

SILICON REGULATES GROWTH, YIELD, PHYSIOLOGICAL RESPONSES AND TISSUE CONCENTRATION OF LEAD IN *BRASSICA CAMPESTRIS* L. GROWN IN LEAD CONTAMINATED SOIL

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Abstract

Heavy metal contamination of agricultural land causes serious problems for the ecosystem. Industrial waste, sludge, petrol, fertilizers, paints, and explosive materials contribute to the occurrence of heavy metals, particularly lead (Pb), in the environment, which causes adverse effects on soil, plant, and human health. The accumulation of Pb interrupts plant growth, which becomes a part of the food chain, thereby compromising food safety. Various methods have been used to overcome the toxicity of Pb. Silicon (Si) can be utilized to protect agricultural plants from the damaging impacts of Pb. *Brassica* species are multipurpose and famous crops cultivated all over the world. Silicon plays a positive role to promote growth, yield, and the physiological attributes of specifically *Brassica campestris* L. cultivated in soils enriched with heavy metals. The main objective of this study was to assess the beneficial role of Si to promote growth, yield and physiological functions of *Brassica campestris* L. cultivated in the Pb-contaminated soil by reducing the Pb concentration in plant tissues. Therefore, a pot experiment was conducted to assess the effect of applied Pb [0 (control), 500 and 1000 mg kg⁻¹ soil] on the growth, some key physiological attributes, and yield of *B. campestris* at varying levels (0, 200 and 400 mg kg⁻¹ soil) of Si application. The results illustrated that Pb toxicity at 1000 mg kg⁻¹ caused a significant reduction of 27% in total chlorophyll contents, 88.4% in relative water contents, 85.7% in membrane stability index, 37.3% in plant height, 20.9% in root dry weights, and 70.2% in grain yield. The Si application improved physiological and growth parameters and reduced the concentration of Pb in plants. Thus, application of Si at 400 mg kg⁻¹ was found to be more effective in alleviating the harmful effects of Pb on the growth and yield of *B. campestris* grown in Pb-contaminated soil by reducing Pb concentration in shoots and grains.

Key words: Chlorophyll, Pb toxicity, Brassica, Silicon.

Abbreviations: Pb: Lead, Si: Silicon, EC: Electrical conductivity, N: Nitrogen, P: Phosphorus, K: Potassium, TCC: Total chlorophyll contents, RWC: Relative water content, SPAD: Soil Plant Analysis Development, MSI: membrane stability index, CRD: completely randomized designed, LSD: Least Significant Difference.

Introduction

Lead (Pb) is a toxic heavy metal that is an essential factor causing environmental pollution. Lead occurs in the environment mainly due to anthropogenic activities. Plant growth and yield are adversely affected in Pb-contaminated soil. Humans are also affected by Pb in a variety of ways. Lead (Pb) can cause hemoglobin synthesis to be disrupted; long-term Pb exposure generates kidney difficulties, excessive blood pressure in grown-ups, and impairments in children's natural and psychological development (Chen *et al.*, 2014).

The earth crust contains Pb in average concentration of 14 mg kg⁻¹ soil (Ghafoor *et al.*, 2012). It is not easy to determine soil Pb levels toxic to different plant species as there is a range of international criteria. It was reported that soil Pb concentration of 30-300 mg kg⁻¹ exerts toxic

effects on plants (Kabata-Pendias & Pendias, 2001; Ramadan & Al-Ashkar, 2007). The Pb threshold level in agricultural soil is 30 mg/kg and the concentrations above the threshold level hinders growth and biomass production of crop plants and have detrimental effects on root and shoot growth and total chlorophyll contents (Ghani *et al.*, 2016; Khan *et al.*, 2021).

