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Ameliorating heavy metal-induced oxidative stress in valerian: The role of melatonin

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Abstract: Heavy metals ubiquitously found in soil and water, represent a serious environmental problem that disrupts plant mineral nutrition homeostasis, osmotic balance and metabolism. The application of some biostimulants can alleviate these disruptions. Melatonin as a signal molecule, and antioxidant plays an important role in plant growth and stress tolerance due to its ability to directly neutralize reactive oxygen and nitrogen species. The reduction or mitigation of heavy metals adverse effects in valerian plants grown in open field conditions using melatonin was investigated in this study. High-pressure liquid chromatography coupled with a fluorescence detector was used to identify and quantify melatonin concentration in valerian root extracts. Also, the physiological and biochemical status of plants under abiotic stress was examined, especially in 100 µM melatonin pre-treated plants. Higher concentrations of endogenous melatonin were measured in roots of Cd and Zn treated plants. Melatonin application alleviated the negative effect of Cd, particularly evident in Cd-melatonin treatment which restored or enhanced bioactive compound levels. Melatonin effectively mitigates Cd and Zn-induced stress in valerian by enhancing both non-enzymatic and enzymatic antioxidant systems and promoting the synthesis of protective compounds. These findings highlight melatonin's potential as a sustainable biostimulant to support plant resilience and productivity in heavy metal-stressed environments.

Keywords: abiotic stress; heavy metals; phytomelatonin; *Valeriana officinalis* L.

INTRODUCTION

Melatonin has been known as a non-toxic and universal molecule, naturally occurring in plants and humans.[1] Melatonin (*N*-acetyl-5-methoxytrytamine), an indoleamine synthesized from tryptophan and secreted by the pineal gland, is also

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found in plant chloroplast and mitochondria, where it participates in several metabolic pathways. It has been found in almost all forms of organisms (invertebrates, algae, fungi and bacteria). The established melatonin concentration in different plant species vary from 2 to 5000 μg/g of dry matter. The measured concentrations are affected by plant genotype, development stage, plant tissue analyzed and environmental conditions (salinity, temperature, ultraviolet light, heavy metals).[2]

Melatonin actively reacts with free radicals and stimulates various physiological, morphological and biochemical plant features, from seed germination to biological yield.[3] Melatonin is involved in development processes, circadian rhythms regulation, promotion of photosynthesis, fruit ripening, chlorophyll preservation and leaf senescence, among others.[2,4] The role of melatonin in reducing plants' stress is achieved by upregulating stress-related genes that scavenge reactive oxygen species (ROS) and improve antioxidant capacity of plants.[5,6]

Abiotic stress, which influences plant growth and reducibility is associated with osmotic and oxidative stress, ionic imbalance and cell metabolism dyshomeostasis.[7] Most heavy metals, assigned as abiotic stressors, cause continuous production of ROS in the chloroplast, mitochondria, and peroxisomes, which can cause oxidative stress in plants and result in the unexpected consequence of heavy metal toxicity.[8]

Valeriana officinalis is a well-known medicinal plant widely used in phytotherapy for its calming, sedative and anxiolytic effects, primarily attributed to its root extracts. It is commonly used to alleviate sleep disorders, anxiety, and nervous tension. In human phytotherapy, polyphenols are valued for their strong antioxidant, anti-inflammatory and potective properties, contributing to the prevention of chronic diseases such as cardiovascular disorders, neurodegenerative conditions and certain cancers.[9]

The negative effect of different heavy metals can be mitigated through exogenous melatonin, directly improving the stress tolerance of different plant species by scavenging ROS, and indirectly, by increasing antioxidant activities, photosynthetic efficiency and metabolite content.[10]

The identification and quantification of endogenous melatonin in valerian leaves and roots, as well as the modulation of secondary metabolites, reactive species detoxification and antioxidant upregulation by exogenous melatonin and heavy metals, cadmium and zinc, have not been carried out so far. To better understand the role of melatonin in plants subjected to the maximum allowed concentrations of cadmium and zinc, a set of experiments was conducted on valerian (*V. officinalis* L.). The assessment of potential protective role of melatonin in mitigating heavy metal stress effects in valerian, enhancing antioxidant defense mechanisms, regulating nutrient and protein metabolism and modulating enzymatic activity related to oxidative stress was determined. The study highlights a strong correlation between phenolic content and antioxidant activity, particularly in leaves.

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Melatonin pre-treatment improved plant tolerance to oxidative stress caused by heavy metals, suggesting its potential role in enhancing valerian resilience and its suitability for phytoremediation in contaminated environments.

