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Salicylic acid restricts mercury translocation by activating strong antioxidant defense mechanisms in sweet pepper (*Capsicum annum* L.)

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ABSTRACT

Mercury (Hg) availability in soil and its absorption in plants is seriously concerned for plant production and human health. Salicylic acid (SA) is one of the major plant hormones involved in plant growth and development under biotic and abiotic stress conditions. So, the experiment was designed to assess the effect of SA on sweet pepper (*Capsicum annum* L.) seedlings grown under different Hg toxicity concentrations. Spraying of 100 μM SA at three different Hg levels, i.e., 0 μM, 50 μM, 100 μM, and 150 μM. The maximum decrease in photosynthetic machinery, plant growth attributes (shoot length, root length, no. of leaves, fresh and dry biomass (shoot and root)), and more accumulation of Hg in leaves, roots, and fruits of sweet pepper. Additionally, SA significantly reduced the reduction in photosynthetic attributes and plant growth, and increased antioxidant enzymes (SOD, POD, and CAT) under Hg toxicity. H_2O_2 was found to be lower in plants treated with SA under Hg toxicity than in non-treated plants. The SA application also restricts the accumulation of Hg in sweet pepper roots, leaves, and fruits. Hg translocation in leaves and fruits was also reduced under SA. These findings provide a novel perspective on Hg accumulation in sweet pepper. They open a door to identify SA signaling pathways to clarify the mechanisms of SA inhibiting Hg accumulation in leaves and fruits.

1. Introduction

There are several metals present in the environment with only a few trace metals accepted by humans, animals, and plants. These metals are cobalt (Co), copper (Cu), iron (Fe), and zinc (Zn). However, even high doses of these metals lead to scale poisoning specific to each metal (Mahbub et al., 2017). Metal extraction from naturally occurring mineral compounds through mining threatens humans, animals, and plants due to high exposure. Metal exposure can come from the household or work environment, food, water, or soil (Bjørklund et al., 2017). Toxic metals affect the lungs, cardiovascular, and nervous systems.

Mercury (Hg) has been prioritized as a high contaminant by several international organizations due to its high toxicity and bioaccumulation in the food chain, and widespread contamination (Li et al., 2020). As per the World Health

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Organization (WHO, 2019), Hg is listed as a toxic chemical causing health concerns. Soil Hg pollution is an increasing environmental issue due to its wide distribution. Based on recent surveys in China, agricultural soil Hg exceeds 1.6% of the national limit (Li et al., 2020).

The Hg is easily absorbed by many plant species (i.e., sweet pepper, spinach, cabbage, cucumber, turnip, onion, carrot etc.) resulting in Hg toxicity in agricultural products (Yu et al., 2018). It is reported that most Hg is found in the roots and less is passed to the shoot (Natasha Shahid et al., 2020). However, even a stunted Hg concentration results in toxic effects in plants like growth retardation, photosynthesis inhibition, reactive oxygen species (ROS) generation, and DNA and protein damage (Natasha Shahid et al., 2020). Marrugo-Negrete et al. (2016) reported that a reduction in biomass was associated with stunted growth in *Jatropha curcas* plants with different Hg concentrations. Teixeira et al. (2018) reported a disturbance in net photosynthesis in higher plants with higher Hg levels. Leaves show chlorotic and necrotic symptoms in addition to stunted growth due to Hg exposure (Xun et al., 2017). Previous studies showed that Hg interrupts normal cell metabolism by attacking all types of biomolecules resulting in triggering oxidative stress in plants with ROS production.

Mercury-induced oxidative stress has been documented in multiple studies (Pirzadah et al., 2018; Tamás et al., 2017; Kim et al., 2017). The increase in ROS production, i.e., hydrogen peroxide (H_2O_2) is due to high toxic metal accumulation. A coping mechanism in plants for ROS's negative effects is to activate their antioxidant defense system. The cellular receptors in plants identify stress and produce immune responses, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) (Khalid et al., 2019, 2020). Hg increases antioxidant enzymes in *Brassica juncea* L. by Shiyab et al. (2009). Long-term Hg exposure enhances antioxidant activity, indicating H_2O_2 scavenging.

Salicylic acid (SA) regulates growth in plants, playing an efficient role in growth and development, flowering and fruit ripening, and photosynthesis (Natasha Shahid et al., 2020). SA is crucial in stimulating immune responses in plants by regulating numerous biochemical and physiological functions related to tolerance to biotic and abiotic stresses (Wani et al., 2016). The application of SA initiates systemic acquired resistance (SAR) in plants, making them more resistant to stresses (Wani et al., 2016).

As a response to Hg toxicity, many strategies have been developed, including the application of SA on plants. Zhou et al. (2009) reported a decrease in Hg-induced stress in the roots of Alfalfa plants treated with SA resulting in increased root growth. SA has been reported to increase the antioxidant machinery in plants by reducing Hg-induced oxidative damage (Sharma et al., 2020). Wang et al. (2021) observed that with the application of SA, heavy metals accumulation and translocation were decreased.

To feed the world, vegetables are the most nutritious products in agriculture, and their consumption is increasing rapidly, but production is decreasing (Khalid et al., 2023). Among different vegetables sweet pepper (*Capsicum annuum* L.) is a majorly produced and consumed vegetable in the world. China is the highest producer of sweet pepper producing nearly half of the total global sweet pepper production with 16.7M metric tons followed by Mexico (2.8M metric tons) and Turkey (2.6M metric tons) (Lo Scalzo et al., 2020). Sweet pepper is rich in antioxidant content with high ascorbic acid and various carotenoid pigments, phenolic compounds, and vitamins (Chen and Kang, 2013). Deepa et al. (2006) reported a wide variation in antioxidant content in sweet pepper highlighting its necessity and importance.

Sweet pepper production is decreasing due to many factors including the accumulation of heavy metals. So, the study was designed to investigate the potential of SA to mitigate or reduce Hg toxicity in sweet pepper seedlings. We aim to assess the potential protective or ameliorative properties of SA against Hg accumulation in the roots, leaves, and fruits of sweet pepper seedlings. Moreover, we need to determine Hg toxicity in sweet pepper. We also need to explore the physiological, growth, and biochemical response of sweet pepper seedlings to different Hg levels.

2. Material and methods

2.1. Plant material

Sweet pepper (*Capsicum annum* L.) cultivar "Milena deniro" seedlings were obtained from Agrico-Qatar. The experiment was conducted in the greenhouse of the Environmental Science Center, Qatar University, Doha – Qatar. Three-week-old seedlings were transplanted in peat moss and irrigated with Hoagland nutrient solutions (Hoagland and Arnon, 1950) to maintain proper growth and development for one week. After one week of transplantation, 90 uniform and homogenous seedlings were selected for the experiment. A factorial experiment based on a completely randomized design (CRD) was performed with two factors (mercury and salicylic acid). Five seedlings were selected in each replication with three replicates and five seedlings in each experimental unit. The seedlings were exposed to mercury toxicity (T0 = 0 μM; T1 = 50 μM; T2 = 100 μM; T3 = 150 μM) through irrigation 2 times a week for up to two months. Moreover, exogenous foliar application of salicylic acid was applied at S0 = 0 μM and S1 = 100 μM on the same day of Hg treatment, respectively. The mean day and night temperatures during the experiment were 28 and 14 °C, respectively, whereas relative humidity fluctuated between 60% and 80% and light (PAR) was used between 300 to 500 μmols/m^2/s.

