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Heavy metal toxicity induced by sewage water treatment in three different vegetables (lettuce, spinach and cabbage) was alleviated by brassinosteroid and silicon supplementation

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ABSTRACT

Purpose: Heavy metal stress due to the application of sewage water is considered a leading factor for constrained plant growth. Therefore, Current study was performed in the field to investigate the interactive influence of two irrigation sources (canal and sewage) and exogenous application of brassinosteroid (BRs) and silicon (Si) at different rates on growth, photosynthetic and physiological attributes of three leafy vegetables (lettuce, cabbage, and spinach). **Methods**: Three treatments were applied such as control, BRs and Si with three replications under split plot factorial design. **Results**: The results indicated that all the growth parameters including fresh biomass of plants and roots, dry biomass of plant and root, leaf area (LA), photosynthetic traits, chlorophyll contents, water use efficiency (WUE), activity of antioxidant enzymes; superoxide dismutase (SOD), peroxidase (POD), Catalase (CAT), ascorbate peroxidase (APX) and uptake of silicon except Cd and Pb contents were improved significantly with the foliar spray of BRs and Si over control treatment under both types of irrigation sources. However maximum improvement in leafy vegetables was recorded when the foliar spray of BRs and Si were applied under canal water irrigation. **Conclusions**: The foliar application of BRs and Si was proved as an ameliorating strategy for improving tolerance mechanisms associated with heavy metal stress. The major restoring mechanism was the restricted translocation of Cd and Pb leading to better growth and physiology under sewage water. Thus the application of BRs and Si reduced the detrimental effect of Cd and Pb led to improve the growth and physiological traits of leafy vegetables under irrigation with sewage water.

1. Introduction

Heavy metal intake through food stuffs is considered a major threat to human health worldwide due to its non-biodegradable and tenacious nature. Heavy metals are toxic elements having density ≥ 4 gcm^{-3} and reduce plant growth by creating the environment vulnerable to the plant population. Intake of these metals by various means of food stuff beyond the normal range in the biological system prompts destabilization of human physiology and affects the functions of major organs related to human physiology such as kidney, liver, brain, liver and bones. Irrigation of sewage water to vegetables, cereals, and fruit crops isthe major cause of heavy metal stress in plant and living organisms (Tariq et al., 2023). Other than sewage water irrigation, species of plants, type of soil and the dynamic intensity of heavy metal stress are also the main factors contributing to the induce metal toxicity in soil and vegetables (Hardaway et al., 2016). Moreover, pesticide and organic and inorganic fertilizer applications also affect the absorbance of nutrients by the plant due to modification in soil properties which could lead to a favorable environment for heavy metal contamination (Rizwan et al., 2021). Among vegetables, leafy vegetables receive more concentrations of heavy metal elements than fruits, cereals, and grain crops (Najmi et al., 2023) which results in a high concentration of impurities in the edible plant parts and subsequently increases in the human body. Metal contaminants are mainly absorbed by plant roots form soil and translocated

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to other plant parts as dissolved ions (Feng et al., 2021). Most of the researchers reported that leafy vegetables including lettuce, spinach cabbage, etc. uptake metal contaminants in higher concentrations after tuber and fruit crops (Christou et al., 2019). Rehman et al. (2017) declared in his research that Cd and Pb were found maximum in green vegetables as compared to other tuber crops. Heavy metal stress encourages stunted plant growth owing to reduced photosynthetic activity, disrupted transpiration and respiration processes and ion homeostasis (Manzoor et al., 2018). Reduction in root: shoot ratio and plant biomass are also due to heavy metal stress. Similarly, deterioration of plant defense systems against stress is also found in plants under the contaminated region. Activation and inhibition of enzymes, membrane damage, and electron transport systems are associated with heavy metal toxicity (Awasthi et al., 2020). Thus the plants suffering stress induced by metal elements contribute to oxidative stress, and physical and biological damage which ultimately destroys plant quality and productivity (Noor et al., 2022). Additionally, protein, lipids, and thylakoid membranes are also retarded in plans due to heavy metal accumulation in plants beyond the limits (Kim et al., 2014). Hence heavy metal contamination is a threatning aspect for plants and animals as well as for human health which could be minimized by applying different techniques (Sadaf et al., 2024). Among various approaches to alleviate the adverse effect of metal contamination, silicon (Si) and brassinosteroid (BRs) could be applied through foliar application to vegetables and other fruit crops.

Silicon (Si) as a beneficial nutrient plays a significant function in reducing negative impacts of abiotic stress (Liang et al., 2015; Deshmukh et al., 2017; Ahanger et al., 2020). Silicon application stimulate the metal detoxification process by altering cellular and biochemical mechanisms during plant growth (Shanmugaiah et al., 2023). The integrity of the positive effect of Si differs plant to plant specie and is more prominent in plants that regulate the maximum proportion of silicon in plant parts (Hosseini et al., 2019). Plant uptake Si by roots from soil solution as silicic acid in the range of 0.1 to 0.5 mmol L^{-1}. Silicon can recover soil physicochemical characteristics by improving water and air transport in soil and subsequently increase nutrient availability by alleviating toxicity of metal ion (Zhu et al., 2019). Thus silicon application also regulates enzymes and gene expressions related to metal transporters (Etesami and Jeong, 2018). Previous researchers reported that plants showed improved growth under heavy metal stress when silicon was applied in optimum concentrations in various crops including, cotton, wheat, maize and penut (Rehman et al., 2021).

Similarly, Brassinosteroids (Brs), a steroid hormone, also influences cell elongation, cell expansion, male fertility and flowering, germination of seed, synthesis of stomata, and structure of plant (Sadaf et al., 2024). Various types of Brs are categorized with respect to the carbon numbers in their structure. Brassinosteroids (Brs) perform different functions in plants to protect against biotic and abiotic stressers (Wang et al., 2014).

By understanding how Brs and Si affect plant responses to WW irrigation, it may be possible to design strategies that maximize crop production while minimizing environmental contamination. This can lead to more efficient and responsible use of WW resources in agricultural settings. The current study's potential benefits include improved crop productivity, enhanced stress tolerance, regulated metal uptake, sustainable waste water (WW) management, and potential applications in various crops and environments. These outcomes have the potential to contribute to more efficient and environmentally conscious agricultural practices, ensuring food security and minimizing the risks associated with wastewater irrigation.

Therefore, this study was aimed to understand the interactive effect of metal stress and foliar spray of Brs and Si on growth and physiological components of plant and on antioxidant enzymatic activities of leafy vegetables (lettuce, spinach, and cabbage) under sewage water irrigation.

2. Material and Methods

2.1. Experiment site

The trial location was situated under semi-arid conditions, with mixed cultivation of orchards and field crops. The weather was characterized by extreme conditions i.e. hot summer and cold winter. Generally, temperatures begin to rise from mid-March and attain a maximum by May-June (mean maximum temperature is about 40 °C. The average rainfall in this area is about 96.2 mm, and the mean relative humidity is 61 %. The field trail was performed in the experimental zone of Horticulture Department, College of Agriculture, University of Sargodha in Punjab province of Pakistan.

