis decreased. Proline improves stress tolerance by maintaining osmotic adjustment.⁸⁴ The decrease in TSP and FAA production under Cd and MPs aligns with previous studies reporting that both of these stresses decrease TSP and FAA synthesis in plants.^{85,86} Cadmium and MPs significantly decreased N uptake and concentration in rice seedlings, which could be important reasons for the decreased TSP and FAA contents. Melatonin application significantly increased the soil TN (Fig. 5) and N accumulation in plant tissues (Table 3), leading to increased TSP and FAA contents because N plays a crucial role in TSP and FAA synthesis. It is also possible that MT enhances the expression of genes encoding stress-responsive proteins and key biosynthetic enzymes. Additionally, MT also reduced oxidative damage and improved nitrogen metabolism (Table 3) and amino acid availability, which ensured cellular stability and improved protein production. Notably, Cd and MPs decreased GA and IAA synthesis, whereas they increased ABA synthesis. These findings align with those of early studies reporting that stress conditions decrease GA and IAA synthesis and increase ABA production, whereas MT restores these hormones to counteract stress conditions.⁸⁷ The combined stress might decrease GA and IAA synthesis through genetic downregulation and reallocation of plant resources. Cadmium toxicity inhibits the activity of enzymes involved in IAA and GA synthesis. Furthermore, MPs increase Cd availability, which induces synergistic stress; therefore, all these changes contribute to a reduction in IAA and GA synthesis. The cellular and oxidative damage caused by Cd and MPs might trigger the activity of ABA biosynthesis genes and enzymes, rapidly increasing ABA synthesis. Increased ABA synthesis increases stomatal closure, whereas MT decreases ABA synthesis by downregulating ABA synthesis genes and upregulating ABA catabolism genes, which leads to a reduction in ABA synthesis.⁸⁸ Moreover, MT enhances GA synthesis and IAA, and positive cross-talk between MT, GA, and IAA has been reported in the literature.⁸⁹ It is possible that MT protected the enzymes involved in the synthesis of these hormones; therefore, it increased IAA and GA synthesis. Moreover, MT improved nitrogen assimilation, which provides metabolic precursors and



ensures better hormone synthesis. Melatonin also decreased ABA synthesis, possibly by directly interfering with ABA biosynthesis pathways and modulating the upstream stress signals that trigger ABA. We also observed that MT scavenged ROS and mitigated cellular damage; therefore, MT might reduce the signaling involved in the activation of ABA synthesis.

Effects of melatonin on cadmium and nutrient accumulation in plant tissues under cadmium and microplastic stress

Microplastics interact with Cd and increase its availability and uptake by plants because of their hydrophobic nature and larger surface area.³⁷ In the present study, MPs increased Cd availability, which is the same as the findings of Huang *et al.*,⁹⁰ who reported that MPs increase Cd availability and accumulation. Microplastics change the soil chemistry and reduce the soil pH, which increases Cd desorption, and they also interact with soil dissolved organic matter, modifying heavy metal complexes and thereby increasing their availability.⁹¹ We observed that MPs decreased the pH, which aligns with the findings of Janczak *et al.*⁹² Moreover, these findings contrast with those of previous studies reporting that MPs increase soil pH.^{93,94} Microplastics generate H⁺ and lactic acid, which are utilized by microbes and lead to a decrease in soil pH.⁹⁵ However, MT increased the soil pH, which was associated with different mechanisms. MT stimulates root secretion and alters ion uptake; therefore, it increases the soil pH. Heavy metals interfere with nutrient uptake and transport, while MPs obstruct the root surface, thereby reducing water and nutrient absorption.^{90,96} We observed that Cd and MPs decreased soil nutrient availability and accumulation. The combined pollution exacerbates nutrient deficiency, thereby impairing growth and yield.⁹⁴ In addition, Cd and MPs also accumulate within the pores of the cell wall, which further restricts water and nutrient flow, resulting in reduced uptake efficiency.⁹⁷ It is also possible that Cd affects membrane permeability and decreases nutrient uptake by altering membrane-bound protein activity.⁹⁸ These processes affect nutrient transporters, thereby decreasing nutrient availability. Cadmium also binds with sulfhydryl groups in proteins, disrupting their function and the structure of nutrient transport proteins and enzymes and decreasing their availability.⁹⁹ However, the adsorption of Cd by MPs largely affects their inherent properties. The chemical compositions and sources of polymers exhibit different adhesion capacities. In particular, the presence of oxygen-containing functional groups on the MP surface induces strong interactions with Cd ions; therefore, the presence of oxygen increases Cd availability to plants. Melatonin treatment significantly enhanced nutrient uptake by improving root growth and possibly fixing Cd in the soil, which reduced the competition between nutrients and Cd and increased nutrient uptake and accumulation in plant tissues. Melatonin also decreases Cd accumulation by decreasing the expression level of Cd uptake genes (*OsNRAMP1* and *OsHMA3*).¹⁰⁰ Genes such as HMA prevent Cd transport from roots to shoots, reduce Cd accumulation from roots to xylem, and play important roles in Cd sequestration in vacuoles.^{1,101} We observed that the level of *OsHMA3*