2.2. Growth attributes

The quantum yield (QY) of dark-acclimated (Fv/Fm) leaves was measured with a chlorophyll fluorometer (FluorPen FP-100, Photon Systems Instruments, Czech Republic) at the end of the experiment. Sweet pepper seedlings were harvested, and root length, shoot length, number of leaves, fresh weight, and dry weight of shoots and roots were measured. For dry weight estimation, the roots, shoots, and leaves were desiccated in an oven at 65 °C till constant weight was achieved. The fresh leaves were crushed in liquid nitrogen to stop its activity immediately and stored in −80 °C for further analysis.

2.3. Enzymatic assays and hydrogen peroxide

Fresh leaf samples were harvested and crushed in liquid nitrogen to analyze enzymatic activity and H_2O_2. Initially, 300 mg of fresh samples were homogenized in sodium phosphate buffer (pH 7.8) and centrifuged at 1500 rpm for 5 min. The supernatant was used to analyze superoxide dismutase (SOD) (EC: 1.15.1.1), catalase (CAT) (EC: 1.11.1.6), and peroxidase (POD) (EC: 1.11.1.7), following Khalid et al. (2020). For SOD activity, the reaction solution contains extract, 75 millimolar ethylenediaminetetraacetic acids, 50 micromolar nitroblue tetrazolium, 50 millimolar sodium phosphate buffer (pH 7.8), 1.3 micromolar riboflavin and 13 millimolar methionine and enzyme extract (Giannopolitis and Ries, 1977). We used the Chance and Maehly (1955) method for CAT and POD activities. The reaction mixture of CAT contains 5.9 millimolar H_2O_2 and sodium phosphate buffer. For POD activity 40 millimolar H_2O_2, 20 millimolar guaiacol, and sodium phosphate buffer.

For H_2O_2 estimation, 300 mg of fresh leaves samples were homogenized in trichloroacetic acid (0.1%) and centrifuged at 12000 rpm for 15 min. The supernatant, 1 molar potassium iodide, and potassium phosphate buffer were used as suggested by Velikova et al. (2000).

2.4. Mercury concentration

Fresh roots, leaves, and fruits of sweet pepper plants and soil (around the plant roots) were collected. The samples were freeze-dried using an AdVantage Pro freeze dryer (SP SCIENTIFIC, USA). Approximately 50 mg of sweet pepper roots, leaves, and fruits were weighed in a nickel boat and analyzed using a Direct Mercury Analyzer (DMA-80 evo, Milestone, Italy). According to US EPA method 7473, the D.M.A. machine was employed for soil, plant tissue, and water samples. The sample is directly introduced into the chamber for drying at a temperature up to 200 °C for 60 s and then ashing at 650 °C for 100 s, in the presence of high-purity oxygen (99.99%) as a carrier and combustion gas. First, mercury and combustion gases are forced through a catalyst bed, where interfering substances such as halogen compounds, sulfur oxides, and nitrogen oxides are retained. Then, Hg0 is selectively trapped in the amalgam while the flammable gas is released. Mercury was then removed from the immobilized amalgam by heating it at 750 °C for about 3s. It was transported to a detector, where the absorption of radiation emitted by the mercury lamp was measured at wavelengths 253.7 nm. This was done for one of two optical path lengths. A dynamic linear calibration curve ranging from low 0.5 to medium 10 ng of Hg was assembled with a high calibration coefficient (R2 = 0.9996). We optimized quality assurance and quality control by utilizing two certified reference materials (CRM). The initial source material comprises NIST 1573a, which pertains to tomato leaves and is procured from the National Institute of Standards and Technology. This material has a certified value of (0.034 ± 0.002). The second reference material is NIST-2709a, which pertains to San Joaquin soil and has a certified value of (0.9 ± 0.2). NIST 1573a and NIST-2709a were found to have observed values of 0.034 ± 0.001 and 0.95 ± 0.002, respectively.

The accumulation coefficient (AC) and the translocation Factor (TF) were calculated as the amount of plant (parts)/soil concentration according to Al-Farraj et al. (2009).

$$AC = \frac{C_{rootorshoot}}{C_{soil}}$$

Moreover, the translocation factor (TF) was calculated to evaluate the transfer of Hg from roots to shoots of the plant.

$$TF = \frac{C_{shoot}}{C_{root}}$$

Where $C_{\text{root orshoot}}$= Concentration of Hg uptake in the plant part (mg/kg^{-1}) and C_{soil} = Concentration in soil (mg/kg^{-1}).

2.5. Statistical analysis

Significant differences between treatments were determined from the analysis of variance by using the general linear model procedure in Statistix 8.1 (Tallahassee Florida, USA) software package, and mean square values were shown in Table 1. LSD test was used to compare the means at the 5% probability level (Gomez and Gomez, 1984). Pearson's correlation and principal component analyses were conducted with Rstat.

3. Results

3.1. Growth attributes

Significant differences were observed in the growth parameters of sweet pepper seedlings grown at different levels of Hg toxicity with the application of SA (Table 1, Fig. 1). The sweet pepper seedlings treated with SA under Hg toxicity showed higher growth than non-treated seedlings. The maximum shoot length, root length, and no. of leaves were observed in the seedlings treated with SA without Hg toxicity, i.e., 18.3 cm, 9.3 cm, and 45.3 respectively (Fig. 1). However, the minimum root length, shoot length and no. of leaves was observed in sweet pepper seedlings grown under 150 μM of Hg without SA application. Moreover, SA application showed no significant difference on shoot length at 150 μM. The

Table 1
Mean square values generated from analysis of variance (ANOVA) from data of exogenous application of salicylic acid on sweet pepper seedling grown under mercury toxicity (p<0.05).

Source	Degree of freedom	Shoot length	Root length	No. of leaves	Quantum yield	Shoot fresh weight
Mercury (T)	**3**	64.15**	442.59**	462.37**	0.29**	79.69**
Salicylic acid (S)	**1**	15.04**	222.04**	135.37**	0.06**	20.14*
T x S	**3**	1.81 ns	12.48 ns	20.48 ns	0.01**	3.99 ns
Error	**16**	0.79	7.83	6.41	0.00	2.54
Total	**23**					
Source	**Degree of freedom**	**Root fresh weight**	**Shoot dry weight**	**Root dry weight**	**Superoxide dismutase**	**Catalase**
Mercury (T)	**3**	11.91**	24.52**	3.07**	665.48**	0.01**
Salicylic acid (S)	**1**	2.9**	7.19**	0.88**	442.04**	0.00**
T x S	**3**	0.55**	1.19**	0.04 ns	59.7**	0.00**
Error	**16**	0.1	0.16	0.01	4.62	0.00
Total	**23**					
Source	**Degree of freedom**	**Peroxidase**	**Hydrogen peroxide**	**Mercury leaves**	**Mercury roots**	**Mercury fruits**
Mercury (T)	**3**	885.83**	2408.49**	86.29**	193456**	5.52**
Salicylic acid (S)	**1**	450.66**	442.04**	7.79**	4910**	0.62**
T x S	**3**	60.33**	79.79 ns	2.57**	3112**	0.4**
Error	**16**	1.45	10.17	0.13	304	0.03
Total	**23**					

ns = Non-significant.
*(p<0.05) Significant.
**(p<0.01) Highly significant.

