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Research Article

Effect of Pb and Cd on the response of some stress genes and the activity of antioxidant enzymes in date palm leaves

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Abstract

This study used 18-month-old palm offshoots irrigated with water containing different concentrations of heavy metals: Pb at 100 and 200 mg/kg soil, and Cd at 3 and 6 mg/kg soil. After three months of treatment, a sample of palm leaves was taken for molecular and chemical tests. The results showed that treatment with Pb and Cd increased their concentrations compared with the control sample, especially at high concentrations. This treatment caused stress to the plants and affected their biochemical and molecular characteristics. The content of H_2O_2 increased significantly in the treatment with 200 mg/kg Pb, reaching the highest level of 1.18±2.67 µmol/g. In contrast, the control sample had the lowest H_2O_2 content (0.90 ± 0.01 µmol/g). Peroxidase activity also increased significantly during treatment, with the highest enzyme activity (40.49 ± 0.26 units/min/g) observed in the Pb 200 mg/kg group. Treatment with Pb at 200 mg/kg also resulted in the highest soluble carbohydrate content (15.25 ± 0.72 mg/g) compared with the control sample (8.63 ± 0.44 mg/g). Photosynthetic pigments, including chlorophylls and carotenoids, were significantly reduced, especially at high treatment concentrations. Treatment with Pb at 200 mg/kg led to decreases in chlorophyll a and b contents (2.17±0.13 and 1.14±0.07 mg/g Fw, respectively) compared with the control (4.14±0.07 and 1.47±0.11 mg/g Fw, respectively). Similarly, Cd treatment at 3 and 6 mg/kg caused decreases in chlorophyll a and b contents compared with the control sample. The content of carotenoids also decreased significantly (1.02 ± 0.03 and 1.16 ± 0.08 mg/g FW, respectively) when treated with high concentrations of Pb and Cd compared with the control sample (1.87 ± 0.14 mg/g FW). Regarding the effect of heavy metal application on gene expression levels of threonine specific kinase/serine (salt tolerance kinases) (*STK*), phosphatidylinositol 4,5-bisphosphate 2 (*PIP2*), pathogenesis-related protein 1 (*PR1*), and superoxide dismutase (*SOD*) genes, treatments with Pb at 100 and 200 mg/kg resulted in the highest expression of the *PIP2* gene (3.39±0.46 and 4.81±0.30-fold, respectively) compared with the control sample.

Keywords: Antioxidant enzymes, Cadmium, Date palm, Gene expression, Lead

Introduction

Heavy elements, as defined by Onakpa *et al.* (2018), are chemical elements or metalloids with a density at least five times greater than that of water. Plants are frequently exposed to biotic and abiotic stresses that harm their well-being. Among these stressors, pollution caused by heavy metals is widely regarded as the most significant, as it negatively affects growth and productivity. Even at low concentrations, heavy metals, especially those with high densities such as Cu and Cd, are highly toxic. Soil contamination by these heavy metals can result from natural activities such as weathering, winds, and volcanic eruptions (Zhang and Wang, 2020).

When plants absorb metals, they tend to accumulate in crops, leafy vegetables, and fruit trees. As a result, the metals are transmitted through the food chain to humans and animals, potentially leading to health problems (Edelstein and Ben-Hur, 2018). The soil in residential areas is often contaminated with Pb and Cd due to various factors such as traffic, waste burning, and industrial activities (Iqbal *et al.*, 2020). Cd is a non-essential and highly toxic heavy element that is present in ecosystems in large quantities due to human

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activities, such as industrial processes and mining. Plants absorb this metal through their roots, and it moves to aerial parts like leaves, causing stress and various morphological, physiological, and molecular changes that affect photosynthesis and chlorophyll synthesis (Wang *et al.*, 2019). Pb is also a toxic metal that is not essential for plants and enters ecosystems through industrial and petroleum activities, car exhaust, and chemical fertilizers (Collin *et al*., 2022). This metal leads to the formation of reactive oxygen species (ROS), damages nucleic acids and lipids, and affects seedling growth (Seneviratne *et al.*, 2019).