EXPERIMENTAL

Valerian seedlings were obtained from a local herb collector. The valerian seedlings were immersed in water and 100 µM melatonin solution for 48 h, in the dark, after which the plants were planted in open field conditions. The soil showed a slightly acidic pH reaction, with pH in KCl being 5.56, suitable for growing most medicinal plants. The experiment included six treatments: *i*) control (valerian seedlings immersed in water for 48 h; *ii*) Cd (15 mg/L cadmium sulfate solution treatment after planting in open field conditions); *iii*) Zn (3 g/L zinc sulfate solution treatment); *iv*) melatonin (valerian seedlings were immersed in a 100 µM melatonin solution, for 48 h, in the dark, prior to planting); *v*) melatonin and Cd (melatonin pre-treatment and cadmium sulfate treatment); *vi*) melatonin and Zn (melatonin pre-treatment and zinc sulfate treatment). Three replicates (9 plants per replicate) were used for each treatment. Plants were sampled at the end of October, lyophilized at –50 °C for 25–30 h (VaCo 2, Zirbus Technology, GmbH, Germany) and stored at 4 °C until extraction. All analyses were performed in triplicate.

Chemicals

All solvents and reagents were of analytical or the highest grade available. Water (HPLC grade), methanol (HPLC grade), ethanol (HPLC grade), melatonin (HPLC grade), quercetin-3--beta-D-glucoside, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, 2,4,6-Tris-(2-pyridyl)-*s*-triazine (TPTZ), Tween 20, phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma–Aldrich. Hydrochloric acid, nitric acid, hydrogen peroxide, acetic acid, sodium hydroxide, zinc(II) hydroxide, cadmium(II) hydroxide, sodium bicarbonate were purchased from Lachner. Gallic acid, Trolox, ferric chloride anhydrous, ferrous sulfate heptahydrate, sodium acetate trihydrate, magnesium carbonate, polyvinylpyrrolidone (PVP), copper(II) chloride, ammonium acetate, sodium dihydrogen phosphate, potassium-sodium tartrate, neocuproine and pyrogallol were purchased from Acros. All solutions were prepared in distilled water.

Melatonin analysis

Melatonin direct extraction with methanol was performed under dark artificial light.[11] Weighted lyophilized valerian roots were mixed with methanol in total volume of 10 mL. After 15 – 17 h of shaking at 4 °C in the dark, 30 min ultrasonic treatment was performed at the same temperature (WiseClean WUC, Witeg GmbH, Germany). Prior to vacuum evaporation (Rotavapor R-215, Buchi Switzerland), the tubes were centrifuged at 6000 rpm for 30 min (Alresa Mod, Digicen). The extracts were dissolved in 1 mL of methanol, filtered (0.45 µm) and analyzed with high-pressure liquid chromatography (HPLC, Agilent 1100 Series) coupled with a fluorescence detector (FLD), reversed phase C18 gravity column (Nucleodur, 3 µm particle diameter, Macherey-Nagel, Germany) and integrated pre-cell as well as programmed mobile phase consisting of 20 % methanol: 80 % water. The flow rate of the analyte was 1.5 mL/min, at 25 °C.

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Heavy metal and macro-elements determination

Perkin–Elmer Analyst 400 atomic absorption spectrophotometer (AAS) was used to determine heavy metal concentrations. Lyophilized valerian leaves and roots were digested with 3 mL of concentrated HNO_3, 3 mL of H_2O_2 and 1 mL of concentrated HCl in closed polytetrafluoroethylene (PTFE) vessels in a microwave oven. All determined metals were atomized in

an oxidizing light blue flame formed by mixture of compressed air (10 L/min) and acetylene (2.5 L/min). Contents of Cd, Zn, Mg and Ca were established respectively at the wave lengths: 228.8, 213.7, 285.2 and 422.7 nm, using the deuterium background for correction of signal for Cd and Zn.

Total phenol and flavonoid determination

Folin–Ciocalteu (FC) colorimetric method in alkaline medium was used to analyze the total phenols from 30 % ethanol extracts.[12] Samples or gallic acid standards (200 μL) were mixed with 1 mL of FC reagent, followed by 800 μL Na_2CO_3 after 5 min. After 2 h incubation in the dark, absorbance was read at 760 nm. The results were expressed as mg of gallic acid equivalents (GAE) per g of extract. Chang *et al*. colorimetric method was used to determine the flavonoid content.[13] Quercetin was used for the calibration curve. Samples or standards were mixed with ethanol, aluminum chloride, potassium acetate and water, then incubated for 30 min at room temperature. Absorbance was measured at 415 nm, with blanks prepared by replacing aluminum chloride with distilled water.

Antioxidant activity

Benzie and Strain method was used, for the determination of ferric reducing antioxidant power.[14] Reduction potential substances react with potassium ferricyanide in forming the ferrocyanide (absorption maximum at 593 nm). Also, the antioxidant capacity of extracts was measured on the basis of the stable 1,1-diphenyl-2-picrylhyorazyl (DPPH) free radical scavenging activity according to the method described by Soler-Rivas and coworkers.[15] Apak *et al*. method was used to measure the cupric reducing antioxidant capacity of plant root and leaf extracts. Trolox was used as a standard, and the reduction potential was expressed as $TEAC_{CUPRAC}$ in mmol equivalents of trolox g dry matter (mmol/L).[16] All experiments were measured using PhotoLab 6600 UV–Vis spectrophotometer (Xylem Analytics Germany Sales GmbH & Co. KG, WTW).

Protein content determination

Protein concentration was determined by Lowry *et al*., and calculated by comparison with a standard curve using BSA as a standard.[17] For protein and enzyme activity determination, lyophilized valerian leaves and roots were powdered in liquid nitrogen and kept at –20 °C to analysis.