2.2. Experiment

The field trial was conducted during October 2016–17. The seeds of 3 different vegetable species were collected from NARC, Islamabad, and sown on ridges. Seeds were dibbled manually by maintaining the proper distance. Three seeds of lettuce (Butter Head) and spinach (Local Sindhi) cabbage (Golden Acre) were sown per dibbled and thinned to a single plant after germination. All these vegetable varieties are mostly preferred in native region for home gardening. In addition, these varieties are high yielding capable to grow under stress conditions and have disease resistance.

Trials were conducted in split-plot two factorial designs where irrigational treatments were assigned in the main plot while vegetables were grown in micro plots. Theplot size was 90 cm × 300 cm and treatments were replicated thrice. Foliar application of Brassinosteroid @ 3.5 μM and Silicon @ 2 mM in case of lettuce and cabbage while Brassinosteroid @ 3.5 μM and Silicon @ 3 mM in case of spinach were applied at 15 days after germination in all three crops. The field was irrigated as per requirement with canal and sewage water according to the treatment plan.

2.3. Collection of data regarding growth parameters

For agronomic attributes, each seedling and its root were harvested after 35 days of plant growth, washed to clean its surface dirt, dried with tissue paper, and weighed by digital electric balance to determine the fresh weight. Harvested seedlings and roots were dried at 70 °C in an oven (KorlKolb 112 SL, Germany) for 48 h to constant mass. The dried weight of the seedling and root was determined through analytical balance. Area of leaf was estimated by using a digital meter (Ll-3000C) known as leaf area (LA) meter. The average leaf area was calculated by the method of (Binkley et al., 2002). The root length was measured with the help of measuring tape.

2.4. Collection of data regarding physiology and antioxidant enzymatic activities

Transpiration rate, Photosynthesis rate and stomatal conductance were determined by an instrument IRGA, CI-340 CID Biosciences, USA. All the gas exchange attributes were measured on top third leaf which was full expandedduring day hours. Water use efficiency (WUE) was measured by using following formula

$$WUE = A/E$$

Where, A = Photosynthetic rate E = transpiration rate

Similarly SPAD meter (502 SPAD spectrum) was used to measure chlorophyll contents of leaf.

The Catalase (CAT) activity was examined by a procedure elaborated by Chance and Maehly (1955). While superoxide dismutase (SOD) was measured by the method of Giannopolitis and Ries (1977). Activity of Peroxidase (POD) enzyme from solution mixture was measured by

Table 1
Foliar Effect of BRs and Si solutions on fresh weight, dry weight and leaf area of leafy vegetables irrigated with canal and sewage water.

Treatments	Water Source	Lettuce			Spinach			Cabbage		
		Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)
Control	CW	42.31 ± 2.60b	4.37 ± 0.27b	43.11 ± 2.65b	66.81 ± 4.10b	6.74 ± 0.41bc	78.83 ± 4.84b	54.56 ± 3.35bc	5.08 ± 0.31b	52.85 ± 3.25bc
	SW	22.90 ± 2.03c	2.86 ± 0.25c	13.15 ± 2.34d	36.19 ± 3.21c	4.41 ± 0.39d	26.30 ± 4.67d	29.56 ± 2.62d	3.33 ± 0.29c	16.12 ± 2.86d
3.5 μM BRs	CW	59.24 ± 3.64a	5.64 ± 0.32a	57.71 ± 4.79a	86.50 ± 5.31a	7.96 ± 0.45ab	101.46 ± 8.42a	76.39 ± 4.69ab	6.55 ± 0.37a	70.74 ± 5.87a
	SW	44.90 ± 2.59b	4.43 ± 0.27b	33.13 ± 2.48c	70.90 ± 4.09b	5.84 ± 0.36c	65.04 ± 4.86bc	57.90 ± 3.34c	5.15 ± 0.32b	40.61 ± 3.04c
2.0 mM Si	CW	62.82 ± 3.54a	5.56 ± 0.31a	57.02 ± 3.21a	87.32 ± 4.92a	8.70 ± 0.49a	99.43 ± 5.60a	81.01 ± 4.56a	6.47 ± 0.36a	69.89 ± 3.93ab
	SW	46.40 ± 2.68b	4.44 ± 0.27b	34.17 ± 2.10bc	66.21 ± 3.82b	6.72 ± 0.41bc	60.60 ± 3.72c	59.83 ± 3.45c	5.16 ± 0.32b	41.88 ± 2.57c

Table 2
Foliar Effect of BRs and Si solutions on Root length, Root fresh weight and Root dry weight of leafy vegetables irrigated with canal and sewage water.

Treatments	Water Source	Lettuce			Spinach			Cabbage		
		Root length (cm)	Root Fresh weight (g)	Root Dry weight (g)	Root length (cm)	Root Fresh weight (g)	Root Dry weight (g)	Root length (cm)	Root Fresh weight (g)	Root Dry weight (g)
Control	CW	7.08 ± 0.43b	18.28 ± 1.12b	1.95 ± 0.12b	8.00 ± 0.49b	19.42 ± 1.19b	2.74 ± 0.17b	7.08 ± 0.43b	17.48 ± 1.07b	2.51 ± 0.15b
	SW	3.44 ± 0.21d	6.90 ± 0.42d	1.05 ± 0.06d	3.88 ± 0.24d	7.33 ± 0.45d	1.48 ± 0.09d	3.44 ± 0.21c	6.60 ± 0.41c	1.35 ± 0.08c
3.5 μM BRs	CW	9.55 ± 0.54a	25.19 ± 1.55a	2.67 ± 0.16a	10.38 ± 0.58a	24.23 ± 1.49a	3.58 ± 0.22a	9.55 ± 0.54a	24.08 ± 1.48a	3.43 ± 0.21a
	SW	6.19 ± 0.34bc	15.13 ± 0.83bc	1.72 ± 0.09bc	7.24 ± 0.40bc	17.58 ± 0.97b	2.48 ± 0.14bc	6.19 ± 0.34b	14.47 ± 0.80b	2.21 ± 0.12b
2.0 mM Si	CW	9.31 ± 0.52a	24.02 ± 1.35a	2.58 ± 0.15a	10.90 ± 0.61a	26.47 ± 1.49a	3.74 ± 0.21a	9.31 ± 0.52a	22.97 ± 1.29a	3.32 ± 0.19a
	SW	5.60 ± 0.31c	14.44 ± 0.80c	1.56 ± 0.09c	6.50 ± 0.36c	13.99 ± 0.77c	2.23 ± 0.12c	5.60 ± 0.31b	13.81 ± 0.76b	2.00 ± 0.11bc

spectrophotometer (Hitachi U-1800) at 470 nm wavelength. The activity of antioxidant enzymes was determined on the basis of protein contents. Activity of Ascorbate peroxidase (APX) enzyme was measured by theprocedure explicated by Mittler and Zilinskas (1991). It was based on the stoichiometric reduction of phosphor molybdenum by ascorbic acid.

2.5. Determination of silicon, cadmium, and lead concentration

Spectrophotometer was used to measure concentration of silicon in plant sample according to the method explained by Elliott and Snyder (1991). Whereas atomic absorption spectrophotometer (AAS) was used to determine the Cadmium (Cd) and lead (Pb) contents in plant samples.