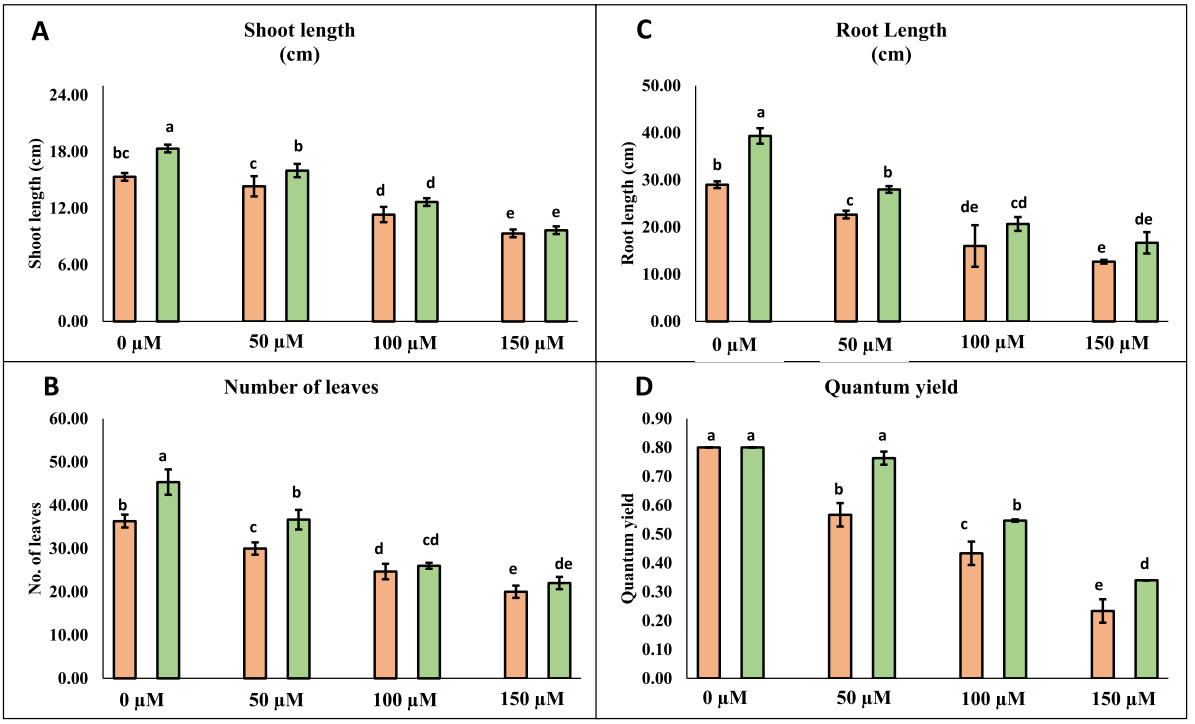

Fig. 1. Measurements of the growth attributes of sweet pepper seedlings grown under different levels of Hg toxicity (0, 50, 100, 150 µM) with SA application (Brown bar = 0 µM SA; Green bar = 100 µM SA). (A) Shoot length; (B) Number of leaves; (C) Root length; (D) Quantum yield (QY). Values are mean ±S.E. at p < 0.05.. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

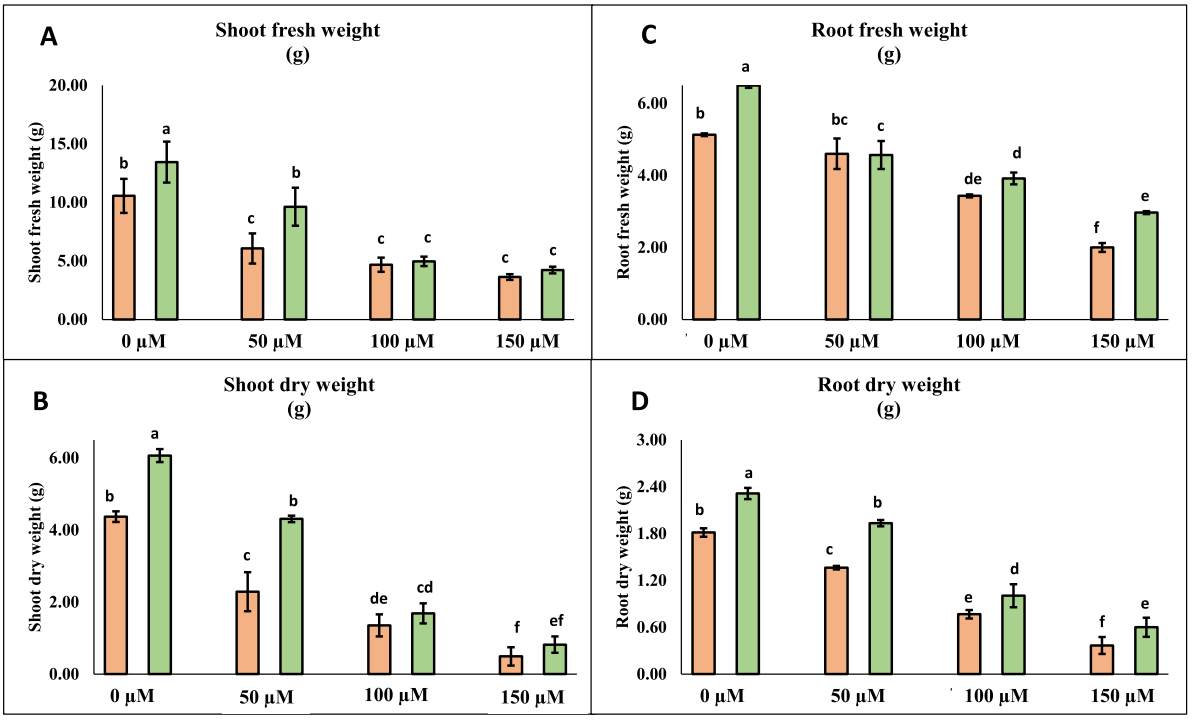

Fig. 2. Measurements of the fresh and dry biomass of sweet pepper seedlings grown under different levels of Hg toxicity (0, 50, 100, 150 µM) with SA application (Brown bar = 0 µM SA; Green bar = 100 µM SA). (A) Shoot fresh weight; (B) Shoot dry weight; (C) Root fresh weight; (D) Root dry weight. Values are mean ±S.E. at $p < 0.05$.. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

maximum quantum yield was observed in control plants, while the minimum quantum yield detected in 150 µM of Hg toxicity without SA application was 0.23 (Fig. 1).