Many key metabolic processes such as electron transport chain, cellular organelles integrity, membrane stability index, PSII connectivity, mineral metabolism, activity of oxygen-evolving complex, and enzymatic activity in plants grown in Pb-contaminated soil are interrupted, resulting in reduced photosynthesis, root and shoot growth, biomass production, water uptake and pigment synthesis (Gupta *et al.*, 2013; Aslam *et al.*, 2021). Furthermore, metal toxicity inhibits physiological processes such as photosynthesis, respiration, transpiration rates, N-

metabolism and mineral nutrition and cell elongation leading to reduced biomass (Morkunas *et al.*, 2018). Plant growth is delayed, and oxidative stress is caused by Pb toxicity (Malar *et al.*, 2014).

Silicon (Si) is the second most abundant element in the earth's crust comprising 60–70% of the soil mass in the form of silicates (Pavlovic *et al.*, 2021). Silicon is not considered necessary for plant growth and development, but it protects plants from abiotic stresses and diseases (Luyckx *et al.*, 2017). Due to its instantaneous functions in encouraging the development of various plant species and reducing metal toxicity, Si has been found as a useful element (Nguyen *et al.*, 2019). Reduced Pb toxicity from Si application is related to lower Pb absorption, and oxidative stress, as well as enhanced uptake of essential nutrients such as nitrogen (N), phosphorus (P), magnesium (Mg), iron (Fe), zinc (Zn), and manganese (Mn), increased antioxidant potential; all these factors work together to protect photosynthesis from Pb effects, thereby resulting in increased biomass (Gu *et al.*, 2011). Silicon helps plants cope with the harmful effects of metals through various mechanisms, including altering metal absorption. Silicon has a strong ability to turn soluble and exchangeable metals in the soil into non-soluble chemical complexes through various processes such as decreased translocation, metal binding to cell walls, and co-precipitation with Si (Elrys *et al.*, 2018). Silicon induces root exudates, resulting in the production of chelated metals and a reduction in vegetable metal absorption (Kroukamp *et al.*, 2016).

Silicon application could improve the growth and yield of brassica growing under Pb stress. *Brassica campestris* grown with effluent irrigation has been found to contain heavy metals (Bortoloti & Baron, 2022). Limited information is available regarding the use of silicon for reducing Pb accumulation in *B. campestris* by lowering its mobility in soil. Therefore, the current study was based on the hypothesis that silicon might be used to immobilize Pb in soil and reduce its accumulation in *Brassica campestris*, thereby enhancing its crop productivity. The present study covered the research gap by determining the effects of Pb on physiological functioning and role of Si for improving growth and yield of *B. campestris* grown in Pb contaminated soil by reducing Pb accumulation. In view of the above-mentioned reports, the current study was designed with the subsequent objective to investigate the impact of Si in enhancing growth, yield, and physiological functions in *Brassica campestris* cultivated in Pb-contaminated soil by lowering Pb concentrations in plant tissues.

Material and Methods

Growth conditions and experimental layout: A pot experiment was conducted in a wire-house of the University of Agriculture Faisalabad (31.4310° North and longitude 73.0695° East). The wire-house had glass-covered roofs with uncontrollable temperature and humidity. The temperature of the wire-house ranged from 28°C (maximum) to 3°C (minimum) during the crop season. The average relative humidity was 66%. The

physico-chemical properties of the soil used in the experiment were determined following Estefan *et al.*, (2013). The soil used in the experiment had 8.21 pH, 3.5 dS m⁻¹ EC, 0.61% organic matter, 33.41% saturation percentage, and sandy clay loam textural class. The present pot study comprised nine treatments. The soils were spiked with Pb as Pb (NO₃)₂ and treated with Si as Ca₂SiO₄. The treatments included T₁= control, T₂ = Si at 200 mg kg⁻¹ soil, T₃ = Si at 400 mg kg⁻¹ soil, T₄ = Pb at 500 mg kg⁻¹ soil, T₅ = Pb at 500 mg kg⁻¹ + Si at 200 mg kg⁻¹ soil, T₆ = Pb at 500 mg kg⁻¹ + Si at 400 mg kg⁻¹ soil, T₇ = Pb at 1000 mg kg⁻¹ soil, T₈ = Pb at 1000 mg kg⁻¹ + Si 200 mg kg⁻¹ soil, and T₉ = Pb at 1000 mg kg⁻¹ + Si 400 mg kg⁻¹ soil. The pots were arranged according to the completely randomized design (CRD) with three replicates of each treatment.