2.6. Statistical analysis

A statistical analysis on collected datawas performed to compare treatment means by using LSD at a 5 % probability level. The software Origin 2023 (Origin Lab, Massachusetts, USA) was used to make graphs and find the Pearson correlation among various growth, physiological, and antioxidant enzymatic activities with silicon, lead, and cadmium concentrations.

3. Results

3.1. Effect on growth attributes of leafy vegetables

Data (Table 1) regarding growth traits of lettuce, spinach, and cabbage showed that sewerage water irrigation reduced fresh weight of shoot, dry weight of shoot and leaf area as compared to canal water, as canal water contains less heavy metals than sewerage water. Mean while spraying BRs and Si on lettuce, cabbage, and spinach improved these parameters under the canal as well as sewage water irrigation. However, all these growth attributes were significantly ($p \leq 0.05$) improved when lettuce, cabbage, and spinach were irrigated with canal water and sprayed with BRs and Si.

Data about the root components (Table 2) of leafy vegetables in terms of root fresh weight, root dry weight and root length had revealed the negative effects of sewage water irrigation on leafy vegetables owing to significant reduction in root attributes while roots of plants irrigated with canal water flourished vigorously. Foliar spray of BRs and Si under canal water irrigation showed promising ($p \leq 0.05$) results by improving root fresh weight, root dry weight and root length while minimum values of root components were observed for plants irrigated with sewage water without BRs and Si application.

3.2. Effect on gas exchange parameters, water use efficiency and chlorophyll contents

The maximum and significant ($p \leq 0.05$) increase in gas exchange attributes in terms of transpiration rate, stomatal conductance and photosynthetic rate (Table 3) and chlorophyll contents and water use efficiency (WUE) (Table 4) of leafy vegetables was observed under canal water irrigation along with leafy spray BRs and Si solutions. Whereas the lowest values were recorded when plants were irrigated with sewerage water without any treatment.

3.3. Effect on enzymatic activity

Data related to the activity of antioxidant enzymes such as SOD,

Table 3
Foliar Effect of BRs and Si solutions on photosynthetic rate, transpiration rate and stomatal conductance of leafy vegetables irrigated with canal and sewage water.

Treatments	Water Source	Lettuce			Spinach			Cabbage		
		Photosynthetic rate (μmol m^{-2} s^{-1})	transpiration rate (mmol $m^{-2}s^{-1}$)	Stomatal conductance (mmol^{-2} s^{-1})	Photosynthetic rate (μmol m^{-2} s^{-1})	transpiration rate (mmol $m^{-2}s^{-1}$)	Stomatal conductance (mmol^{-2} s^{-1})	Photosynthetic rate (μmol m^{-2} s^{-1})	transpiration rate (mmol $m^{-2}s^{-1}$)	Stomatal conductance (mmol^{-2} s^{-1})
Control	CW	28.51 ± 1.76a	16.08 ± 0.99b	1.17 ± 0.06b	22.65 ± 1.39bc	10.33 ± 0.63b	1.04 ± 0.06b	27.42 ± 1.68a	16.14 ± 0.99bc	1.17 ± 0.06bc
	SW	17.16 ± 1.05b	6.93 ± 0.43d	0.50 ± 0.03d	13.59 ± 0.83d	4.45 ± 0.27d	0.45 ± 0.03d	16.45 ± 1.01b	6.95 ± 0.43d	0.50 ± 0.03e
3.5 µM BRs	CW	32.04 ± 1.97a	20.18 ± 1.24a	1.46 ± 0.09a	28.50 ± 1.75ab	12.59 ± 0.77a	1.33 ± 0.08a	30.71 ± 1.89a	20.26 ± 1.24ab	1.46 ± 0.09ab
	SW	31.00 ± 1.71a	12.51 ± 0.69c	0.85 ± 0.05c	23.43 ± 1.29c	9.18 ± 0.51bc	0.92 ± 0.05bc	29.70 ± 1.64a	12.56 ± 0.69c	0.85 ± 0.05d
2.0 mM Si	CW	32.96 ± 1.86a	20.99 ± 1.18a	1.52 ± 0.09a	29.78 ± 1.68a	12.61 ± 0.71a	1.39 ± 0.08a	31.59 ± 1.78a	21.08 ± 1.19a	1.52 ± 0.09a
	SW	31.19 ± 1.72a	12.71 ± 0.73c	0.90 ± 0.06c	24.26 ± 1.34c	8.32 ± 0.48c	0.76 ± 0.05c	29.89 ± 1.65a	12.76 ± 0.74c	0.90 ± 0.06 cd

POD, CAT and APX in lettuce, cabbage and spinach plants revealed (Figs. 1 and 2) that enzymatic activity was significantly ($p \leq 0.05$) influenced byBRs and Si under both types of irrigation sources. BRs and Si mitigated the negative effects of metal stress and aggaravated the activity of antioxidant enzymes. The minimum enzymatic activity was recorded when plants were irrigated with sewerage water without BRs and Si whereas plants irrigated with canal water or sewerage water showed higher activity under the foliar spray of BRs and Si solutions.

3.4. *Effect on silicon, cadmium, and lead uptake*

Data about concentration of Cd and Pb in vegetable plants showed (Fig. 3) increased content of Cd and Pb in plants were observed when sewerage water was applied as an irrigation source while minimum heavy metal (Cd and Pb) concentration was observed when irrigated with canal water. However, minimum Cd concentration was recorded in plants when irrigated with canal water along with a foliar spray of BRs and Si while plants irrigated with sewerage water and no foliar spray of BRs and Si showed maximum uptake of Cd and Pb concentration.

The results regarding silicon contents in leafy vegetables showed (Fig. 4) that foliar spray of Si improved the Si contents significantly ($p \leq 0.05$) and followed by BRs. The increase in Si concentration decreased the detrimental effects of Cd and Pb found in sewerage water. Minimum Si concentration was measured in plants when irrigated with sewerage water without foliar spray of Si. On the other hand, plants irrigated with canal water and foliar spray of silicon (Si) significantly improved Si contents.

3.5. *Pearson association between growth and physiological parameters, metal concentration, and antioxidant enzymatic activities*

The data revealed that the shoot fresh and dry weight, chlorophyll content, and root length were significantly positively associated with POD, SOD, CAT, and APX in all the tested leafy vegetables (Figs. 4, 5, and 6). However, the Pb and Cd concentration was negatively related to the growth parameters and antioxidant enzymatic activities in spinach, cabbage, and lettuce. Silicon concentration was not significantly associated with shoot fresh and dry weight, root length, and chlorophyll contents but it was negatively associated with the concentration of Pd and Cd.

4. Discussion

4.1. *Growth attributes of vegetables*

The stunted growth of all leafy vegetables was owing to the upregulation of heavy metals, particularly Cd and Pb under sewage water irrigation. The improvement in growth attributes in terms of fresh weight of shoot and roots and dry weight of shoot and roots, root length, and leaf area were attributed to the foliar spray of BRs and silicon. Foliar application of BRs accelerated cell division and cell elongation controlling the growth of plants under a heavy metal stress (Shafi et al., 2023). Likewise, Si foliar application also improved photosynthetic apparatus and nutrient absorption by confining the translocation of Cd an Pb which subsequently were associated with improved growth in plants (Hussain et al., 2019). Si spray also contributed to the intermodal elongation of shoots of leafy vegetables under metal contamination stress (Kabir et al., 2016).