Spectrophotometric analysis of peroxidase activity (E.C. 1.11.1.7)

For the peroxidase activity determination, modified Teisseire and Guy method was used.[18] The increase in absorbance at 430 nm (ε_{430} = 12 mM^{-1} cm^{-1}) was monitored. The reaction was initiated by adding 3.43 mM H_2O_2 to a mixture containing 50 μL of sample, 10.3 mM pyrogallol and Na phosphate buffer (pH 6.4) at 37 °C. The activity is expressed as μmol/(mg protein min).

Statistical analysis

The data were analyzed using SPSS Statistics, v. 23.0. Analysis of variance (ANOVA) was conducted and significance of differences among treatments and time dependence were tested using the least significant difference (*LSD*). Differences were significant at the $*p < 0.05$ probability level.

RESULTS AND DISCUSSION

Having ascertained that melatonin could effectively ameliorate Cd and Zn--induced phytotoxicity in valerian, the actions were taken to estimate whether this

toxicity had an effect on melatonin biosynthesis. Fig. 1 shows the results of endogenous melatonin in the roots of valerian after treatment with exogenous melatonin (100 μM) and heavy metals. Valerian, as a plant with exceptional medicinal properties, showed significant concentrations of melatonin in roots, with values of 3.14±0.102 μg/g dry weight and 2.75±0.006 μg/g.

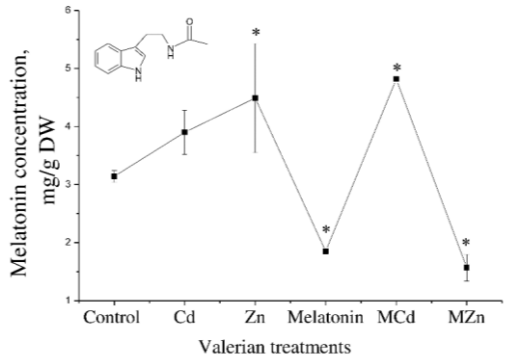

Fig. 1. Melatonin in valerian roots under heavy metal treatment and melatonin pre-treatment. M – melatonin; *Tukey test; $p < 0.05$.

The results indicate that melatonin concentration in the roots increased in response to heavy metal treatments, both in the presence and absence of exogenous melatonin. However, this increase was not observed in the Zn and melatonin treatment, suggesting a possible interaction that limits melatonin accumulation under this specific condition. As shown in Fig. 1, the highest concentration was observed in the roots of plants treated with Zn ions, and with melatonin and Cd ions (4.491 and 4.879 μg/g), which are 30 and 35 % higher concentrations compared to the control roots. There was a decrease in endogenous melatonin content in samples pre-treated with melatonin. This observation aligns with earlier findings, suggesting a complex regulation of melatonin biosynthesis in response to external stress factors like heavy metals.

There are lots of evidences that confirm a great influence of heavy metal ions on the content of compounds with hormonal function. Melatonin synthesis occurs in parallel with melatonin degradation in the chloroplasts and cytoplasm, and the resulting melatonin metabolite, 2-hydroxymelatonin, also acts as a signaling molecule for the induction of defense genes. Melatonin content in paddy rice shoots was increased under the influence of Cd, suggesting that melatonin could play a crucial role in adjusting the response of different parts of the plant to Cd.[19] In

wheat seedlings, exogenous melatonin increased endogenous melatonin and, as a result, enhanced root and shoot growth under cadmium toxicity.[20]

Valerian shows a significant ability to accumulate cadmium in both roots and leaves (Fig. 2A). Higher concentrations of Cd were observed in all three treatments with exogenously added melatonin (melatonin, melatonin+Zn, melatonin+Cd).

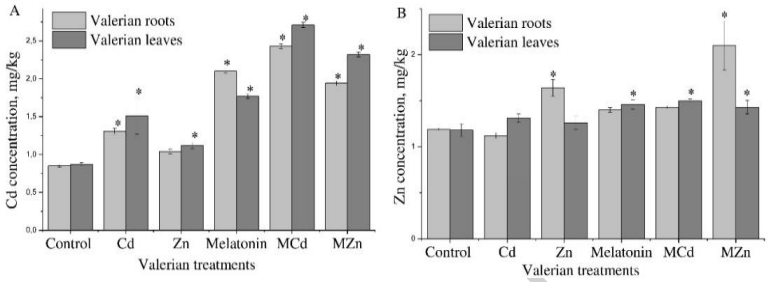

Fig. 2. Cd (A) and Zn (B) concentration in valerian roots and leaves. The data are presented as means of three replicates. M – melatonin; *Tukey test; $p < 0.05$.

The accumulation of Cd in valerian organs treated with Zn was increased compared to the control (0.851 and 0.868 mg/kg in root and leaf) with amounts to 1.038 mg/kg in root and 1.121 mg/kg in valerian leaf. Cd ions showed a greater possibility of translocation, which is a prerequisite for efficient phytoextraction and accumulation of metals in the aerial parts of plants. Melatonin also showed a positive effect in plants pre-treated with this hormone in terms of the accumulation of larger amounts of Cd. Increased accumulation occurs in examined plant species treated with both Zn and Cd. Thus, the concentration of Cd in the roots of plants treated with Zn and melatonin was 2.431 mg/kg of valerian root, which is twofold higher compared to treated plants with zinc without melatonin (1.310 mg/kg). A similar increased accumulation of Cd was observed in the leaves.