The seeds of *Brassica campestris* L. were acquired from the Ayub Agricultural Research Institute Faisalabad. Three seeds were sown in each pot. Calcium silicate was used as a Si source. The recommended doses of N = 90 kg ha⁻¹, P = 60 kg ha⁻¹ and K = 50 kg ha⁻¹, as urea, diammonium phosphate, and potassium sulphate, respectively, were used according to the recommendation of Anon., (2020). Irrigation of the crop was accomplished as and when required.

Measurement of physiological responses: After 60-days of seed germination, physiological responses like total chlorophyll contents, membrane stability index (MSI) and relative water contents (RWC) were determined.

Total chlorophyll contents (TCC in terms of SPAD values) of *Brassica campestris* leaves with a portable SPAD-502 meter (Minolta, Osaka, Japan) were determined. Three completely grown mustard leaves were detached from each pot, and average SPAD measurements were taken between 9:00 and 10:00 am. The TCC was determined from the leaf tip to the leaf base and then averaged following Iqbal *et al.*, (2017).

For relative water content (RWC), young leaf with complete growth from the top of the plant was taken and RWC was determined according to the method of Barrs & Weatherley (1962).

For the determination of leaf membrane stability index (MSI), the method given by Sairam (1994) was used.

Measurement of growth responses: Plants were harvested at maturity, grain were separated, after taking the shoot and grain weight, the samples initially air dried and then oven dried for 72 hours in forced air oven at 65 + 5°C and subsequently ground with grinding mill.

Plant growth was determined by measuring plant height, shoot and root length, shoot fresh and dry weight, root fresh and dry weight, number of pods and grain yield.

Determination of Pb concentration in shoot, grain and post experiment soil: The wet digestion technique was utilized to determine the Pb concentration in shoot and grain samples according to the method of Helrich *et al.*, (1990). The AB-DTPA extractable Pb in post-experiment soil was determined according to Soltanpour (1985).

Statistical analysis

Statistix 10.1 was used to do statistical analysis on the data collected. The effect of Si on *Brassica* growth, yield, and Pb absorption was investigated applying Analysis of Variance. To compare mean values, the LSD test at $p \leq 0.05$ was performed (Steel *et al.*, 1997; Naz *et al.*, 2022).

Results

Plant physiological responses: The plant physiological parameters such as TCC (Fig. 1a), RWC (Fig. 1b) and MSI (Fig. 1c) were significantly affected ($p \leq 0.05$) by Pb pollution, Si amendments and their interactive effect. The TCC, RWC and MSI of the plants were reduced by increasing the levels of Pb toxicity in soil. The Si application enhanced TCC, RWC and MSI in normal as well as Pb contaminated soil conditions. At 1000 mg kg^{-1} Pb toxicity in soil, Si application of $200 \text{ and } 400 \text{ mg kg}^{-1}$ enhanced TCC by 17.6% and 87.9%, RWC by 44.6% and 59.2%, and MSI by 54.2% and 85.7% respectively, as compared to the respective controls. The Si application at 400 mg kg^{-1} was more effective in ameliorating the negative impacts of Pb on the physiological parameters of the mustard plants.