4.2. *Gas exchange parameters of vegetables and chlorophyll contents*

Transpiration rate, Photosynthetic rate, stomatal conductance and chlorophyll contents were downregulated under sewage water irrigation due to the absorption of metal ions (Cd and Pb) in high concentration rather than other essential nutrients. Uptake of Cd and Pb in high concentration inhibited the exchange of CO_2 and O_2 in plant parts by

Table 4
Foliar Effect of BRs and Si solutions on WUE and chlorophyll contents of leafy vegetables irrigated with canal and sewage water.

Treatments	Water Source	Lettuce		Spinach		Cabbage	
		Water use efficiency (WUE)	chlorophyll contents	Water use efficiency (WUE)	chlorophyll contents	Water use efficiency (WUE)	chlorophyll contents
Control	CW	2.67 ± 0.15c	51.70 ± 2.85b	4.65 ± 0.26c	26.40 ± 1.46b	2.47 ± 0.15d	56.10 ± 3.09ab
	SW	3.95 ± 0.24b	23.63 ± 2.09c	6.27 ± 0.39c	12.06 ± 1.07c	3.95 ± 0.24bc	25.64 ± 2.27c
3.5 µM BRs	CW	2.97 ± 0.17ab	63.76 ± 3.59a	4.98 ± 0.30bc	32.56 ± 1.83a	2.92 ± 0.17 cd	69.19 ± 3.89ab
	SW	4.88 ± 0.27b	50.57 ± 3.06b	7.10 ± 0.40a	26.05 ± 1.58b	4.48 ± 0.27ab	54.87 ± 3.33ab
2.0 mM Si	CW	3.10 ± 0.17a	64.78 ± 3.65a	5.40 ± 0.30ab	33.08 ± 1.86a	3.22 ± 0.17b	70.30 ± 3.96a
	SW	5.26 ± 0.32a	49.37 ± 2.85b	7.65 ± 0.47a	25.44 ± 1.47b	5.26 ± 0.32a	53.57 ± 3.09b

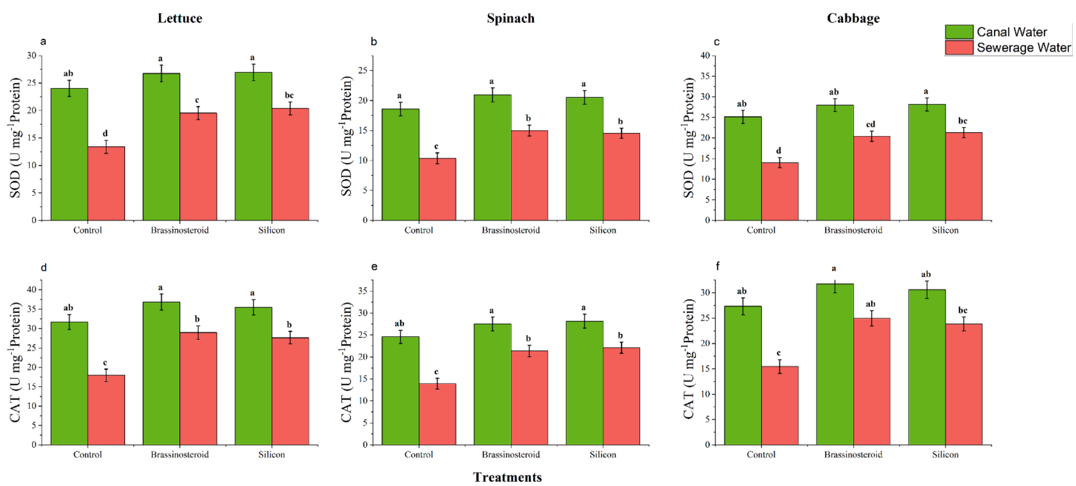

Fig. 1. Foliar effect of BRs and Si on superoxide dismutase (SOD) and catalase activity (CAT) of leafy vegetables irrigated with canal and sewage water. Means sharing same letter (s) do not differ significantly at $p \leq 0.05$.

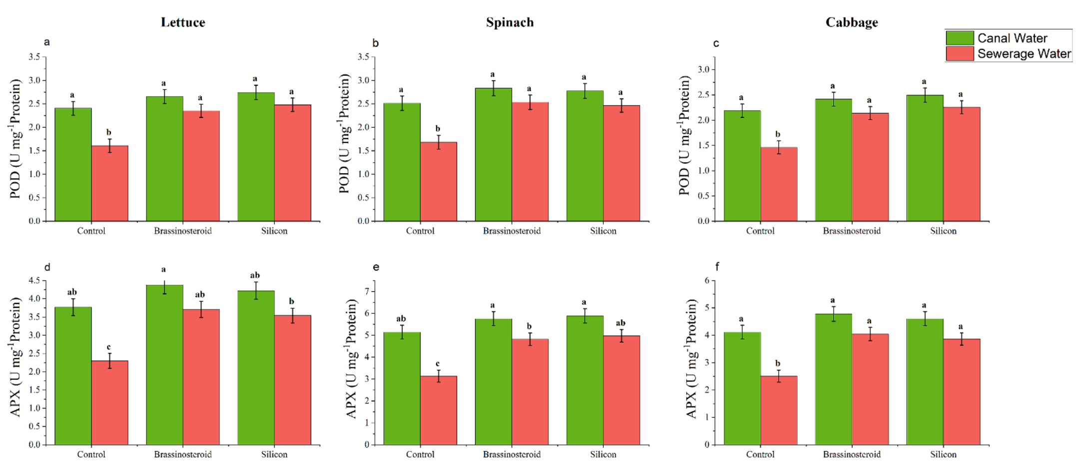

Fig. 2. Foliar effect of BRs and Si on peroxidase dismutase (POD) and ascorbate peroxidase (APX) activity of leafy vegetables irrigated with canal and sewage water. Means sharing same letter (s) do not differ significantly at $p \leq 0.05$.

increased chlorophyllase activity leading to disrupted stomatal conductance and transpiration rate which were imperative for the process of photosynthesis (Paunov et al., 2018; Hajihashemi et al., 2020). The upregulation in these gas exchange attributes was observed under BRs and Si application and they improved photosynthetic pigment by adjusting ion homeostasis and CO_2 fixation by reducing Cd and Pb ions and activity of chlorophyllase enzymes in plant tissues leading to encourage exchange parameters and chlorophyll contents in plant leaves (Adrees et al., 2015; Sonjaroon et al., 2018). BRs and Si foliar application to leafy vegetables prevented the accumulation of toxic elements and increased availability of essential ions in plants to improve photosynthetic rate by replacing central atom (Mg^{2+}) of photosynthetic apparatus which is directly associated with increased stomatal conductance, transpiration rate and chlorophyll pigment (Agami, 2013; Shu et al., 2013).