The date palm (*Phoenix dactylifera* L.) belongs to the Arecaceae family and is a perennial plant. It is known for its tolerance to certain conditions, such as water stress, salinity, and drought. As a result, it possesses genes that confer resistance to unfavorable conditions. Heavy metal pollution is one of the major abiotic stresses affecting palm trees, particularly in urbanized areas due to increased human pollution (Rekik *et al.*, 2019). These pressures not only impact the plants and their productivity but also transmit pollutants to humans through fruit consumption, thereby affecting human health (Mawari *et al*., 2022). The date palm's tolerant nature and widespread availability make it a valuable biological monitor for pollution. Therefore, this research aims to identify the mechanisms of date palm tolerance to heavy metals (Cd and Pb) and to use them to assess pollution levels in the region.

Materials and methods

The laboratory experiment involved using 18-month-old palm (Al-Khadhrawi) offshoots. This research was carried out in a completely randomized design with two treatments of lead and cadmium in 4 repetitions and three concentrations. Lead in the form of lead acetate in concentrations of 0, 100, and 200 and cadmium in the form of cadmium chloride in concentrations of 0, 3, and 6 mg/kg of soil were added to the soil every other day as a spray. After 85 days of treatment, the leaves were collected (Abass *et al*., 2018). The palm trees were washed and sterilized with 70% ethyl alcohol, and the concentration of heavy metals in the leaves was determined through acid digestion of the fresh leaves as described by Jones (1984).

Assay of biochemical characteristics: Chlorophyll a and b, total chlorophyll, and carotenoids were estimated using the method developed by Arnon (1949). Chlorophyll was extracted in 80% acetone. The extracts were centrifuged at 3000 g, and the absorbance of the supernatant was measured at 663.2 and 646.8 nm with a UV–VIS spectrophotometer. Chlorophyll a and b contents were calculated using the following formulas:

Chl a= $(12.25A_{663.2}–2.79A_{646.8})$ × volume of supernatant (ml) × dilution factor/sample mass (g)

Chl b = $(21.21A_{646.8}–5.1A_{663.2})$ × volume of supernatant (ml)× dilution factor/sample mass (g)

A: Absorbance at a specific wavelength.

Car (mg/l) = 1000A480 – 1.8Chl a-85.02Chl b/198

The method described by Watanabe *et al*. (2000) was employed to estimate soluble carbohydrates. The phenol-sulfuric method was used to determine soluble sugars. After homogenizing 0.5 g of leaf tissue, 1 ml from each sample was taken, and then 1 ml of 5 % phenol and 5 ml of 98% sulfuric acid were added. After coolness and complete color emergence of the solutions, the sugar contents were maintained spectrophotometrically at 485 nm. H_2O_2 content of leaf tissues was determined following the method described by Sergiev *et al*. (1997). The amount of H_2O_2 was determined based on the reaction of H_2O_2 with potassium iodide (KI). In this method, 0.5 g of fresh tissue was extracted in 5 ml of trichloroacetic acid (TCA) (0.1% w/v). The extract was centrifuged at 12,000 g for 15 min. 0.5 ml of the supernatant was mixed with 0.5 ml of potassium phosphate buffer (10 mM; pH 7.0) and 1 ml of KI (1 M). The reaction mixture was placed in the dark at room temperature for 1 h, and absorbance was measured at 390 nm. Peroxidase (POX) activity was assayed according to the method described by Kim and Yoo (1996). Five milliliters of the assay mixture for the peroxidase activity contained 125 M of phosphate buffer, pH 6.8, 50 M of pyrogallol, 50 mM of H_2O_2, and 1 ml of the 20 times-diluted enzyme extract. This was incubated for 5 min at 25ºC, after which the reaction was stopped by adding 0.5 ml of 5% (v/v) H_2SO_4. The amount of purpurogallin formed was determined by taking the absorbance at 420 nm.

Quantitative real-time polymerase chain reaction assay (reverse transcription): Quantitative Reverse Transcription Real-Time PCR (RT-qPCR) was performed to measure the mRNA levels of the genes listed in Table 1 and determine their expression levels. The *ACTIN* gene was used as a reference gene to calculate gene expression.