It is important to note that of the total amount of ions that are bound by the roots, only a part is absorbed into the cells. Melatonin may enhance the effectiveness of valerian in phytoremediation, particularly in the context of Cd contamination. By promoting the accumulation and translocation of Cd, melatonin could support the use of valerian in soil decontamination efforts, especially in slightly polluted soils.

Zinc belongs to a group of moderate mobility within plant tissues. In the case when its concentration in the soil is low, the intensity of transmission from older to younger parts of the plant was extremely weak. In case the concentration in the external environment is high, it accumulates in the roots.[21] The study demonstrates that melatonin, both exogenously applied and naturally present in valerian, plays a

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significant role in ameliorating the phytotoxicity induced by heavy metals. The results suggest that melatonin enhances the concentration of endogenous melatonin in roots, especially under the influence of Cd, which may contribute to the plants defense mechanisms against heavy metal stress.

Potato weed is a hyper accumulator of Cd with high Cd tolerance. Under conditions of low Cd concentration, melatonin not only improved the activity of antioxidant enzymes, but also improved the transfer of Cd to the cell wall and vacuoles, removing Cd away from sensitive parts of the cell, and accelerating its absorption.[22]

The influence of melatonin and heavy metals on the content of macro elements is shown in Fig. 3. The results showed the presence of a high concentration of Mg (Fig. 3A), and even higher Ca contained in valerian leaves (Fig. 3B).

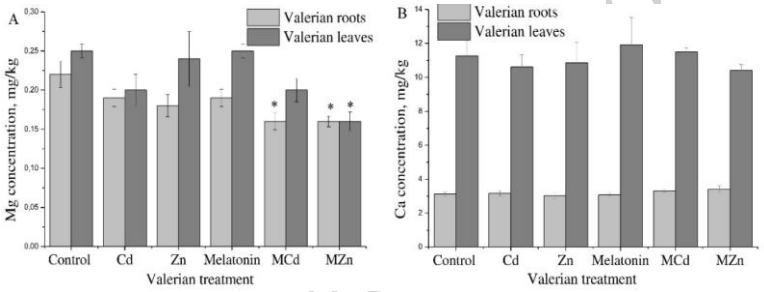

Fig. 3. Mg (A) and Ca (B) concentration in valerian roots and leaves. The data are presented as means of three replicates. M – melatonin; *Tukey test; $p < 0.05$.

Under higher Cd levels, macronutrients are somewhat decreased. Because Cd and mineral nutrients share identical pathways for transport, they have similar effects on balance at an ionic level.[10] Exogenously added melatonin did not prominently affect the changes in Mg and Ca concentrations compared to the control. A statistically significant decrease in magnesium concentration occurred in plants pre-treated with melatonin and treated with Cd and Zn. The reduction referred to both analyzed plant organs, compared to the control. Ca levels remained relatively stable across treatments, with no significant changes. The heavy metal interaction with soil matrix is decisive for the phytoremediation concept. In principle, soil particles sorption reduces the activity of metals in the system. Higher capacity of cation exchange leads to higher sorption and immobilization of metals. In acidic soils, the desorption of metals bound in the soil solution is stimulated due to the participation of H^+.[23]

The uptake of metals into plant roots is a complex process involving the transfer of metals from the soil to the root surface and within the root cells. Understanding the uptake process is difficult due to the complex nature of the rhizosphere, which is in continuous dynamic change in interaction with the plant root, the soil that creates it, and the microorganisms that live within the rhizosphere.[23]

The results of spectrophotometric determination of total phenols and flavonoids concentration in ethanol extracts of valerian roots and leaves are shown in Table I. The concentration of phenolic compounds in valerian root ranged from 26 to 37 mg/g of lyophilized root material, while in its leaves the values ranged from 70 mg/g in melatonin pre-treated plants to 95 mg/g of lyophilized leaf in melatonin and Zn treated plants. Treatment of valerian with Cd ions had an inhibitory effect on the content of total phenolic compounds in the roots of this plant, although the reduction was not statistically significant.