4.3. Water use efficiency of vegetables

A decline in water use efficiency (WUE) by leafy vegetables was recorded under sewage water irrigation which is attributed to low

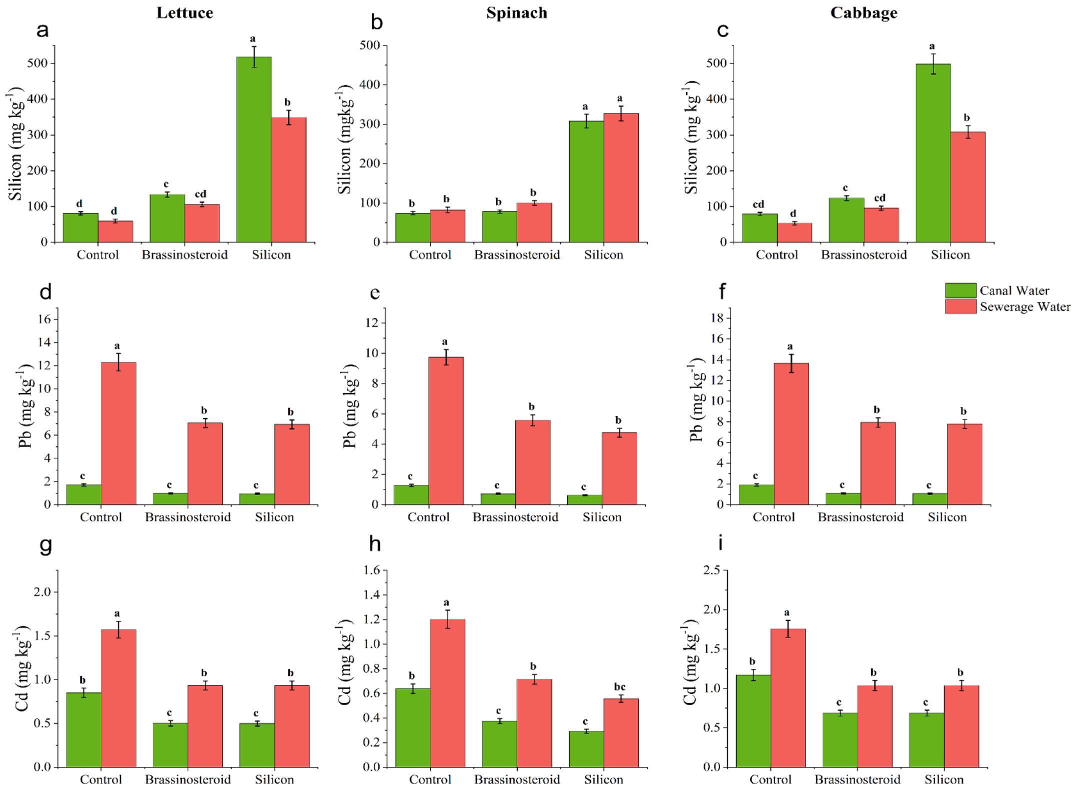

Fig. 3. Foliar effect of BRs and Si on Si, Pb and Cd concentration of leafy vegetables irrigated with canal and sewage water. Means sharing same letter (s) do not differ significantly at $p \leq 0.05$.

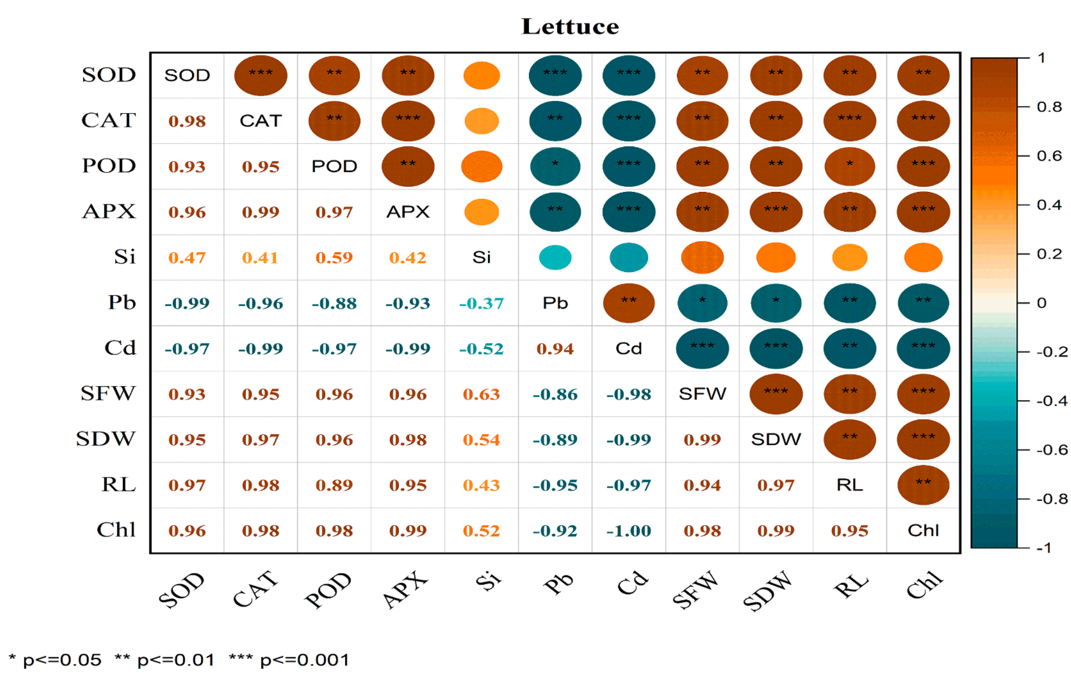

Fig. 4. Correlation between antioxidant enzymatic activities, growth parameters and metal concentration in lettuce. Superoxide dismutase (SOD), catalase (CAT) peroxidase (POD), Ascorbate peroxidase (APX), Si (silicon), Pb (lead), Cd (cadmium), SFW (shoot fresh weight), SDW (shoot dry weight), RL (root length) and Chl (chlorophyll contents).

relative water contents due to the toxic concentration of metal elements. Accumulation of Cd and Pb caused damage to plant plasma membrane due to the induction of reactive oxygen species (ROS) leading to a decrease in water transporters and subsequently low water use efficiency (Kaur et al., 2018). Foliar spray of BRs and Si reversed the loss of relative water contents by improving the membrane stability index owing to the limited supply of heavy metal ions. These also contributed to reducing oxidative damage to the plasma membrane by lowering toxic elements and upregulation of essential elements to increase membrane stability and relative water contents (Jan et al., 2018). Under