Using candidate gene-specific primers designed with the Primer3Plus online software (Rozen and Skaletsky, 2000) and verified using the NetPrimer and Beacon Designer programs (Table 1), quantitative real-time polymerase chain reaction (qPCR) amplification was performed according to Zhang *et al*. (2021b).

Manufacturing of cDNA: We utilized the GoScript Reverse Transcription System kit (Promega) to generate cDNA complementary to the RNA.

Quantitative real-time PCR (qRT-PCR) test: Relative gene expression was studied by real-time qPCR using a Rotor-Gene Q instrument (Qiagen). The PCR mixture (0.01 ml) contained 10 μM of each primer, 50 ng of the cDNA template, and 0.005 ml of SYBR Premix Ex Taq (TaKaRa Bio). The thermal cycle conditions were as follows: 95ºC for 10 min + 40 cycles of 95ºC for 5 s + 58-62ºC for 10 s + 72ºC for 20 s. The amplification of the target was justified using analysis of melting curves and electrophoresis of the PCR product on the agarose gel. All reactions were replicated

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Table 1. Target stress genes in date palm

Gene name	Gene Description	Tm Forward	Primer sequence (5´→ 3´) Forward	Tm Reverse	Primer sequence (5´→3´) Revers	efficiency
1-STKs	Serine threonine protein kinases STY8	52.1	TATGGCGGCTTATCTTTTGG	55.5	CTTGTTCGGAAGAGGAGGTG	99 %
2-PIP2	Aquaporin PIP2-4	56.9	TCCCACGTCCCGGTTTT	56.0	GGACCATGAACACCGCAAA	100%
3-ACTIN (ref)	Actin	57.9	TCAATGTGCCTGCCATGTATGT	58.2	GCGGCCGCTAGCATAGAG	93%
SOD	dismutase superoxide	57.5	TGGTTTGGGATTACTCGCCC	57.4	GCTCTTTGCCAGCCAGAGTA	94%
4-PR1	Pathogenesis related protin 1	57.2	GCAGACTCATACACTCTGGTGG	60.4	ACTCCATTGCACGTGTTCGCAG	95%

twice. The ΔΔCT procedure was used to determine the gene relative expression (Livak and Schmittgen, 2001).

Calculation of results by the Livak method:

1-Calculate ΔCt for each sample

ΔCt for a sample was calculated as follows:

2- ΔCt = Ct (target gene) – Ct (reference gene)

The reference gene was a gene that is stably expressed across all conditions being compared.

Calculate ΔΔCt: Calculate the ΔΔCt for each experimental condition (Conditions B, C, etc.) relative to the reference condition (Control condition A) as follows:

ΔΔCt = ΔCt (Condition B) – ΔCt (Control condition)

Calculate fold change in gene expression: Calculate the fold change in gene expression for each experimental condition relative to the reference condition using the formula:

Fold change = 2^ (-ΔΔCt)

Total nucleic acids were extracted from plant tissues treated with different concentrations of heavy metal pollutants using the Total RNA Isolation Kit (Promega). The extraction process followed the protocol provided with the kit.

The primer efficiency was calculated for each primer by using the equation :

E = -1+10(-1/slope). It was noted that the amplification efficiencies range from 90% to 100% for all primers.

Results

The effect of treatment with Pb and Cd on their accumulation in palm leaves: The results presented in Figures 1 and 2, as well as Table 2, indicate that watering the plants with these two elements and subjecting them to treatment for 85 days increased their concentrations compared with the control sample. The concentrations of Pb and Cd exhibited a significant increase, particularly at higher treatment levels. The concentration of Pb was 64.98±0.97 mg/kg, whereas the control sample had a concentration of 1.90±0.07 mg/kg. Similarly, for Cd, the highest concentration was observed in the 6 mg/kg treatment, reaching 9.40±0.60 mg/kg.

The effect of Cd and Pb treatments on the level of gene expression: The results shown in Table 3 demonstrate the impact of adding Pb and Cd on gene expression levels for the genes *STK*, *PIP2*, *PR1*, and *SOD*. Statistically significant differences were observed, particularly with Pb treatment at both low and high concentrations, resulting in the highest gene expression for the *PIP2* gene compared to the control sample. The gene expression levels of the *PIP2* gene at concentrations of 100 and 200 mg/kg were 3.39±0.46-fold and 4.81±0.30-fold, respectively. On the other hand, Cd treatment resulted in the highest gene expression for the *STK* genes at a concentration of 6 mg/kg, with a fold change of 1.66 ± 0.94 compared to the control sample's fold change of (0.41 ± 0.02).