The fresh and dry weights were observed to significantly decrease with the increase of mercury treatment (Table 1, Fig. 2). The maximum shoot fresh weight, root fresh weight and root dry weight was measured in control sweet pepper seedlings treated with SA application (Fig. 2). However, the minimum shoot dry weight, root fresh weight, and root dry weight was observed in 150 µM Hg toxicity without SA. The minimum shoot fresh weight was observed to be 100 and 150 µM Hg with and without SA application (Fig. 2).

3.2. Antioxidative enzymes and H_2O_2

SOD, POD, and CAT activities were significantly increased with increasing Hg toxicity (Table 1). The maximum increase of SOD, POD, and CAT was observed under 100 µM Hg with SA application (Fig. 3). However, the minimum SOD, POD, and CAT were observed in control plants. No significant difference was observed in control plants with or without SA application. Moreover, at 150 µM of Hg toxicity, antioxidant enzymes start decreasing (Fig. 3). On the other hand, the H_2O_2 concentration was significantly higher in plants treated with higher Hg toxicity (Fig. 3). The maximum H_2O_2 content was noted in sweet pepper seedlings grown under 150 µM of Hg toxicity without SA application. SA significantly decreases the H_2O_2 content in the leaves of sweet pepper seedlings under Hg toxicity (Fig. 3).

3.3. Mercury concentration

Mercury concentration in the leaves, roots, and fruits was higher with increased Hg toxicity (Table 1). The highest concentration in leaves, roots, and fruits was observed in 150 µM without SA 10.55 mg/kg, 454.3 mg/kg, and 2.65 mg/kg respectively. Lowest Hg concentration in leaves, roots, and fruits was observed in control plants at 0.52, 0.68, and 0.03 mg/kg (Fig. 4). The maximum AC of Hg was observed under 150 µM of Hg toxicity from root/soil with SA application (Table 2), while the minimum AC was observed at 50 µM Hg toxicity with SA application. The translocation factor (TF) of Hg during Hg toxicity was observed at the maximum in fruit/leaves at 150 µM without SA. The SA application under Hg toxicity showed less Hg translocation in sweet pepper leaves, roots, and fruits (<1) to be considered as non-hyperaccumulator (Table 2). This observation illustrates the diminished capacity of roots to uptake Hg in the presence of SA. In their study, Yang et al. (2020) conducted an evaluation of the transfer factor (TF) in eleven species of leafy

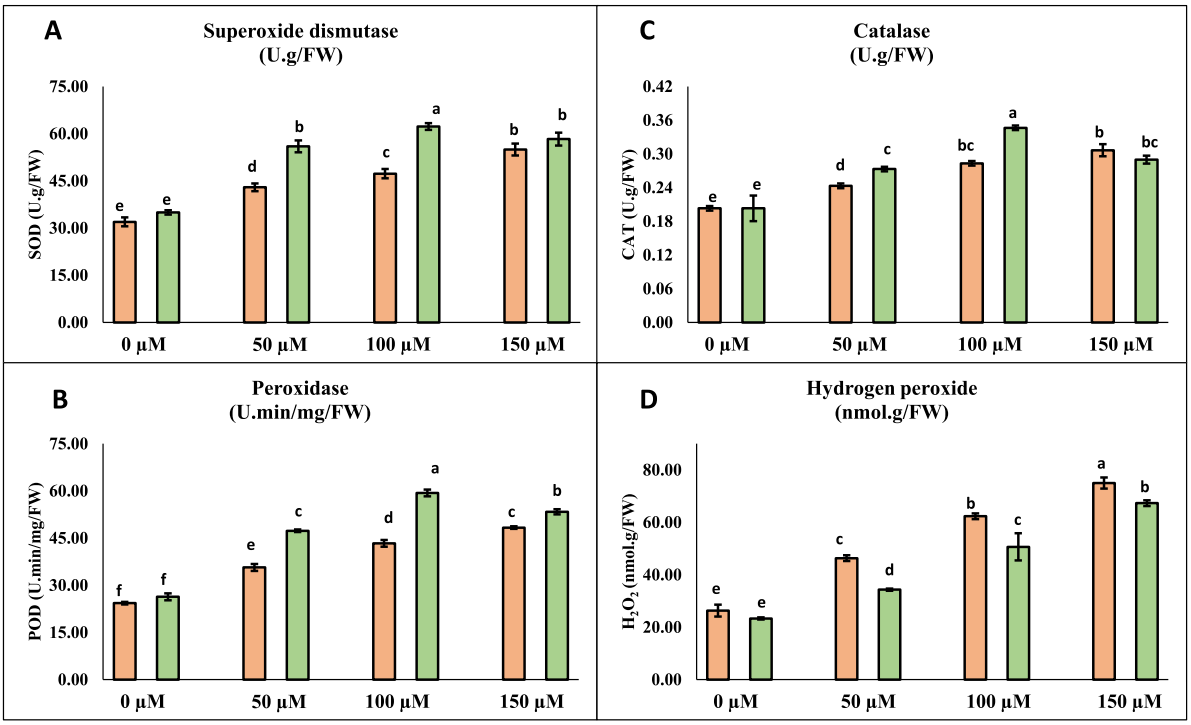

Fig. 3. Measurements of the antioxidative enzymes and hydrogen peroxidase in the leaves of sweet pepper seedlings grown under different levels of Hg toxicity (0, 50, 100, 150 µM) with SA application (Brown bar = 0 µM SA; Green bar = 100 µM SA). (A) Superoxide dismutase; (B) Peroxidase; (C) Catalase; (D) Hydrogen peroxide. Values are mean ±S.E. at $p < 0.05$.. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
The accumulation coefficient (AC) and translocation factor (TF) of different mercury concentrations on leaves, roots, and fruits of sweet pepper.

Salicylic acid (µM)	Mercury (µM)	Soil concentration (mg kg $^{-1}$)	Accumulation Coefficient (AC)			Translocation Factor (TF)	
			Root/Soil	Leaves/Soil	Fruits/Soil	Leaves/Roots	Fruits/Leaves
0.00	**Control**	0.07	7.95	7.95	0.52	0.71	0.07
	50.00	4.35	13.06	0.44	0.03	0.03	0.08
	100.00	8.92	17.38	0.58	0.08	0.03	0.15
	150.00	14.87	30.55	0.71	0.18	0.02	0.25
100.00	**Control**	0.07	9.81	7.48	0.67	0.76	0.09
	50.00	4.35	13.59	0.40	0.02	0.03	0.06
	100.00	8.92	14.96	0.41	0.06	0.03	0.14
	150.00	14.87	24.17	0.52	0.11	0.02	0.20

Soil reference material (NIST2709a) was analyzed as a Quality control sample with mean value 0.84 ± 0.01 mg/kg (the certified value is 0.90 mg/kg).

vegetables, namely, tung choy, spinach, leek, fennel, coriander, wutatsai, pakchoi, chicory, Chinese flowering cabbage, lettuce, and crown daisy. The study investigated the impact of varying concentrations of Hg and soil on the TF, and the findings were consistent with the present study, indicating a TF value of less than one. Hussain et al. (2023) reported similar findings, indicating that the TF of Hg in pepper was below the threshold value of 1, which is considered an optimal benchmark for a non-hyperaccumulator, in response to varying soil types. Mei et al. (2021) conducted a study to assess the phytotoxicity of Hg and observed an inverse correlation between Hg concentrations and the translocation factor (TF) in cotton seedlings. The results of our study are consistent with the observed translocation factor of Hg at concentrations of 50µM and 100µM, which were determined to be 0.05 and 0.06, respectively.