TABLE I. Total phenol and flavonoid content (mg/g) in valerian roots and leaves under different treatments; $^{a, b, c}$ – different letters indicate statistically significant difference; *Tukey test, $p < 0.05$

Treatment	Total phenol content		Flavonoid content	
	Roots	Leaves	Roots	Leaves
Control	27.54 ± 0.661^{ab}	83.79 ± 0.457^{d}	3.04 ± 0.085^{a}	45.34 ± 0.972^{b}
Cd	26.58 ± 0.613^{a}	73.67 ± 0.581^{a}	3.23 ± 0.167^{a}	35.03 ± 1.972^{a}
Zn	37.31 ± 0.144^{d}	77.44 ± 0.687^{c}	5.14 ± 0.285^{b}	45.08 ± 1.047^{b}
Melatonin	31.67 ± 0.977^{c}	69.43 ± 0.688^{b}	5.37 ± 0.259^{b}	35.37 ± 0.591^{a}
Cd + Melatonin	31.11 ± 0.681^{bc}	77.66 ± 0.622^{c}	4.88 ± 0.294^{b}	52.69 ± 0.740^{c}
Zn + Melatonin	28.36 ± 0.808^{abc}	94.36 ± 0.547^{e}	6.61 ± 0.235^{c}	56.62 ± 0.538^{c}

Heavy metal concentration is considered a crucial parameter that affects the response of plants in secondary metabolism production. Lower levels of heavy metals enhance the production and higher concentrations inhibit the synthesis of secondary metabolites in plants.[24] Treatments induced different responses in valerian leaves. Namely, under the influence of Cd and Zn ions, and with melatonin and Cd ions, there was a statistically significant decrease in the phenol content compared to the control. Plants pre-treated with melatonin, with and without zinc contamination, show a higher content of total phenols.

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The content of flavonoids in valerian roots ranged from 3 to 7 mg/g of lyophilized plant material, while in valerian leaves, flavonoid concentrations were from 35 to 57 mg/g. The valerian leaf shows a seven to fifteen times higher concentration of flavonoids than the root. A statistically significant increase in the concentration of flavonoids in valerian root occurs in all included treatments, except for the treatment with Cd ions which inhibited the production of these secondary metabolites. Zinc and melatonin treatment showed the highest flavonoid content in valerian leaves, followed by cadmium and melatonin treatment. Cd reduced

flavonoid levels substantially. Variations in the content of phenolic compounds in plants result from a large number of factors, which, in addition to genetic factors, include the area of cultivation as well as numerous environmental factors. Biotic and abiotic stress (pathogens, viruses, mechanical damage, temperature extremes, UV radiation, imbalance in mineral nutrition, heavy metal and herbicide pollution, drought, salinity) cause an increase in the level of phenolic compounds in vegetative shoots and roots.[25] The addition of melatonin improved the anthocyanin content in tomato plants under Ni stress and in rosemary herb under Cr stress.[26] Melatonin has many physiological functions in plants, and the most researched function is the prevention of oxidative damage caused by various abiotic stressors such as salinity,[27] low temperatures[28] and the toxic effects of cadmium. The content of total phenols, flavonoids and proanthocyanides gradually improved with melatonin treatment in berries.[29]

In the results of the CUPRAC test, significantly higher ability of valerian leaves to reduce Cu ions was observed (two to three times) compared to the root (Fig. 4A).

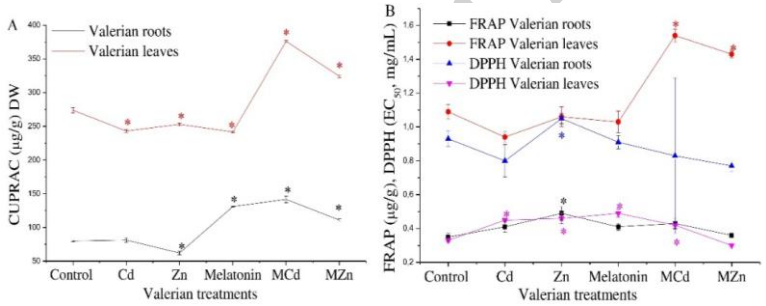

Fig. 4. Antioxidant activity estimated by CUPRAC (A), FRAP and DPPH (B) test. The data are presented as means of three replicates; *Tukey test, $p < 0.05$.

In all five treatments, the ability to reduce Cu^{2+} in valerian roots was significantly increased, while in leaves, the reduction ability was reduced in plants treated with Cd and Zn ions (243.05 and 253.05 µg/g) compared to the control (273.93 µg/g). Exogenous melatonin in combination with heavy metal treatment also shows a significant positive effect on the reduction of Cu ions, in both roots and leaves.

According to the test results, the ability to neutralize DPPH radicals in valerian roots treated with Cd ions is higher (0.8 mg/mL) compared to untreated roots (0.93 mg/mL) (Fig. 4B). Exogenous melatonin also increased the ability to neutralize DPPH radicals in valerian root (0.91 mg/mL) compared to the control. Likewise, the roots of plants pre-treated with melatonin show a greater ability to neutralize DPPH radicals with an increased concentration of Cd (0.83 mg/mL) and Zn (0.77

mg/mL) ions, which indicates an increase in antioxidant capacity in situations of oxidative stress caused by cadmium ions. The valerian leaf, with higher content of phenolic compounds found, shows a several times greater ability to neutralize DPPH radicals than the root. Valerian leaves grown on soil contaminated with cadmium (0.45 mg/mL) and zinc (0.46 mg/mL) ions, as well as plants pre-treated with exogenous melatonin (0.49 mg/mL), show a significantly reduced ability to neutralize DPPH radicals.