Effect of Cd and Pb treatments on some biochemical indices, chlorophyll and carotenoids: The results in Table 4 show a significant decrease in the content of chlorophyll pigments (a, b, and total) and carotenoids in palm leaves after being treated with heavy metals for 85 days. Treatment with a concentration of 200 mg/kg resulted in a reduction of chlorophyll a and b from 4.14 ± 0.07 and 1.47 ± 0.11 mg/g, respectively, in the comparison sample to 2.17 ± 0.13 and 1.14 ± 0.07 mg/g. This reduction was not significantly different from the concentration of Pb at 100 mg/kg. In the case of Cd treatment at two concentrations, 3 and 6 mg/kg, it decreased the concentration of chlorophyll a and b compared to the control sample.

As for carotenoids, the results in the table indicate a significant decrease in this pigment compared to the control sample, which had a recorded value of 1.87 ± 0.01 mg/g. It decreased to 1.02 ± 0.03 mg/g and 1.16 ± 0.08 mg/g when treated with high concentrations of Pb and Cd, respectively. The low concentrations of both treatments also led to an increase in this pigment in the leaves compared to the high concentration.

The effect of Cd and Pb treatments on H_2O_2 content and POX activity: The results in Table 5 and Figure 1 indicate a statistically significant increase in H_2O_2 content compared with the control sample following the treatment. The Pb treatment exhibited the highest H_2O_2 concentration at 200 mg/kg, with 2.67 ± 1.18 µmol/g. Conversely, the control sample showed the lowest H_2O_2 concentration at 0.90 ± 0.01 µmol/g.

Regarding POX enzyme activity, Table 5 demonstrates a significant increase in enzyme activity following treatment. All treatments and concentrations

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Table 2. The effect of Cd and Pb treatments on their concentration in palm leaves after 85 days of treatment

Lead	Treatment	Lead	Treatment
1.90±0.07^{c}	Control	1.3±0.08^{c}	Control
35.34±0.79^{b}	Lead (100 mg/kg)	6.90±0.86^{b}	Cadmium (3 mg/kg)
64.98±0.97^{a}	Lead (200 mg/kg)	9.40±0.60^{a}	Cadmium (6 mg/kg)

Different letters indicate a significant difference at P ≤ 0.05 between the samples and the control

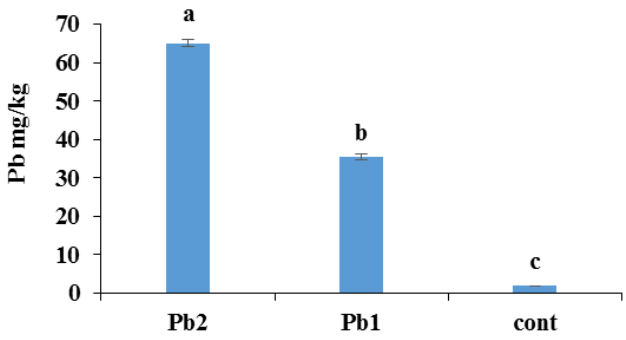


Figure 1. Effect of Pb treatment with different concentrations on its accumulation inside palm leaves: Control sample, Pb1 treated at 100 mg/kg, and Pb2 treated at 200 mg/kg. Different letters indicate significant differences at P< 0.05.

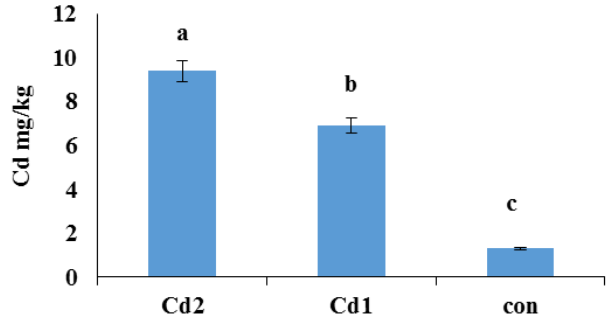


Figure 2. Effect of Cd treatment with different concentrations on its accumulation inside palm leaves: Control sample, Cd1 treated at 3 mg/kg, and Cd2 treated at 6 mg/kg. Different letters indicate significant differences at P< 0.05.