3.4. Pearson's correlation

The quantum yield, no. of leaves, shoot length, root length, fresh and dry weight of shoot and roots were significant and positively correlated with each another, while H_2O_2 was negatively significant correlated with them (Table 3). Mercury concentration in leaves, roots and fruits were negatively correlated with growth attributes, however, positively correlated with antioxidative enzymes and H_2O_2.

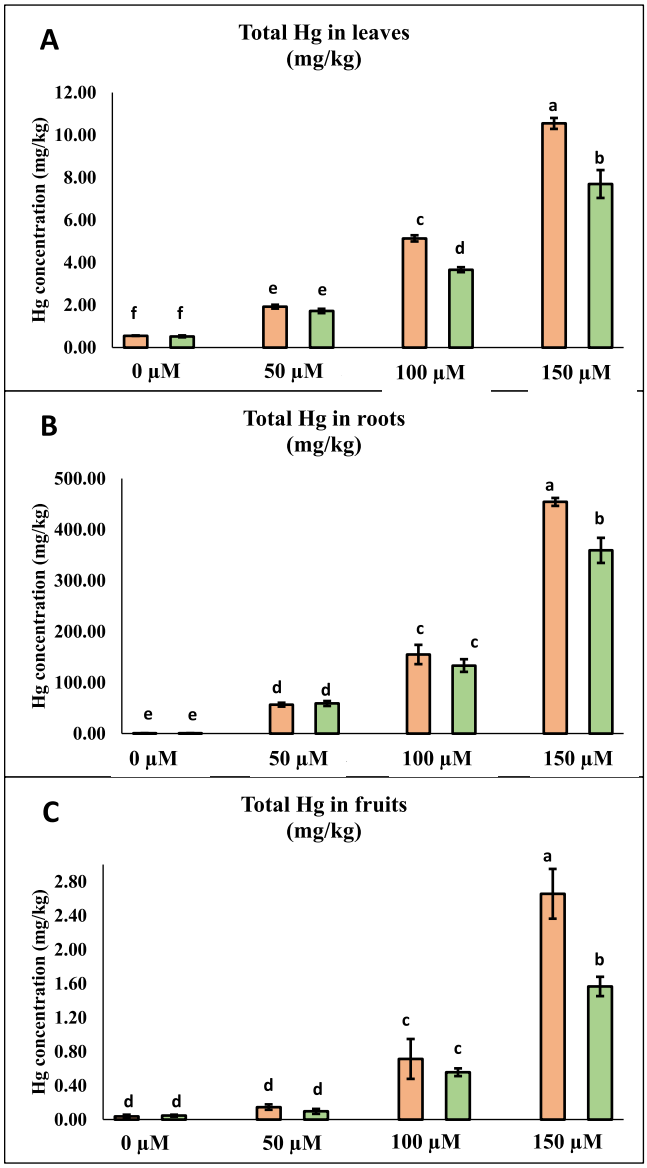

Fig. 4. Measurements of the total Hg concentrations in the leaves, roots, and fruits of sweet pepper seedlings grown under different levels of Hg toxicity (0, 50, 100, 150 µM) with SA application (Brown bar = 0 µM SA; Green bar = 100 µM SA). (A) Total Hg in leaves; (B) Total Hg in roots; (C) Total Hg in fruits. Values are mean ±S.E. at $p < 0.05$.. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5. Principal component analysis

Principal component analysis was conducted for different studied attributes of salicylic acid on sweet pepper seedlings grown under mercury toxicity. Analysis clearly showed separation due to application of salicylic acid and mercury toxicity. F1 separated different toxicity level of mercury, whereas F2 separated application of salicylic acid. The first two components with Eigenvalues >1 contributed 94.2% of the variance in the studied attributes (Table 4 and Fig. 5). A variation of 85.01% was noted, and the attributes that contributed positively to F1 were H_2O_2, mercury in leaves, roots, and fruits while, quantum yield and all growth attributes contributed negatively. For F2, a variability of 9.18% was observed, and SOD, POD, and CAT contributed positively. Fifteen variable groups over four planes were studied, which showed negative and positive factor space in 2D (Fig. 5). SOD, POD, and CAT laid on the positive planes of both axes, which shows positive correlation, whereas the other 12 variables showed negative association with at least one parameter by occurring in the negative axis.

Table 3
The Pearson's correlation matrix of exogenous application of salicylic acid on sweet pepper seedling grown under mercury toxicity.

	RL	NOL	SL	SFW	SDW	RFW	RDW	SOD	POD	CAT	H2O2	QY	ML	MR	MF
RL	1														
NOL	0.988	1.000													
SL	0.964	0.980	1.000												
SFW	0.975	0.985	0.937	1.000											
SDW	0.982	0.995	0.966	0.994	1.000										
RFW	0.966	0.958	0.965	0.918	0.936	1.000									
RDW	0.971	0.988	0.989	0.963	0.984	0.952	1.000								
SOD	−0.639	−0.669	−0.623	−0.697	−0.663	−0.695	−0.625	1.000							
POD	−0.683	−0.719	−0.670	−0.748	−0.715	−0.714	−0.679	0.990	1.000						
CAT	−0.738	−0.767	−0.703	−0.796	−0.763	−0.750	−0.737	0.938	0.966	1.000					
H2O2	−0.949	−0.958	−0.967	−0.940	−0.959	−0.952	−0.979	0.655	0.691	0.725	1.000				
QY	0.916	0.936	0.954	0.911	0.939	0.926	0.969	−0.593	−0.629	−0.670	−0.991	1.000			
ML	−0.847	−0.865	−0.921	−0.803	−0.846	−0.931	−0.909	0.606	0.617	0.644	0.944	−0.955	1.000		
MR	−0.803	−0.833	−0.897	−0.764	−0.811	−0.906	−0.873	0.617	0.617	0.617	0.911	−0.928	0.990	1.000	
MF	−0.755	−0.778	−0.848	−0.697	−0.751	−0.869	−0.832	0.513	0.511	0.548	0.866	−0.897	0.979	0.981	1

RL = Root length; NOL = No. of leaves; SL = Shoot length; SFW = Shoot fresh weight; SDW = Shoot dry weight; RFW = Root fresh weight; RDW = Root dry weight; SOD = Superoxide dismutase; POD = Peroxidase; CAT = Catalase; H2O2 = Hydrogen peroxide; QY = Quantum yield; ML = Mercury in leaves; MR = Mercury in roots; MF = Mercury in fruits.

Table 4
Principal Components for studied attributes of salicylic acid on sweet pepper seedlings under mercury toxicity.