Valerian plants grown on soil contaminated with cadmium and zinc ions show an increased root reduction capacity (0.41 and 0.49 μg/g, respectively, Fig. 4B). Exogenous melatonin also caused a significant increase in reducing power (0.41 μg/g) compared to the control root (0.35 μg/g). Unchanged reducing capacity is observed only in valerian roots treated with zinc and melatonin. In contrast to the roots, a slightly reduced reducing capacity of valerian leaves occurs in plants treated with Cd and Zn (0.94 and 1.06 μg/g), as well as in those pre-treated with melatonin (1.03 μg/g). The increase in the concentration of cadmium and zinc in plants pre-treated with melatonin influenced the increase in the reducing capacity of valerian leaves.

The research results show increased antioxidant activity of plants, especially in the leaf in all applied biochemical tests. The increased antioxidant activity of the leaves of both plant species can be attributed to the increased content of total phenols, compared to the root. There is a very high correlation between the content of phenol and the ability to reduce Fe^{3+} and Cu^{2+}, as well as to neutralize DPPH radicals. The antioxidant activity of melatonin is enhanced in the roots of the analyzed plants, as the root is probably the most frequently mentioned plant organ in earlier research as a potential site of melatonin biosynthesis. The results suggest that melatonin might boost the plants' reducing capacity, enhancing their ability to counteract oxidative damage and potentially assisting in metal detoxification processes.

An increased content of soluble proteins is observed in valerian roots treated with Cd (21.70 mg/g) and Zn (21.70 mg/g) ions compared to the control (11.76 mg/g) as shown in Fig. 5A.

Pre-treatment with melatonin slightly increased the protein content of valerian root, but the increase was not statistically significant. However, melatonin pre--treatment and cadmium ions significantly increased the protein content of valerian roots. Zn with melatonin did not have the same effect. In the valerian leaf, on the other hand, in all six treatments, a statistically significant increase in soluble proteins concentration was observed, compared to the control. There was no increase in peroxidase activity in the leaves (Fig. 5B). It is possible that the defensive activity of peroxidases is based on the protection of plants in the roots, where a statistically significant increase in enzyme activity is observed. Also, the applied

concentration of cadmium ions might not have been toxic for these very resistant plant species.

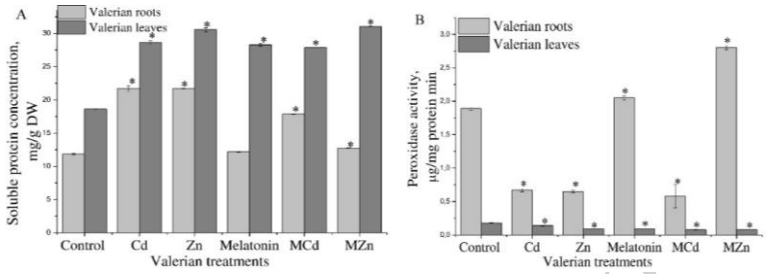

Fig. 5. Total soluble protein concentration (A) and peroxidase activity (B) in valerian roots and leaves under heavy metal treatment and melatonin pre-treatment. The data are presented as means of three replicates; * Tukey test, $p < 0.05$.

Enzyme activity in the leaves was reduced in all applied treatments compared to untreated plants. In general, the most significant increase in peroxidase activity occurs in valerian roots pre-treated with melatonin, plants grown on soil contaminated with zinc ions (2.80 μmol/(mg protein min)).

Melatonin enhances plant metabolism and antioxidant enzymes activity and initiates the ascorbate–glutathione cycle to counteract the effects of heavy metal stress.[30] Heavy metal stress disrupts the balance between reactive oxygen species generation and detoxification by the antioxidative protection system in plants.[31] Plants resist stress-induced ROS production and related adversities by directly neutralizing and removing them or indirectly by controlling the uptake, transport, translocation, and sequestration of heavy metals. In the earlier study, authors showed melatonin may act as a free-radical scavenger and broad-spectrum antioxidant, protecting plant tissues from oxidative damage. It could also stimulate antioxidant enzyme production or enhance the activity of other antioxidants to further protect the plant.[32] The increase of endogenous melatonin mitigates cadmium toxicity by balancing H_2O_2 homeostasis and activating antioxidant defense systems in wheat.[33] Melatonin treated plants improve their growth and yield under heavy metal stress conditions. Exogenous melatonin applications can be the trigger for endogenous melatonin production in plants, thereby building up heavy metal tolerance.[2,34] Melatonin application methods vary, including pre-treatment of roots or foliar application just before stress exposure, with some studies exploring repeated applications. Interestingly, seed priming with melatonin has shown lasting effects on stress tolerance, even with a single application, especially for crops like rice, soybean and cucumber, often grown in large-scale agriculture. However,

more research is needed to understand the long-term effects of melatonin treatment and its impact on unpredictable stress conditions.

CONCLUSION

Cadmium and zinc exposure induce significant physiological and biochemical stress in *Valeriana officinalis*, disrupting antioxidative balance, reducing phenolic and flavonoid contents and impairing nutrient homeostasis, particularly in leaves. The application of melatonin alleviates these stress effects and enhances the plant defense mechanisms by elevating antioxidant activity, increasing peroxidase enzyme levels, promoting the accumulation of protective compounds and enhancing soluble protein levels. The heavy metal concentration and exposure duration could be the controlling factors for the synthesis of endogenous melatonin. Melatonin might help to confer the heavy metal stress tolerance in valerian due to the increase of endogenous melatonin under Cd and Zn stress conditions, and modulated metal uptake.