Table 3. Effect of Cd and Pb treatments on the level of gene expression (Fold change)

SOD	*PR1*	*PIP2*	*STKs*	Treatment
0.55±0.16^{d}	0.33±0.01^{d}	0.36±0.46^{d}	0.41±0.02^{c}	Control
0.65±0.25^{d}	0.46±0.45^{d}	0.83±0.03^{d}	0.45±0.01^{c}	Cd (3 mg/kg)
1.38±0.49^{c}	1.29±0.94b^{c}	1.36±0.45^{c}	1.66±0.94^{b}	Cd (6 mg/kg)
2.46±0.42^{b}	1.99±0.20a^{b}	3.39±0.46^{b}	1.87±0.20^{ab}	Pb (100 mg/kg)
3.38±0.48^{a}	2.62±0.44^{a}	4.81±0.30^{a}	2.82±0.44^{a}	Pb (200 mg/kg)

Different letters indicate a significant difference at P ≤ 0.05 between the samples and the control

Table 4. Effect of Cd and Pb treatments on chlorophyll and carotenoid content

Carotenoid (mg/g Fw)	Total Chl (mg/g Fw)	Chl. b (mg/g Fw)	Chl. a (mg/g Fw)	Treatment
1.87±0.14^{a}	5.56±0.07^{a}	1.47±0.11^{cd}	4.14±0.07^{a}	Control
1.48±0.06^{bc}	4.40±0.1^{bc}	1.54±0.04^{bc}	2.85±0.06^{b}	Cd (3 mg/kg)
1.16±0.08^{d}	4.51±0.13^{b}	1.24±0.15?	2.53±0.25^{c}	Cd (6 mg/kg)
1.65±0.05^{ab}	3.90±0.02^{cd}	1.55±0.09^{ab}	2.34±0.08^{d}	Pb (100 mg/kg)
1.02±0.03^{d}	3.32±0.14^{e}	1.14±0.07^{d}	2.17±0.13^{e}	Pb (200 mg/kg)

Different letters indicate a significant difference at P ≤ 0.05 between the samples and the control

showed high enzyme activity, with the Pb treatment at 200 mg/kg recording 40.49 ± 0.26 units/min/g.

The results presented in Figure 3 indicate that the soluble carbohydrate content was significantly higher in

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Table 5. Effect of Cd and Pb treatments on POX activity, H_2O_2, and soluble carbohydrate content

Soluble carbohydrates (mg/g)	POX activity (units/min/g)	H_2O_2 (µmol/ g)	Treatment
8.63±0.44^{d}	21.59±0.76^{e}	0.90±0.01^{e}	Control
12.90±0.47^{c}	26.83±0.80^{d}	1.86±0.91^{cd}	Cd (3 mg/kg)
14.89±0.93^{ab}	36.88±0.91^{b}	2.31±0.73^{b}	Cd (6 mg/kg)
13.87±0.78^{b}	31.71±0.89^{c}	1.81±0.29^{d}	Pb (100 mg/kg)
15.25±0.72^{a}	40.49±0.26^{a}	2.67±1.18^{a}	Pb (200 mg/kg)

Different letters indicate a significant difference at P ≤ 0.05 between the samples and the control

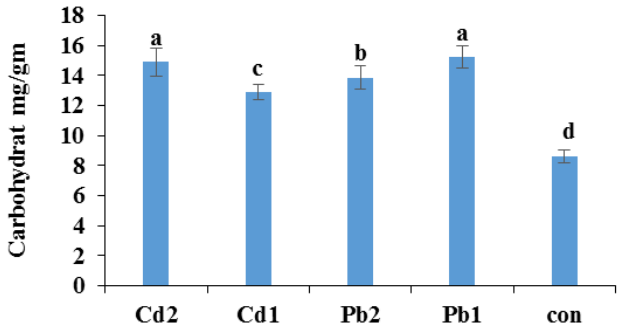


Figure 3. Effect of Cd and Pb treatments on the soluble carbohydrate content of date palm leaves. Different letters indicate significant differences at P< 0.05.

the treated samples compared with the control sample. Specifically, the treatment with Pb at 200 mg/kg showed the highest increase, with a value of 15.25 ± 0.72 mg/g, whereas the control sample recorded a value of 8.63 ± 0.44 mg/g. A similar trend was observed for Cd in comparison with the control sample.