Variables	F1	F2
Root Length	−0.269	0.044
Number of Leaves	−0.273	0.026
Shoot length	−0.273	0.121
Shoot fresh weight	−0.267	−0.053
Shoot dry weight	−0.271	0.017
Root fresh weight	−0.273	0.059
Root dry Weight	−0.274	0.098
SOD	0.209	0.529
POD	0.218	0.519
CAT	0.227	0.469
H2O2	0.275	−0.104
QY	−0.270	0.177
Mercury leaves	0.262	−0.200
Mercury roots	0.255	−0.193
Mercury fruits	0.241	−0.279
Eigenvalue	12.752	1.378
Variability (%)	85.013	9.187
Cumulative %	85.013	94.200

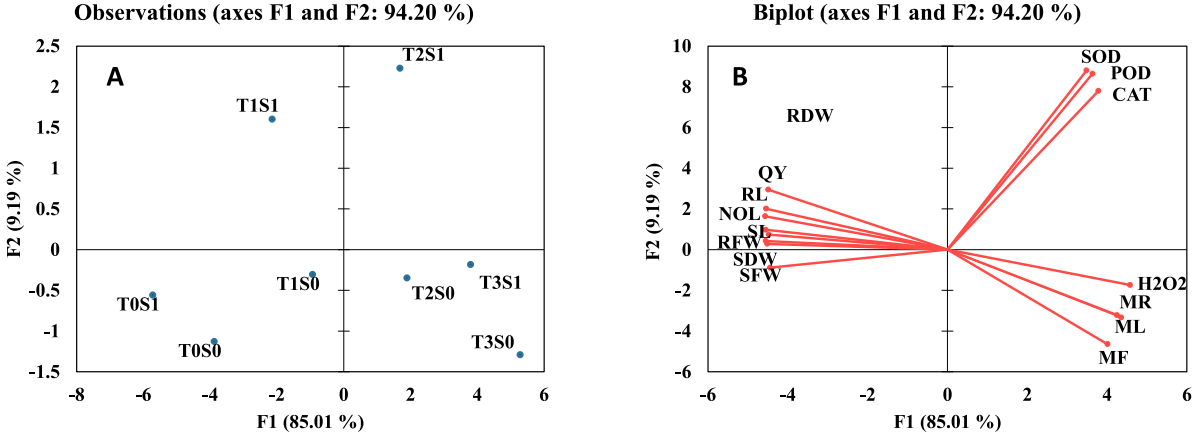

Fig. 5. Principal Component Analysis (PCA) representing the application of SA (0 and 100 μm) in sweet pepper seedlings grown under different levels of Hg toxicity (0, 50, 100, 150 μM), plotted with the contribution of each parameter on the two PCA axes (A) and all the physiological and biochemical parameters measured (B). Principal Component Analysis (PCA)-Variable's correlation of seedlings.

4. Discussion

Plants readily absorb mercury ions from the soil. Plants that accumulate Hg^{2+} in their roots develop oxidative stress due to a rapid mobilization of Hg^{2+} into their aerial branches (Jameer Ahammad et al., 2018). Hg can inhibit photosynthesis, reduce growth, and cause chlorosis, necrosis, and leaf drop. In addition, the metabolism of essential nutrients uptake

can be interfered by Hg toxicity, leading to nutrient deficiencies and reduced yields. Furthermore, Hg toxicity can cause oxidative stress and damage to the plant's DNA, which can lead to mutations and even cell death (Cargnelutti et al., 2006; Xu et al., 2020). To cope with the negative effect of heavy metals including Hg, SA is found to be effective (Wang et al., 2021). SA's role in signaling molecules is crucial the development and mediation of plant responses to heavy metal stress (Roya et al., 2019). In this study, the increase in the concentration of Hg significantly decreases the growth and development of sweet pepper seedlings, while the application of SA under Hg toxicity restricts the decrease in growth attributes (Figs. 1 and 2). Pearson's correlation and principal component analysis also suggested that Hg concentration and growth attributes (shoot length, root length, no. of leaves, shoot and root fresh weight, and shoot and root dry weight) were negatively correlated with each other (Table 3, Fig. 5). Xu et al. (2020) also observed that Hg toxicity restricts the growth and development of ginger plan ts. However, the external application of SA mitigates Hg stress and showed fewer fluctuations in plant growth attributes (Zhou et al., 2009; Safari et al., 2019; Dar et al., 2023. The photosystem II (PSII) is known as the primary indicator of plants under stress conditions, we indicated the PSII values as quantum yield (Fv/Fm). The ratio of Fv/Fm is an effective indicator of the plant health and its ability to tolerate heavy metal stress. Additionally, some genotypes are more sensitive to pressure and show a more significant decrease in Fv/Fm (Dąbrowski et al., 2023), mainly affected by electron acceptor concentrations (NADP+ available at the acceptor side of PSI) (Maxwell and Johnson, 2000). A number of investigations suggest that sweet pepper seedling QY is decreasing from increased Hg toxicity, yet SA inhibits this decrease and maintains QY, making them tolerable under Hg toxicity (Fig. 2). The application of SA enhanced the effective QY of PSII by increasing the efficiency of the oxygen-evolving complex and enhancing the fraction of open PSII reaction centers (q*p*), which resulted in a higher electron transport rate (Moustakas et al., 2023).

When plants showed a decrement in their photosynthetic machinery cells accumulate ROS (e.g., H_2O_2) that damages chloroplast and mitochondrial metabolism, resulting in cell death (Gill and Tuteja, 2010; Khalid et al., 2022). As plants that suffer from greater stress have more ROS, our findings demonstrate that sweet pepper seedlings with very high Hg toxicity exhibit more H_2O_2, while SA significantly decreases the H_2O_2 content (Fig. 3). ROS must be produced and expelled in equilibrium as part of plant metabolism. To maintain equilibrium in response to ROS overproduction under unfavorable conditions, plants produce antioxidizing enzymes and osmolytes (Hussain et al. 2018; Khalid et al., 2020, 2022). Plants use SOD as one of their first defense mechanisms under such conditions. Superoxide ions are scavenged and converted to H_2O_2 by SOD, and H_2O_2 is scavenged equally by CAT and POD. In our experiment, we observed a significant increase in SOD, POD, and CAT activity in sweet pepper seedlings when exposed to Hg toxicity (Fig. 3). The increase was greater in the seedlings treated with SA. Hu et al. (2023) observed that, by increasing heavy metal toxicity, antioxidant enzymes also increased. Sihag et al. (2019) indicated that, by applying SA, under chromium toxicity, the plants exhibited more antioxidative enzymes as compared to the one without SA applications. Furthermore, SOD, POD, and CAT were the only attributes in the positive axis, as both were positively correlated with Hg toxicity and SA application (Fig. 5). The accumulation of Hg was increased in leaves, roots, and fruits by increasing Hg concentration in soil. The accumulation of Hg was lower in seedlings treated with SA. The SA application restricts the accumulation of Hg at 150 μM in leaves, roots and fruits of sweet pepper However, Hg accumulation was not significantly affected when treated with 50 and 100 μM in roots and fruits. However, at 100 μM in leaves the SA hinders the transportation of Hg ions to the leaves (Fig. 4). Wang et al. (2021) also observed that with the application of SA, heavy metals accumulation and translocation was decreased.