Growth responses: The growth including plant height (Fig. 2a), shoot fresh and dry weights (Fig. 2b and 2c), root fresh and dry weights (Fig. 2d and Fig. 2e), and root length (Fig. 2f) were significantly affected ($p \leq 0.05$) by Pb spiking, Si amendments and their interactive effect. The plant height, shoot and root fresh and dry weights were reduced as the concentration of Pb increased in soil. The application of Si at $200 \text{ and } 400 \text{ mg kg}^{-1}$ resulted in enhanced plant height (24.4% and 36.3%), shoot fresh weight (5.70% and 36.3%), root fresh weight (57.1% and 90%), shoot dry weight (48.3% and 57.3%), root dry weight (16.2% and 20.9%) and root length (10.75% and 30.1%) when plants were exposed to Pb contamination in soil at 1000 mg kg^{-1} .

Yield responses: The yield parameters including number of siliquae (Fig. 3a) and seed yield (Fig. 3b) were affected significantly ($p \leq 0.05$) by Pb spiking, Si amendments and their interactive effect. The number of siliquae was significantly reduced (19.8% and 24.8%) due to $500 \text{ and } 1000 \text{ mg kg}^{-1}$ Pb toxicity in soil, respectively. However, the application of Si was found very useful in alleviating the negative impacts of Pb on the number of siliquae of the mustard plants. It was found that the number of siliquae was enhanced by 17.4% and 23.2% in response to $200 \text{ and } 400 \text{ mg kg}^{-1}$ Si application, respectively, in soil contaminated with 1000 mg kg^{-1} Pb. As for as seed yield is concerned, there was 16% and 43.6% reduction in seed yield at $500 \text{ and } 1000 \text{ mg kg}^{-1}$ Pb in soil. However, the application of $200 \text{ and } 400 \text{ mg kg}^{-1}$ Si enhanced the seed yield by 56% and 72%, respectively, at 1000 mg kg^{-1} Pb spiking. Similar to growth parameters, the application of Si at 400 mg kg^{-1} was found more effective in improving the yield parameters of mustard.