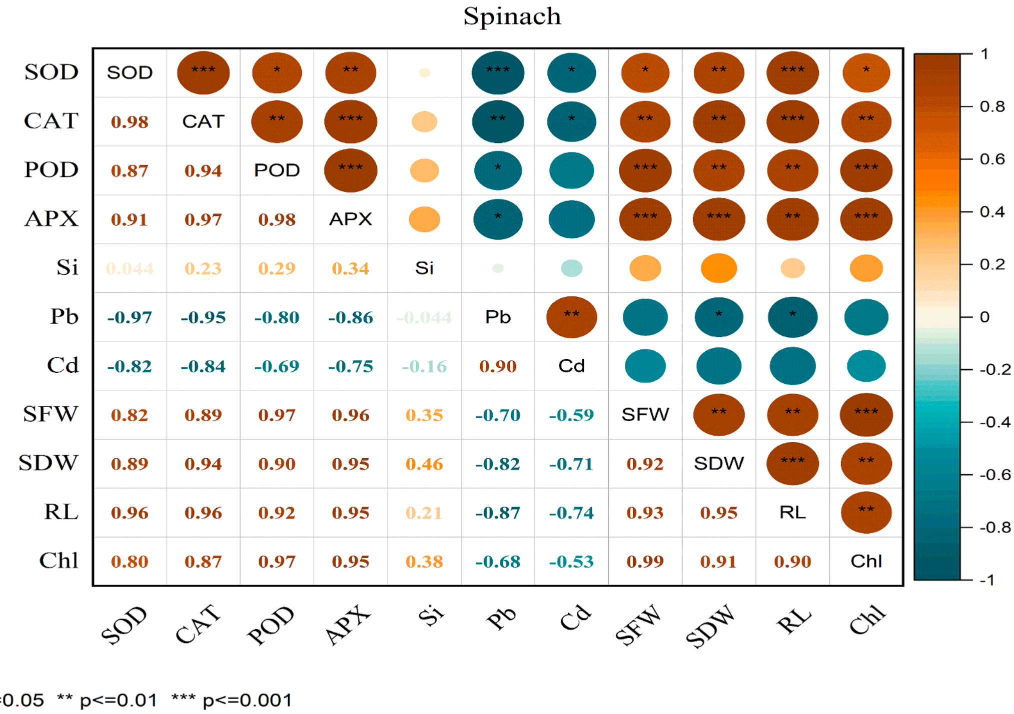

Fig. 5. Correlation between antioxidant enzymatic activities, growth parameters and metal concentration in spinach. Superoxide dismutase (SOD), catalase (CAT) peroxidase (POD), Ascorbate peroxidase (APX), Si (silicon), Pb (lead), Cd (cadmium), SFW (shoot fresh weight), SDW (shoot dry weight), RL (root length) and Chl (chlorophyll contents).

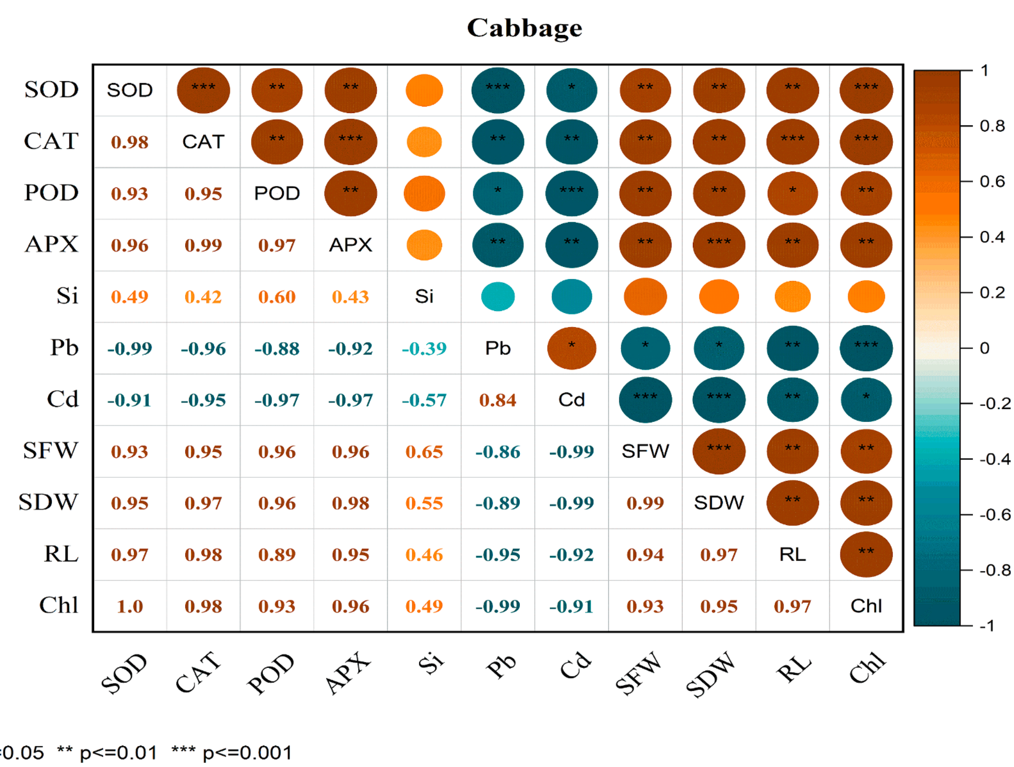

Fig. 6. Correlation between antioxidant enzymatic activities, growth parameters and metal concentration in cabbage. Superoxide dismutase (SOD), catalase (CAT) peroxidase (POD), Ascorbate peroxidase (APX), Si (silicon), Pb (lead), Cd (cadmium), SFW (shoot fresh weight), SDW (shoot dry weight), RL (root length) and Chl (chlorophyll contents).

stress environment foliar spray of BRs also increased osmolytes, particularly proline and Glycinebetain (GB) which improved water potential in plant tissues (Ahmad et al., 2015). All these processes are associated with improved WUE in leafy vegetables under both canal water and sewage water irrigation.

4.4. Activity of antioxidant enzymes in vegetables

The activity of antioxidants such as SOD, POD, CAT and APX in leafy vegetables decreased under sewage water irrigation while increased under canal water irrigation. Generally, the antioxidants activity increased under stress environment but our results are contradictory with the findings of other researchers such as (Fulekar et al., 2012) and (Garcia-Caparros et al., 2021). This might be due to the fact that in some situations protein misfolding happens under heavy metal stress that can decrease the activities of the enzymes. Moreover, for the activation of antioxidant enzymes, plants must be genetically capable of producing and activating antioxidant enzymes. Thus in some conditions, the plants are unable to activate the antioxidant system due to the lack of genetic capabilities (Tamás et al., 2014). Thus foliar application of BRs and Si is attributed to improve antioxidant enzymatic activities under both types of irrigation sources; canal water and sewage water. Under heavy metal stress, BRs and Si augmented their activity by lowering translocation of Cd metal through chelating agents (phytochelatins, phenols) and activating metal regulation through metal transporter within plants (Jan et al., 2018). The increase in activity of antioxidants caused a reduction in ROS species including hydrogen peroxide (H_2O_2) and singlet oxygen (O_2) and subsequently upregulated plant metabolic functions (Rahman et al., 2017).