Melatonin acts as a potent bio-stimulant and stress-mitigating agent, improving antioxidant capacity, biochemical composition and nutrient balance in valerian under heavy metal exposure. These findings highlight melatonin's potential as a sustainable and effective strategy to enhance plant resilience and phytochemical quality in contaminated environments. Gaining deeper insight into melatonin-mediated signaling and responses could help maintain crop productivity in soils contaminated with heavy metals. However, additional research is essential to fully uncover the roles of melatonin and enable its effective and sustainable application in agriculture.

ИЗВОД

МЕЛАТОНИН КАО МОДУЛАТОР ТОКСИЧНОСТИ ТЕШКИХ МЕТАЛА И АНТИОКСИДАТИВНА ЗАШТИТА У ВАЛЕРИЈАНИ

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Тешки метали, свеприсутни у земљишту и води, као озбиљан еколошки проблем, ремете хомеостазу минералне исхране биљака, осмотску равнотежу и метаболизам. Примена неких биостимуланата може ублажити поремећај. Мелатонин као сигнални молекул и антиоксидант игра важну улогу у расту биљака и толеранцији на стрес због своје способности да директно неутралише реактивне врсте кисеоника и азота. У овом раду је испитано смањење или ублажавање штетних ефеката тешких метала код биљака валеријане узгајаних на отвореном пољу употребом мелатонина. Течна хроматографија високог притиска са флуоресцентним детектором коришћена је за идентификацију и квантификацију концентрације мелатонина у екстрактима корена валеријане. Такође, испитан је физиолошки и биохемијски статус биљака под абиотским стресом, посебно код биљака

претходно третираних мелатонином од 100 μM. Веће концентрације ендогеног мелатонина измерене су у корену биљака третираних Cd и Zn, са сличним резултатима у концентрацијама протеина. Примена мелатонина ублажила је негативан ефекат Cd, што је посебно очигледно код третмана Cd-мелатонином који је обновио или повећао нивое биоактивних једињења. Мелатонин ефикасно ублажава стрес изазван Cd и Zn код валеријане побољшавајући и неензимске и ензимске антиоксидативне системе и промовишући синтезу заштитних једињења. Ови налази истичу потенцијал мелатонина као одрживог биостимуланса за подршку отпорности и продуктивности биљака у окружењима оптерећеним тешким металима.

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REFERENCES

1. M.A. Altaf, Y. Hao, H. Shu, M. A. Mumtaz, S. Cheng, M. N. Alyemeni, P. Ahmad, Z. Wang, *J. Hazard. Mater.* **454** (2023) 131468 (https://doi.org/10.1016/j.jhazmat.2023.131468)
2. E. Hodžić, S. Galijašević, M. Balaban, S. Rekanović, H. Makić, B. Kukavica, D. Mihajlović, *Turkish J. Chem.* **45** (2021) 737 (https://doi.org/10.3906/kim-2012-7)
3. E. Hodžić, M. Balaban, B. Kukavica, S. Rekanovic, in *Melatonin: Production, Functions and Benefits*, A. R. Martínez, F. L. Muñoz, J. Egea, Eds., Nova Science Publishers, Hauppauge, New York, 2021 (ISBN: 978-1-53619-031-1)
4. N. Zhang, H. J. Zhang, B. Zhao, Q. Q. Sun, Y. Y. Cao, R. Li, et al., *J. Pin. Res.* **56** (2014) 39 (https://doi.org/10.1111/jpi.12095)
5. M. H. Siddiqui, S. Alamri, Q. D. Alsubaie, H. M. Ali, A. A. Ibrahim, A. Alsadon, *Ecotox. Environ. Safety* **180** (2019) 656 (https://doi.org/10.1016/j.ecoenv.2019.05.043)
6. R. Colombage, M. B. Singh, P. L. Balla, *Intern. J. Mol. Sci.* **24** (2023) 7447 (https://doi.org/10.3390/ijms24087447)
7. F. Godoy, K. Olivos-Hernández, C. Stange, M. Handford, *Plants* **10** (2021) 186 (https://doi.org/10.3390/plants10020186)
8. T. Chen, Y. Su, *Human Ecol. Risk Assess.: An Int. J.* **24** (2018) 1550 (https://doi.org/10.1080/10807039.2017.1416580)
9. C. H. Shekhar, H. Kırmızıbekmez, *Phytochem. Rev.* (2024) (https://doi.org/10.1007/s11101-024-10061-x)
10. Md. N. Hoque, Md. Tahjib-Ul-Arif, A. Hannan, N. Sultana, S. Akhter, Md. Hasanuzzaman, F. Akter, Md. S. Hossain, Md. A. Sayed, Md. T. Hasan, M. Skalicky, X. Li, M. Brestič, *Intern. J. Mol. Sci.* **22** (2021) 11445 (https://doi.org/10.3390/ijms222111445)
11. M. B. Arnao, J. Hernandez-R, *Phytochem. Anal.* **20** (2009) 14 (https://doi.org/10.1002/pca.1083)
12. V. L. Singleton, R. Orthofer, R. M. Lamuela-Raventos, *Meth. Enzym.* **299** (1999) 152 (https://doi.org/10.1016/S0076-6879(99)99017-1)
13. C. C. Chang, M. H. Yang, H. M. Wen, J. C. Chern, *J. Food Drug Anal.* **10** (2002) 178 (https://doi.org/10.38212/2224-6614.2748)
14. I. F. F. Benzie, J. J. Strain, *Meth. Enzym.* **299** (1999) 15 (https://doi.org/10.1016/S0076-6879(99)99005-5)
15. C. Soler-Rivas, J. C. Espin, H. J. Wichers, *Phytochem. Anal.* **11** (2000) 330 (https://doi.org/10.1002/1099-1565(200009/10)11:5<330::AID-PCA534>3.0.CO;2-G)