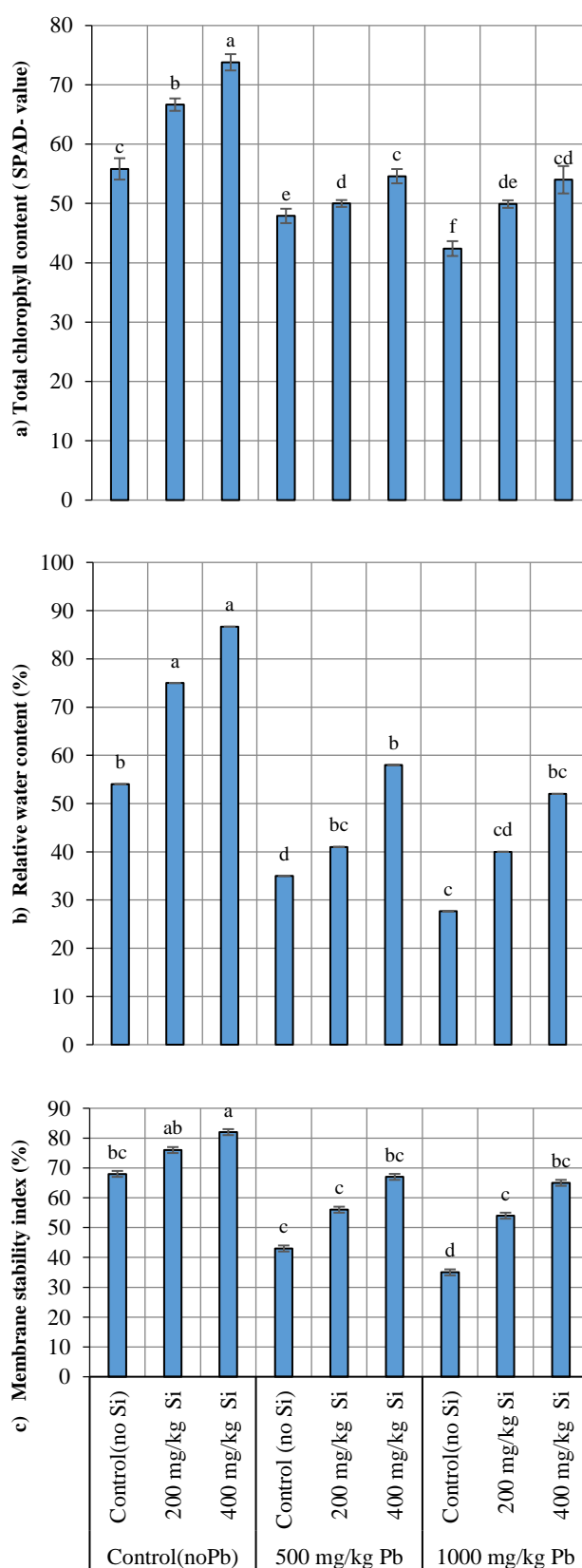


Fig. 1(a, b, c). *Brassica campestris* physiological responses (a = Total chlorophyll contents (SPAD-value), b = Relative water content %, c = Membrane stability index %) as affected by applied Si in Pb-contaminated soil (Means + SE, $n=3$). [LSD values for treatments interactions: total chlorophyll contents, Pb \times Si = 4.12; relative water content, Pb \times Si = 2.61; membrane stability index, Pb \times Si = 3.21].