4.5. Silicon, cadmium, and lead contents in vegetables

Increased concentration of silicon under stressed conditions is attributed to the adjustment of ion homeostasis by foliar application of Si and BRs in leafy vegetables (Wani et al., 2017). Furthermore, silicon application prompted to formation of silicates in cytoplasm leading to restricting the mobility of Cd and Pb in plants. Foliar application of Si also increased silicon contents by inducing silicon-containing compounds such as cysteine, glutathione, and methionine (Ji et al., 2017). Whereas transportation of Cd and Pb diminished due to increased absorption of other essential elements including P, K, Mg, Ca and Si, within plant tissues (Ahmad et al., 2018) through the antagonistic effect of nutrient ions. Thus depressing effect of Br and Si on Cd and Pb concentrations was mediated by ion homeostasis and the antagonistic effect of nutrient ions with each other (Waisi et al., 2017). Moreover, the induction of chelating agents like phenols and phytocelatins (PC) by BRs and Si provided favorable conditions for osmotic adjustment to vegetable plants under heavy metal stress (Cao et al., 2017).

5. Conclusion

All the measured attributes regarding growth, physiological, biochemical and yield of leafy vegetables had decreased significantly when irrigated with sewage water as compared to canal water. However, foliar application of BRs and Si solution in recommended doses under both irrigation sources had delivered a substantial contribution in minimizing the overwhelming affects of Cd and Pb stress due to sewage water irrigation. Foliar application of BRs and Si was proved as an ameliorating strategy for heavy metal stress by improving tolerance mechanisms associated with heavy metal stress. The major ameliorating mechanism in this strategy was restricted translocation of Cd and Pb leading to improved growth and silicon contents. Our study was limited to only two heavy metals Cd and Pb due to their dominance in sewage water. This strategyfurther could be applied under different ecological zones of Pakistan in the future.

6. Compliance with ethical standards

Not applicable.

7. Declaration of funding

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Author contributions

All authors contributed in research conception. RMB and SAJ drafted the experimental design and MTJ did the experimental work. AS, BAP and AA analyzed the data and helped the writing of this paper. RS, RMB, SAJ and AS revised the manuscript.

CRediT authorship contribution statement

Rashad Mukhtar Balal: Writing – review & editing, Methodology, Conceptualization. **Syed Ayyaz Javed:** Writing – review & editing, Methodology, Conceptualization. **Muhammad Tauseef Jaffar:** Investigation, Conceptualization. **Anam Sadaf:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Bilal Ahamad Paray:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Rattandeep Singh:** Writing – review & editing, Conceptualization.

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.

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Appendix A. Supplementary data

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References

Adrees, M., Ali, S., Rizwan, M., Zia-ur-Rehman, M., Ibrahim, M., Abbas, F., Farid, M., Qayyum, M.F., Irshad, M.K., 2015. Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: A review. Ecotoxicol. Environ. Saf. 119, 186–197.

Agami, R.R., 2013. Alleviating the adverse effects of NaCl stress in maize seedlings by pretreating with salicylic acid and 24-epibrassinolide. South Afri J Bot 88, 171–177.

Ahanger, M.A., Bhat, J.A., Siddiqui, M.H., Rinklebe, J., Ahmad, P., 2020. Integration of silicon and secondary metabolites in plants: a significant association in stress tolerance. J. Exp. Bot. 71, 6758–6774.

Ahmad, P., Sarwat, M., Bhat, N.A., Wani, M.R., Kazi, A.G., Tran, L.S.P., Czern, 2015. Alleviation of cadmium toxicity in Brassica juncea L. PLoS One 10, e0114571.

Ahmad, P., Ahanger, M.A., Egamberdieva, D., Alam, P., Alyemeni, M.N., Ashraf, M., 2018. Modification of osmolytes and antioxidant enzymes by 24-epibrassinolide in chickpea Seedlings under mercury (Hg) toxicity. J. Plant Growth Regul. 37, 309–322.

Awasthi, G., Singh, T., Awasthi, A., Awasthi, K.K., 2020. Arsenic in mushrooms, fish, and animal products. Arsenic in Drinking Water and Food 307–323.

Binkley, D., Stape, J.L., Ryan, M.G., Barnard, H.R., Fownes, J., 2002. Age-related decline in forest ecosystem growth: an individual-tree, stand-structure hypothesis. Ecosystems 5, 58–67.

Cao, Z., Yue, Y., Zhong, H., Qiu, P., Chen, P., Wen, X., Wang, S., Liu, G., 2017. The cationic dye removal by novel SiZn composites prepared from zinc ash. J Taiwan Institute Chem Engineer 71, 464–473.

Chance, B., Maehly, A., 1955. [136] Assay of catalases and peroxidases.

Christou, A., Papadavid, G., Dalias, P., Fotopoulos, V., Michael, C., Bayona, J.M., Piña, B., Fatta-Kassinos, D., 2019. Ranking of crop plants according to their potential to uptake and accumulate contaminants of emerging concern. Environ. Res. 170, 422–432.

Deshmukh, R.K., Ma, J.F., Bélanger, R.R., 2017. Role of silicon in plants. Frontiers Media SA 1858.

Elliott, C.L., Snyder, G.H., 1991. Autoclave-induced digestion for the colorimetric determination of silicon in rice straw. J. Agric. Food Chem. 39, 1118–1119.

Etesami, H., Jeong, B.R., 2018. Silicon (Si): review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants. Ecotoxicol. Environ. Saf. 147, 881–896.

Feng, R., Wang, L., Yang, J., Zhao, P., Zhu, Y., Li, Y., Zheng, S., 2021. Underlying mechanisms responsible for restriction of uptake and translocation of heavy metals (metalloids) by selenium via root application in plants. J. Hazard. Mater. 402, 123570.

Fulekar, M., Sharma, J., Tendulkar, A., 2012. Bioremediation of heavy metals using biostimulation in laboratory bioreactor. Environ. Monit. Assess. 184, 7299–7307.

Garcia-Caparros, P., De Filippis, L., Gul, A., Hasanuzzaman, M., Ozturk, M., Altay, V., Lao, M.T., 2021. Oxidative stress and antioxidant metabolism under adverse environmental conditions: a review. Bot. Rev. 87, 421–466.

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases: I. Occurrence in Higher Plants. Plant Physiol. 59, 309–314.

Hajihashemi, S., Mbarki, S., Skalicky, M., Noedoost, F., Raeisi, M., Brestic, M., 2020. Effect of wastewater irrigation on photosynthesis, growth, and anatomical features of two wheat cultivars (Triticum aestivum L.). Water 12, 607.

Hardaway, C.J., Sneddon, J., Sneddon, E.J., Kiran, B., Lambert, B.J., McCray, T.C., Bowser, D.Q., Douvris, C., 2016. Study of selected metal concentrations in sediments by inductively coupled plasma-optical emission spectrometry from a metropolitan and more pristine bayou in Southwest Louisiana, United States. Microchem. J. 127, 213–219.

Hosseini, S.A., Naseri Rad, S., Ali, N., Yvin, J.-C., 2019. The ameliorative effect of silicon on maize plants grown in Mg-deficient conditions. Int. J. Mol. Sci. 20, 969.

Hussain, I., ParveenA, R., R, A., Ma, I., M, R., S, A., Z, I., M, 2019. Exogenous silicon modulates growth, physiochemicals and antioxidants in barley (Hordeum vulgare L.) exposed to different temperature regimes. Silicon 11, 2753–2762.