Statistical analysis: Mean values were obtained from three replicates for each experiment. The data were analyzed using SPSS 26, and the one-way ANOVA followed by Tukey's test at a significance level of P ≤ .05 was used to determine statistical differences among means. Finally, graphs were plotted using Microsoft Excel 2016.

Discussion

The results presented in Table 1 demonstrate a statistically significant increase in Pb and Cd concentrations after 85 days of treatment compared with the control sample. This increased accumulation of these metals in the aerial parts is consistent with previous studies on palm plants (Zouari *et al.*, 2016), tomato plants (Salem *et al.*, 2016), and bean plants (Saleh *et al.*, 2020). The absorption of heavy metals from the soil can occur through two mechanisms: active transport, which requires energy, and passive transport, which requires minimal energy. In both cases, the metals are absorbed and transported into the inner tissue of the root epidermis (Guo *et al.*, 2016). Transpiration, the process of water movement in plants, plays a crucial role in mineral uptake by roots and their transport to the aerial parts. Additionally, chelating agents and root secretions containing amino and organic acids facilitate the absorption process, allowing elements to move through the xylem (Thakur *et al.*, 2016).

Plant genes associated with stress and resistance to abiotic conditions influence various biochemical traits in plants. These traits include photosynthesis, water balance, energy production, and other indicators of plant growth and survival (Zhang *et al.*, 2021a). Along with other plant immunity genes, *STK* genes play a crucial role in plant defense mechanisms. *STKs*, which are part of a large protein family, transmit signals in stressed plants through phosphorylation (Sanchita *et al.*, 2014). The STK group consists of different types of protein kinases, including mitogen-activated protein kinase (MAPK), calmodulin-dependent protein kinase (CAM), protein kinases A (PKA) and C (PKC), and phosphorylase kinase (Phk) (Zhou *et al.*, 2023). Overexpression of the *STK* gene enhances a plant's ability to tolerate water stress by regulating the expression of four stress-related genes: *OsABAR1*, *OSBZ8*, *Os3BGlu6*, and *OsSik1* (Zhou *et al.*, 2023). Cd and Pb have shown profound effects on these MAPKs in several species. Up-regulation of MPK3 and MPK6 in response to these heavy metals provides insight into their roles in metal homeostasis, either by regulating downstream metal transporters or chelators involved in responses to Cd and Pb (Siddhi *et al.*, 2018).

Biotic and abiotic stress factors affect water balance within plant cells, influencing ion transport and the transpiration process. In response to these stresses, plants activate *AQPs* (Aquaporin) genes, which are responsible for transporting water, uncharged molecules, and glycerol across membranes (Sun *et al.*, 2024). Plant aquaporins can be classified into different subgroups, including plasma membrane intrinsic proteins (*PIPs*), tonoplast intrinsic proteins (*TIPs*), nodulin-like intrinsic proteins (*NIPs*), and small basic

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essential intrinsic proteins (*SIPs*). *PIPs* are further divided into two subclasses, *PIP1* and *PIP2* (Bezerra-Neto *et al*., 2019; Sabir *et al*., 2020). A study by Zhang *et al.* (2013) demonstrated that overexpression of these genes in rice and tomato enhances plants' ability to withstand abiotic stress conditions.