16. R. Apak, K. Güçlü, B. Demirata, M. Özyürek, S. E. Çelik, B. Bektaşoğlu, K. Işıl Berker, D. Özyurt, *Molecules* **12** (2007) 1496 (https://doi.org/10.3390/12071496)
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193** (1951) 265 (https://doi.org/10.1016/s0021-9258(19)52451-6)
18. H. Teisseire, V. Guy, *Plant Sci.* **153** (2000) 65 (https://doi.org/10.1016/S0168-9452(99)00257-5)
19. Y. Jiang, S. Huang, L. Ma, L. Kong, S. Pan, X. Tang, H. Tian, M. Duan, Z. Mo, *Antioxidants* **11** (2022) 776 (https://doi.org/10.3390/antiox11040776)
20. W. Zeng, S. Mostafa, Z. Lu, B. Jin, *Front. Plant Sci*. **13** (2022) 847175 (https://doi.org/10.3389/fpls.2022.847175)
21. A. Baran, *Ecol. Chem. Eng., A* **19** (2012) 669 (https://doi.org/10.2428/ecea.2012.19(07)069)
22. Y. Tang, L. Lin, Y. Xie, J. Liu, G. Sun, H. Li, Z. Liu, Z. Huang, Z. He, L. Tu, *Intern. J. Phytorem*. **20** (2018) 295 (https://doi.org/10.1080/15226514.2017.1374341)
23. H. Obata, M. Umebayashi, *J. Plant Nutr*. **20** (1997) 97 (https://doi.org/10.1080/01904169709365236)
24. M. H. Ibrahim, Y. Chee Kong, N. A. Mohd Zain, *Molecules* **22** (2017) 1623 (https://doi.org/10.3390/molecules22101623)
25. L. Chalker-Scott, *Adv. Bot. Res*. **37** (2002) 103 (https://doi.org/10.1016/S0065-2296(02)37046-0)
26. M. S. Jahan, S. GuO, A. R. Baloch, J. Sun, S. Shu, Y. Wang, G. J. Ahammed, K. Kabir, R. Roy, *Ecotox. Environ. Saf.* **197** (2020) 110593 (https://doi.org/10.1016/j.ecoenv.2020.110593)
27. J. Li, J. Liu, T. Zhu, C. Zhao, L. Li, M. Chen, *Internat. J. Mol. Sci.* **20** (2019) 1735 (https://doi.org/10.3390/ijms20071735)
28. J. Li, J. Xie, J. Yu, J. Lyv, J. Zhang, D. Ding, N. Li, J. Zhang, E. P. Bakpa, T. Yang, T. Niu, F. Gao, *Front. Plant Sci*. **13** (2022) 998293 (https://doi.org/10.3389/fpls.2022.998293)
29. Y. Xu, Y. Li, K. G. Maffucci, L. Huang, R. Zeng, *Molecules* **22** (2017) 2080 (https://doi.org/10.3390/molecules22122080)
30. M. Moustafa-Farag, A. Mahmoud, M. B. Arnao, M. S. Sheteiwy, M. Dafea, M. Soltan, A. Elkelish, M. Hasanuzzaman, S. Ai, *Antioxidants* **9** (2020) 809 (https://doi.org/10.3390/antiox9090809)
31. S. Singh, P. Parihar, R. Singh, V. P. Singh, S. M. Prasad, *Front. Plant Sci.* **6** (2016) 1143 (https://doi.org/10.3389/fpls.2015.01143)
32. E. Hodžić, M. Balaban, N. Šuškalo, S. Galijašević, D. Hasanagić, B. Kukavica, *J. Serb. Chem. Soc.* **84** (2019) 11 (https://doi.org/10.2298/JSC180504070H)
33. J. Ni, Q. Wang, F. A. Shah, W. Liu, D. Wang, S. Huang, S. Fu, L. Wu, *Molecules* **23** (2018) 799 (https://doi.org/10.3390/molecules23040799)
34. S. Menhas, X. Yang, K. Hayat, T. Aftab, J. Bundschuh, M. B. Arnao, Y. Zhou, P. Yhou, *J. Plant Gr. Regul*. **41** (2022) 922 (https://doi.org/10.1007/s00344-021-10349-8).