Jan, S., Alyemeni, M.N., Wijaya, L., Alam, P., Siddique, K.H., Ahmad, P., 2018. Interactive effect of 24-epibrassinolide and silicon alleviates cadmium stress via the modulation of antioxidant defense and glyoxalase systems and macronutrient content in Pisum sativum L. seedlings. BMC Plant Biol. 18, 146.

Ji, X., Liu, S., Juan, H., Bocharnikova, E.A., Matichenkov, V.V., 2017. Effect of silicon fertilizers on cadmium in rice (Oryza sativa) tissue at tillering stage. Environ SciPollu Res 24, 10740–10748.

Kabir, A.H., Hossain, M.M., Khatun, M.A., Mandal, A., Haider, S.A., 2016. Role of silicon counteracting cadmium toxicity in alfalfa (Medicago sativa L. Front Plant Sci 7.

Kaur, R., Yadav, P., Thukral, A.K., Sharma, A., Bhardwaj, R., Alyemeni, M.N., Wijaya, L., Ahmad, P., 2018. Castasterone and Citric Acid Supplementation Alleviates Cadmium Toxicity by Modifying Antioxidants and Organic Acids in Brassica juncea. J. Plant Growth Regul. 37, 286–299.

Kim, Y.H., Khan, A.L., Kim, D.H., Lee, S.Y., Kim, K.M., Waqas, M., Lee, I.J., 2014. Silicon mitigates heavy metal stress by regulating P-type heavy metal ATPases, Oryzasativalow silicon genes, and endogenous phytohormones. BMC Plant Biol. 14, 1–13.

Liang, Y., Nikolic, M., Bélanger, R., Gong, H., Song, A., Liang, Y., Song, A., 2015. History and introduction of silicon research. In: Silicon in Agriculture: From Theory to Practice, pp: 1–18.

Manzoor, J., Sharma, M., Wani, K.A., 2018. Heavy metals in vegetables and their impact on the nutrient quality of vegetables: A review. J. Plant Nutr. 41, 1744–1763.

Mittler, R., Zilinskas, B.A., 1991. Purification and characterization of pea cytosolic ascorbate peroxidase. Plant Physiol. 97, 962–968.

Najmi, A., Albratty, M., Al-Rajab, A.J., Alhazmi, H.A., Javed, S.A., Ahsan, W., Rehman, Z. u., Hassani, R., Alqahtani, S.S., 2023. Heavy metal contamination in leafy vegetables grown in Jazan region of Saudi Arabia: assessment of possible human health hazards. Int. J. Environ. Res. Public Health 20, 2984.

Noor, I., Sohail, H., Sun, J., Nawaz, M.A., Li, G., Hasanuzzaman, M., Liu, J., 2022. Heavy metal and metalloid toxicity in horticultural plants: Tolerance mechanism and remediation strategies. Chemosphere 135196.

Paunov, M., Koleva, L., Vassilev, A., Vangronsveld, J., Goltsev, V., 2018. Effects of different metals on photosynthesis: cadmium and zinc affect chlorophyll fluorescence in durum wheat. Int. J. Mol. Sci. 19, 787.

Rahman, M.F., Ghosal, A., Alam, M.F., Kabir, A.H., 2017. Remediation of cadmium toxicity in field peas (Pisumsativum L.) through exogenous silicon. Ecotoxicol. Environ. Saf. 135, 165–172.

Rehman, M.U., Ilahi, H., Adnan, M., Wahid, F., Rehman, F.U., Ullah, A., Raza, M.A., 2021. Application of silicon: A useful way to mitigate drought stress: An overview. Curr. Res. Agric. Farm. 2, 9–17.

Rehman, Z.U., Khan, S., Brusseau, M.L., Shah, M.T., 2017. Lead and cadmium contamination and exposure risk assessment via consumption of vegetables grown in agricultural soils of five-selected regions of Pakistan. Chemosphere 168, 1589–1596.

Rizwan, M.S., Imtiaz, M., Zhu, J., Yousaf, B., Hussain, M., Ali, L., Hu, H., 2021. Immobilization of Pb and Cu by organic and inorganic amendments in contaminated soil. Geoderma 385, 114803.

Sadaf, A., Balal, R.M., Jaffar, M.T., Javed, S.A., Javaid, M.M., 2024. Influence of brassinosteroid and silicon on growth, antioxidant enzymes, and metal uptake of leafy vegetables under wastewater irrigation. Int. J. Phytorem. 26 (6), 936–946.

Shafi, Z., Shahid, M., AlGarawi, A.M., Zeyad, M.T., Marey, S.A., Hatamleh, A.A., Wang, S., Singh, U.B., 2023. The Exogenous Application of 24-Epibrassinolide (24-EBL) Increases the Cd and Pb Resilience in Zea mays (L.) by Regulating the Growth and Physiological Mechanism. Applied Biochemistry and Biotechnology, 1-25.

Shanmugaiah, V., Gauba, A., Hari, S.K., Prasad, R., Ramamoorthy, V., Sharma, M.P., 2023. Effect of silicon micronutrient on plant's cellular signaling cascades in stimulating plant growth by mitigating the environmental stressors. Plant Growth Regul.

Shu, S., Yuan, L., Guo, S., Sun, J., Yuan, Y., 2013. Effects of exogenous spermine on chlorophyll fluorescence, antioxidant system and ultrastructure of chloroplasts in Cucumissativus L. under salt stress. Plant PhysiolBioch 63, 209–216.

Sonjaroon, W., Jutamanee, K., Khamsuk, O., Thussagunpanit, J., Kaveeta, L., Suksamrarn, A., 2018. Impact of brassinosteroid mimic on photosynthesis, carbohydrate content and rice seed set at reproductive stage under heat stress. Agriculture and Natural Resources 52, 234–240.

Tamás, M.J., Sharma, S.K., Ibstedt, S., Jacobson, T., Christen, P., 2014. Heavy metals and metalloids as a cause for protein misfolding and aggregation. Biomolecules 4, 252–267.

Tariq, Y., Ehsan, N., Riaz, U., Nasir, R., Khan, W.A., Iqbal, R., Ali, S., Mahmoud, E.A., Ullah, I., Elansary, H.O., 2023. Assessment of Heavy Metal (oid) s Accumulation in Eggplant and Soil under Different Irrigation Systems. Water 15, 1049.

Waisi, H., Petkovic, A., Nikolic, B., Jankovic, B., Raicevic, V., Lalevic, B., Giba, Z., 2017. Influence of 24-epibrassinolide on seedling growth and distribution of mineral elements in two maize hybrids. Chem Indus 71, 201–209.

Wang, X.H., Shu, C., Li, H.Y., Xq, H., Wang, Y.X., 2014. Effects of 0.01% brassinolide solution application on yield of rice and its resistance to autumn low-temperature damage. Acta Agric Jiangxi 26, 36–38.

Wani, A.S., Tahir, I., Ahmad, S.S., Dar, R.A., Nisar, S., 2017. Efficacy of 24-epibrassinolide in improving the nitrogen metabolism and antioxidant system in chickpea cultivars under cadmium and/or NaCl stress. SciHorticul 225, 48–55.

Zhu, Y.X., Gong, H.J., Yin, J.L., 2019. Role of silicon in mediating salt tolerance in plants: a review. Plants 8, 147.