When a plant is exposed to biotic and abiotic stresses that exceed its capacity to tolerate and eliminate them, ROSs are produced. The *SOD* gene family plays a crucial role in protecting the plants from biotic and abiotic stress conditions and also contributes to plant growth and development. SOD enzyme acts as the first line of defense against the harmful effects of ROS by converting O^{2-} into water molecules and hydrogen peroxide (Su *et al.,* 2021). In higher plants, the *SOD* gene family is categorized into three groups based on cellular localization: Mn-SOD is found in the mitochondria and peroxisomes, while Cu-Zn SOD is located in the chloroplasts (Zheng *et al*., 2023). Hajbagheri *et al*. (2022) showed that treatment of *Pimpinella anisum* L. with NO + Cd caused a significant induction of *SOD* gene transcription, increasing it by 11.3-fold. Analysis of cis-acting elements in *SOD* gene promoters revealed that *NtSOD* expression is regulated by plant hormones, defense- and stress-related responses, and light. In addition, multiple transcription factors and miRNAs are predicted to be involved in the regulation of *NtSOD* gene expression (Huo *et al*., 2022). Previous studies have demonstrated that overexpressing SOD in tobacco plants enhances their resistance to salt stress (Pan *et al.,* 2019).

The results presented in Table 4 indicate that treatment with heavy metals leads to a significant decrease in chlorophylls (a, b, and total) and carotenoids in palm leaves, particularly at high concentrations. This finding is consistent with numerous studies highlighting the detrimental impact of heavy metals on plants, including the reduction of chlorophyll concentration (Zhang *et al*., 2020a). The decrease in chlorophyll may be attributed to the inhibitory effect of heavy metals on proteins, as well as their ability to reduce the levels of proteins involved in the Calvin cycle, such as Rubisco (Ahmad *et al.,* 2021). This adversely affects the biosynthesis of essential enzymes, including protochlorophyllide reductase and δ-aminolevulinic acid dehydratase. Furthermore, heavy metals interfere with chlorophyll synthesis by mimicking nutrient cations such as P, leading to competition with Cd and As for absorption and substitution of P and Zn. Additionally, heavy metals interact with the salt-sulfhydryl groups of basic proteins, disrupting their structure (Zhang *et al.,* 2020b).

Regarding carotenoids, the results showed a significant decrease in this pigment compared with the control sample due to the influence of heavy metals. These findings are consistent with several studies, including the effects of Cd and Zn stress on tobacco leaves (Zhang *et al.,* 2020b), Ni and Pb stress on wheat (Saleh *et al*., 2020), and Ni stress on palm trees (Zouari *et al.,* 2016). However, some studies have reported an increase in carotenoid accumulation in the leaves of certain plants. This increase may be attributed to factors such as the concentration and nature of the heavy element, the duration of exposure, as well as the plant species and its accumulation capacity (Zhang *et al*., 2020a).

H_2O_2 is considered a signaling molecule and a regulator of plant growth at low concentrations, but at high concentrations, it generates ROS radicals, acting as a cellular oxidant that can lead to cell death (Cerny *et al.,* 2018). The present findings indicate an accumulation of H_2O_2 at higher concentrations compared with the control sample due to heavy metal stress. These results are consistent with the studies conducted by Nogueirol *et al.* (2016), Wang *et al.* (2016), and Nazir and Khan (2020).

The results indicated an increase in the content of soluble carbohydrates when plants were treated with high concentrations of heavy metals, with a statistically significant difference compared with the control sample. When plants are exposed to stress, they require more energy. One mechanism employed by plants to conserve energy is the accumulation of carbohydrates (Abass *et al.,* 2016). This finding is consistent with numerous studies reporting an increase in carbohydrate accumulation due to heavy metal stress, such as the effect of Cd on *Brassica juncea* plant (Kapoor *et al.*, 2016), Cd and Ni on tobacco plant (Aldoobie and Beltagi, 2013), and Cd and Mercury on Morvared and Falat bread wheat cultivars (Jahanbakhsh Godehkahriz *et al*., 2023).

Conclusion

The study concludes that treatment with high concentrations of Pb and Cd led to an increase in their accumulation in the plant, causing abiotic stress. This resulted in an increase in soluble carbohydrate content and a decrease in photosynthetic pigments, including chlorophyll and carotenoids. Peroxide concentration and peroxidase enzyme activity also increased, and along with the expression levels of the target genes. These findings indicate that heavy metals affect numerous biochemical processes in plants, and plant genetic and molecular changes can be used to assess the damage caused by environmental heavy metal pollution.

Acknowledgments

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