5. Conclusion

Hg toxicity caused a serious reduction in plant growth and development. The application of SA significantly decreases the Hg toxicity in sweet pepper seedlings. SA showed less decrease in PSII, plant length (shoot and root), fresh and dry biomass (shoot and root), and no. of leaves as compared to non-SA plants. SA also restricts the production of H_2O_2 by producing more SOD, POD, and CAT activity under Hg toxicity. SA restricts the accumulation of Hg in the roots and leaves of sweet pepper plants and hinders the translocation of Hg from roots to fruits. In the future transcriptome analysis in plants under Hg toxicity along with SA signaling pathways to clarify the mechanisms of SA inhibiting Hg accumulation in fruits.

CRediT authorship contribution statement

Muhammad Fasih Khalid: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. **Ahmed Abou Elezz:** Investigation, Formal analysis, Methodology, Writing – review & editing. **Muhammad Zaid Jawaid:** Formal analysis, Writing – review & editing. **Talaat Ahmed:** Supervision, Resources, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data collected during the study presented within the article.

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References

Al-Farraj, A.S., Al-Otabi, T.G., Al-Wabel, M.I., 2009. Accumulation coefficient and translocation factor of heavy metals through ochradenus baccatus plant grown on mining area at Mahad AD'Dahab Saudi Arabia. WIT Trans. Ecol. Environ. 459–468. http://dx.doi.org/10.2495/ECO090421.

Bjørklund, G., Dadar, M., Mutter, J., Aaseth, J., 2017. The toxicology of Mercury: Current research and emerging trends. Environ. Res. 159, 545–554. http://dx.doi.org/10.1016/j.envres.2017.08.051.

Cargnelutti, D., Tabaldi, L.A., Spanevello, R.M., Jucoski, G.de.Oliveira., Battisti, V., Redin, M., Linares, C.E.B., Dressler, V.L., de Moraes Flores, E.M., Nicoloso, F.T., Morsch, V.M., 2006. Mercury toxicity induces oxidative stress in growing cucumber seedlings. Chemosphere 65 (6), 999–1006. http://dx.doi.org/10.1016/j.chemosphere.2006.03.037.

Chance, B., Maehly, A.C., 1955. Assay of catalases and peroxidases. In: 10.1016/S0076-6879(55)02300-8. Methods Enzymol. 2, 764–775.

Chen, L., Kang, Y.-H., 2013. Anti-inflammatory and antioxidant activities of red pepper (*Capsicum annuum L.*) stalk extracts: Comparison of pericarp and placenta extracts. J. Function. Food. 5 (4), 1724–1731. http://dx.doi.org/10.1016/j.jff.2013.07.018.

Dąbrowski, P., Keutgen, A.J., Keutgen, N., Sierka, E., Baczewska-Dąbrowska, A.H., Mojski, J., Pawluśkiewicz, B., Sieczko, L., Kalaji, H.M., 2023. Photosynthetic efficiency of perennial ryegrass (*Lolium perenne L.*) seedlings in response to Ni and Cd stress. Sci. Rep. 13 (1), 1–16. http://dx.doi.org/10.1038/s41598-023-32324-x.

Dar, S.A., Lone, R., Tyub, S., Kamili, A.N., Nawchoo, I.A., 2023. Crosstalk of ethylene and salicylic acid in the amelioration of toxic effects of heavy metal stress in mustard. In: Plant Phenolics in Abiotic Stress Management (173–193). Springer Nature Singapore, Singapore, http://dx.doi.org/10.1007/978-981-19-6426-8_9.

Deepa, N., Kaur, C., Singh, B., Kapoor, H.C., 2006. Antioxidant activity in some red sweet pepper cultivars. J. Food Composit. Anal. 19 (6–7), 572–578. http://dx.doi.org/10.1016/j.jfca.2005.03.005.

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases I. occurrence in higher plants. Plant. Physiol. 59, 309–314. http://dx.doi.org/10.1104/pp.59.2.309.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930. http://dx.doi.org/10.1016/j.plaphy.2010.08.016.

Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agricultural research. John Wiley & sons.

Hoagland, D.R., Arnon, D.J., 1950. The water culture method for growing plants without soil calif. Agric. Exp. State Circ. 347, 1–3.

Hu, Y., Wang, H., Jia, H., Peng, M., Zhu, T., Liu, Y., Wei, J., 2023. Effects of cd treatment on morphology, chlorophyll content and antioxidant enzyme activity of Elymus nutans Griseb. a native plant in Qinghai-Tibet Plateau. Plant Signal. Behav. 18 (1), 2187561. http://dx.doi.org/10.1080/15592324.2023.2187561.

Hussain, S., Jianjun, Y., Hussain, J., Zandi, P., Xing, X.Subhanullah., Liandong, Z., Yu, T., Ali, A., Kebin, Z., 2023. The rhizospheric transformation and bioavailability of mercury in pepper plants are influenced by selected Chinese soil types. Environ. Geochem. Health 45, 41–52. http://dx.doi.org/10.1007/s10653-022-01209-9.

Jameer Ahammad, S., Sumithra, S., Senthilkumar, P., 2018. Mercury uptake and translocation by indigenous plants. Rasayan J. Chem. 11, 1–12. http://dx.doi.org/10.7324/RJC.2018.1111726.

Khalid, M.F., Huda, S., Yong, M., Li, L., Li, L., Chen, Z.H., Ahmed, T., 2023. Alleviation of drought and salt stress in vegetables: crop responses and mitigation strategies. Plant Growth Regul. 99, 177–194. http://dx.doi.org/10.1007/s10725-022-00905-x.

Khalid, M.F., Hussain, S., Ahmad, S., Ejaz, S., Zakir, I., Ali, M.A., Ahmed, N., Anjum, M.A., 2019. Impacts of abiotic stresses on growth and development of plants. In: Plant tolerance to environmental stress. CRC Press, pp. 1–8.

Khalid, M.F., Hussain, S., Anjum, M.A., Ahmad, S., Ali, M.A., Ejaz, S., Morillon, R., 2020. Better salinity tolerance in tetraploid vs diploid Volkamer lemon seedlings is associated with robust antioxidant and osmotic adjustment mechanisms. J. Plant Physiol. 244, 153071. http://dx.doi.org/10.1016/j.jplph.2019.153071.

Khalid, M.F., Morillon, R., Anjum, M.A., Ejaz, S., Rao, M.J., Ahmad, S., Hussain, S., 2022. Volkamer lemon tetraploid rootstock transmits the salt tolerance when grafted with diploid kinnow mandarin by strong antioxidant defense mechanism and efficient osmotic adjustment. J. Plant Growth Regul. 41 (3), 1125–1137. http://dx.doi.org/10.1007/s00344-021-10367-6.

Kim, Y.-O., Bae, H.-J., Cho, E., Kang, H., 2017. Exogenous glutathione enhances Mercury tolerance by inhibiting mercury entry into plant cells. Front. Plant Sci. 8, http://dx.doi.org/10.3389/fpls.2017.00683.

Li, Y., Guan, J., Zhao, J., Li, B., Li, Y., -feng, Gao, Y., 2020. Comparative study of the effects of different chelating ligands on the absorption and transport of mercury in maize (zea mays l.). Ecotoxicol. Environ. Saf. 188, 109897. http://dx.doi.org/10.1016/j.ecoenv.2019.109897.