increased under Cd and MT treatments, indicating its role in mitigating Cd accumulation. Moreover, other genes, including *Nramp1* and *LCT1*, play crucial roles in Cd transport.¹⁰² We found that MT decreased *OsNRAMP1*, leading to decreased Cd uptake, which aligns with the findings of previous studies, indicating that MT decreased Cd uptake by downregulating gene expression.^{37,103,104}

Conclusion

The findings revealed that the combination of cadmium and microplastic significantly decreased the rice yield and biomass. The presence of microplastics in the growing media promoted cadmium accumulation, which induced oxidative stress and decreased photosynthetic pigments, hormone synthesis, and water and nutrient uptake, leading to a decrease in growth and yield. Melatonin treatment increased the rice grain yield (64.74%) and biomass yield (25.39%) through multiple pathways. Melatonin decreased cadmium uptake and accumulation, which was achieved through the downregulation of *OsNRAMP1* (45.67%) and *OsHMA3* (37.82%). Melatonin also improves plant nutrient balance by increasing nutrient accumulation, which maintains better chlorophyll synthesis, antioxidant, and hormonal balance, leading to better growth and yield. Melatonin can alleviate the toxic impacts of cadmium and microplastics on rice by modulating plant function and maintaining nutritional homeostasis and gene expression. Furthermore, research should clarify the impacts of melatonin on soil nutrient cycling, soil bacterial and fungal populations, and plant transcriptomic and metabolomic responses in cadmium- and microplastic-polluted soils. In the future, multipoint verification can be carried out across different soil types (saline-alkali, red soil, black soil, *etc.*) and seasons (early and late rice) to evaluate the universality of the effects of melatonin in mitigating cadmium and microplastic toxicity. Furthermore, studies can track the morphology of soil cadmium, the aging characteristics of microplastics, and the safety of subsequent crops and assess long-term environmental risks. Additionally, field studies are needed to explore the roles of melatonin in counteracting cadmium and microplastic toxicity. The observed changes in gene expression levels do not directly confirm ion flux; rather, they reflect a regulatory response, which needs validation through transcriptomic and proteomic studies. Furthermore, the adsorption dynamics of cadmium by microplastics are largely affected by microplastic type, size, surface chemistry, and aging. Future studies should aim to predict the environmental behavior of microplastics.

Author contributions

Xie Zhiming: writing the original draft and methodology. Muhammad Aamer: conceptualization, investigation. Fahd Rasul: supervision, project administration. Abdul Ghafour, reviewing and editing. Muhammad Munir, review and editing. Hassan Ali-Dinar, data curation Muhammad Aamer.



Conflicts of interest

There are no conflicts to declare.

Abbreviations

Cd	Cadmium
MT	Melatonin
MPs	Microplastics
N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
Chl	Chlorophyll
Cart	Carotenoid
RWC	Relative water contents
EL	Electrolyte leakage
MDA	Malondialdehyde
H ₂ O ₂	Hydrogen peroxidase
IAA	Indole acetic acid
GA	Gibberellin
ABA	Abscisic acid
APX	Ascorbate peroxidase
POD	Peroxidase
CAT	Catalase
SOD	Superoxide dismutase
TN	Total nitrogen
AP	Available phosphorus
AK	Available potassium
RL	Root length
RFW	Root fresh weight
RDW	Root dry weight
PH	Plant height

Data availability

The data supporting the conclusions of this article are included within the article.

Supplementary information (SI) is available. See DOI: <https://doi.org/10.1039/d5ra10106a>.

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