Lo Scalzo, R., Campanelli, G., Paolo, D., Fibiani, M., Bianchi, G., 2020. Influence of organic cultivation and sampling year on quality indexes of sweet pepper during 3 & nbsp;years of production. Euro. Food Res. Technol. 246 (6), 1325–1339. http://dx.doi.org/10.1007/s00217-020-03492-1.

Mahbub, K.R., Krishnan, K., Naidu, R., Andrews, S., Megharaj, M., 2017. Mercury toxicity to terrestrial biota. Ecol. Indic. 74, 451–462. http://dx.doi.org/10.1016/j.ecolind.2016.12.004.

Marrugo-Negrete, J., Durango-Hernández, J., Pinedo-Hernández, J., Enamorado-Montes, G., Díez, S., 2016. Mercury uptake and effects on growth in Jatropha curcas. J. Environ. Sci. 48, 120–125. http://dx.doi.org/10.1016/j.jes.2015.10.036.

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. J. Exp. Bot. 51, 659–668. http://dx.doi.org/10.1093/jexbot/51.345.659.

Mei, L., Zhu, Y., Zhang, X., Zhou, X., Zhong, Z., Li, H., Li, Y., Li, X., Daud, M.K., Chen, J., Zhu, S., 2021. Mercury-induced phytotoxicity and responses in upland cotton (Gossypium hirsutum L.) seedlings. Plants 10, 1494. http://dx.doi.org/10.3390/plants10081494.

Moustakas, M., Sperdouli, I., Moustaka, J., Şaş, B., Işgören, S., Morales, F., 2023. Mechanistic insights on salicylic acid mediated enhancement of photosystem ii function in oregano seedlings subjected to moderate drought stress. Plants 12 (3), 518. http://dx.doi.org/10.3390/plants12030518.

Natasha Shahid, M., Khalid, S., Bibi, I., Bundschuh, J., Khan Niazi, N., Dumat, C., 2020. A critical review of mercury speciation bioavailability, toxicity and detoxification in soil-plant environment: ecotoxicology and health risk assessment. Sci. Total Environ. 711, 134749. http://dx.doi.org/10.1016/j.scitotenv.2019.134749.

Pirzadah, T.B., Malik, B., Tahir, I., Irfan, Q.M., Rehman, R.U., 2018. Characterization of mercury-induced stress biomarkers in fagopyrum tataricum plants. Int. J. Phytoremediat. 20 (3), 225–236. http://dx.doi.org/10.1080/15226514.2017.1374332.

Roya, Z., Rashid, J., Fatemeh, R., 2019. Role of salicylic acid and hydrogen sulfide in promoting lead stress tolerance and regulating free amino acid composition in *Zea mays* l. Acta. Physiol. Plant. 41, 94. http://dx.doi.org/10.1007/s11738-019-2892-z.

Safari, F., Akramian, M., Salehi-Arjmand, H., Khadivi, A., 2019. Physiological and molecular mechanisms underlying salicylic acid-mitigated mercury toxicity in lemon balm (*Melissa officinalis* l.). Ecotoxicol. Environ. Saf. 183, 109542. http://dx.doi.org/10.1016/j.ecoenv.2019.109542.

Sharma, A., Sidhu, G.P., Araniti, F., Bali, A.S., Shahzad, B., Tripathi, D.K., Brestic, M., Skalicky, M., Landi, M., 2020. The role of salicylic acid in plants exposed to heavy metals. Molecules 25 (3), 540. http://dx.doi.org/10.3390/molecules25030540.

Shiyab, S., Chen, J., Han, F.X., Monts, D.L., Matta, F.B., Gu, M., Su, Y., Masad, M.A., 2009. Mercury-induced oxidative stress in Indian mustard (*Brassica juncea* L.). Environ. Toxicol. 24 (5), 462–471. http://dx.doi.org/10.1002/tox.20450.

Sihag, S., Brar, B., Joshi, U.N., 2019. Salicylic acid induces amelioration of chromium toxicity and affects antioxidant enzyme activity in *Sorghum bicolor L.* Int. J. Phytoremediat. 21 (4), 293–304. http://dx.doi.org/10.1080/15226514.2018.1524827.

Tamás, L., Mistrík, I., Zelinová, V., 2017. Heavy metal-induced reactive oxygen species and cell death in barley root tip. J. Exp. Bot. 140, 34–40. http://dx.doi.org/10.1016/j.envexpbot.2017.05.016.

Teixeira, D.C., Lacerda, L.D., Silva-Filho, E.V., 2018. Foliar mercury content from tropical trees and its correlation with physiological parameters in situ. Environ. Pollut. 242, 1050–1057. http://dx.doi.org/10.1016/j.envpol.2018.07.120.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151, 59–66. http://dx.doi.org/10.1016/S0168-9452(99)00197-1.

Wang, F., Tan, H., Huang, L., Cai, C., Ding, Y., Bao, H., Chen, Z., Zhu, C., 2021. Application of exogenous salicylic acid reduces cd toxicity and Cd accumulation in rice. Ecotoxicol. Environment. Saf. 207, 111198. http://dx.doi.org/10.1016/j.ecoenv.2020.111198.

Wani, A.B., Chadar, H., Wani, A.H., Singh, S., Upadhyay, N., 2016. Salicylic acid to decrease plant stress. Environ. Chem. Lett. 15 (1), 101–123. http://dx.doi.org/10.1007/s10311-016-0584-0.

WHO, 2019. Mercury and health. world health organization. https://www.who.int/news-room/fact-sheets/detail/mercury-and-health, (Accessed 24 January 2023).

Xu, J., Zhang, J., Lv, Y., Xu, K., Lu, S., Liu, X., Yang, Y., 2020. Effect of soil mercury pollution on ginger (zingiber officinale roscoe): growth, product quality, health risks and silicon mitigation. Ecotoxicol. and Environ. Saf. 195, 110472. http://dx.doi.org/10.1016/j.ecoenv.2020.110472.

Xun, Y., Feng, L., Li, Y., Dong, H., 2017. Mercury accumulation plant cyrtomium macrophyllum and its potential for phytoremediation of mercury polluted sites. Chem. 189, 161–170. http://dx.doi.org/10.1016/j.chemosphere.2017.09.055.

Yang, B., Gao, Y., Zhang, C., Zheng, X., Li, B., 2020. Mercury accumulation and transformation of main leaf vegetable crops in cambosol and ferrosol soil in China. Environ. Sci. Pollut. Res. 27, 391–398. http://dx.doi.org/10.1007/s11356-019-06798-0.

Yu, H., Li, J., Luan, Y., 2018. Meta-analysis of soil mercury accumulation by vegetables. Sci. Rep. 8, 1261. http://dx.doi.org/10.1038/s41598-018-19519-3.

Zhou, Z.S., Guo, K., Elbaz, A.A., Yang, Z.M., 2009. Salicylic acid alleviates mercury toxicity by preventing oxidative stress in roots of Medicago sativa. Environ. Exp. Bot. 65 (1), 27–34. http://dx.doi.org/10.1016/j.envexpbot.2008.06.001.