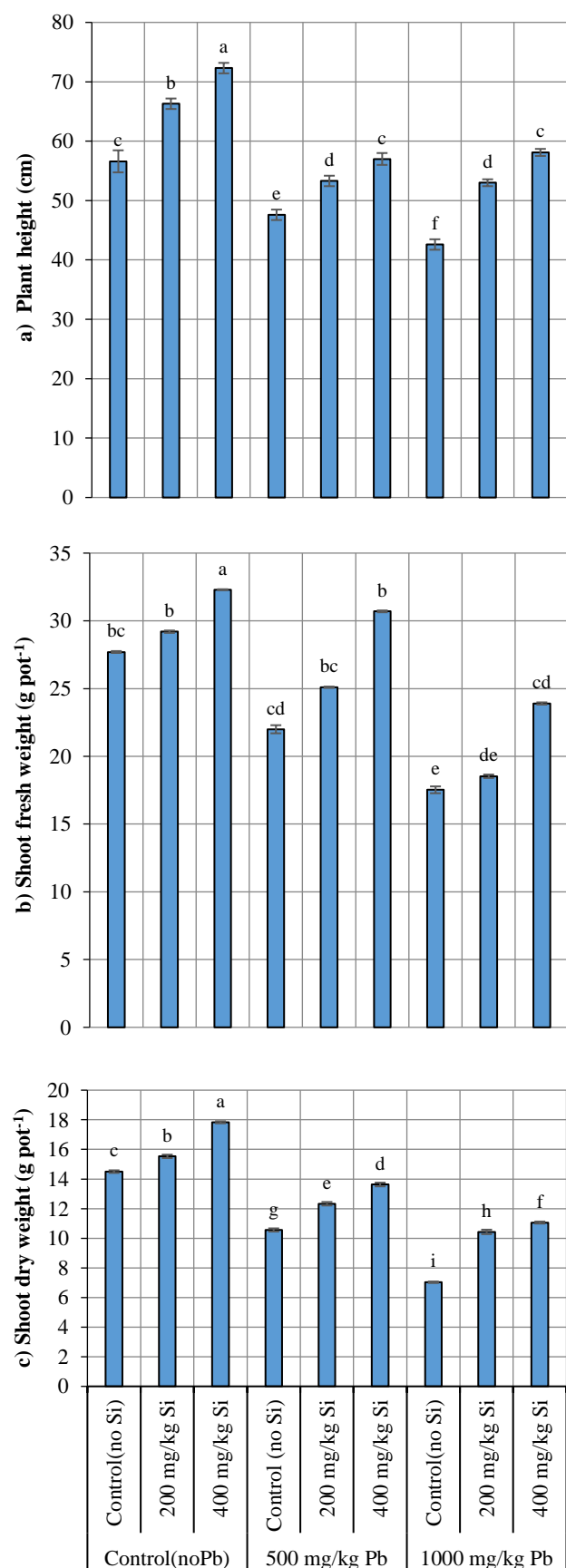


Fig. 2(a, b, c). *Brassica campestris* growth responses (a = plant height, b = shoot fresh weight, c = shoot dry weight) as affected by applied Si in Pb-contaminated soil (Means + SE, $n = 3$). [LSD values for treatments interactions: plant height, Pb \times Si = 3.08; shoot fresh weight, Pb \times Si = 2.14; shoot dry weight, Pb \times Si = 0.17].

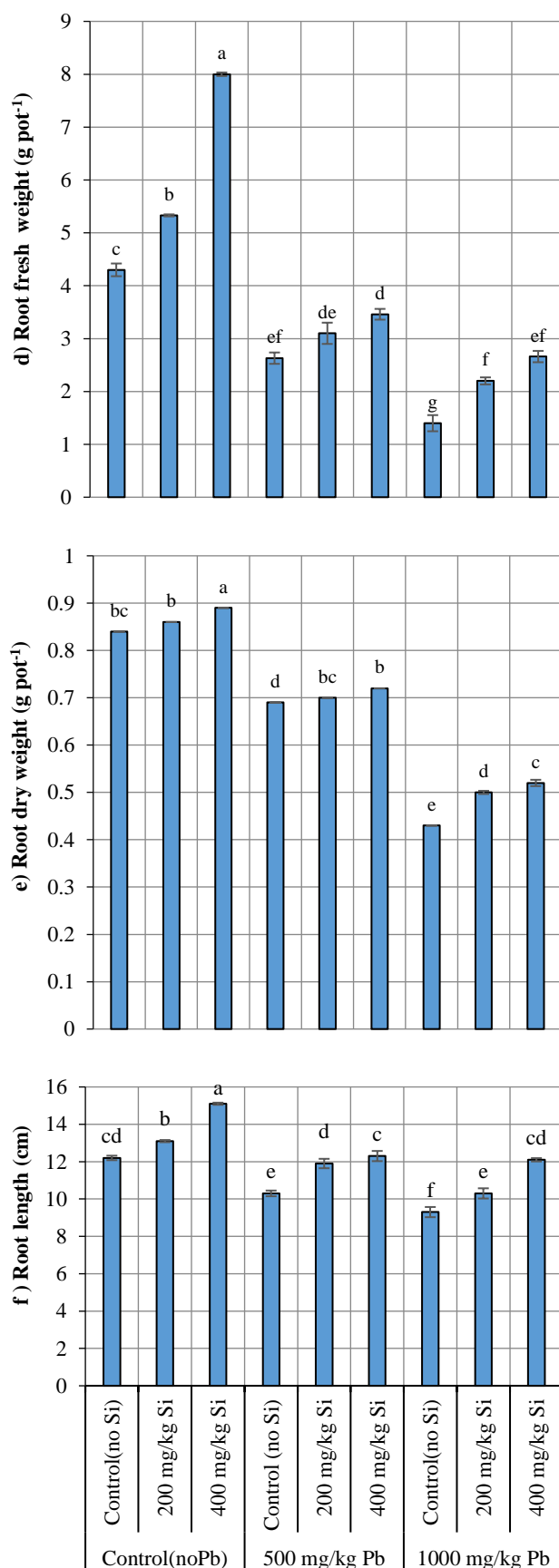


Fig. 2(d, e, f). *Brassica campestris* growth responses (d = root fresh weight, e = root dry weight, f = root length) as affected by applied Si in Pb-contaminated soil (Means + SE, $n = 3$). [LSD values: root fresh weight Pb \times Si = 2.1; root dry weight Pb \times Si = 0.47; root length Pb \times Si = 0.57].

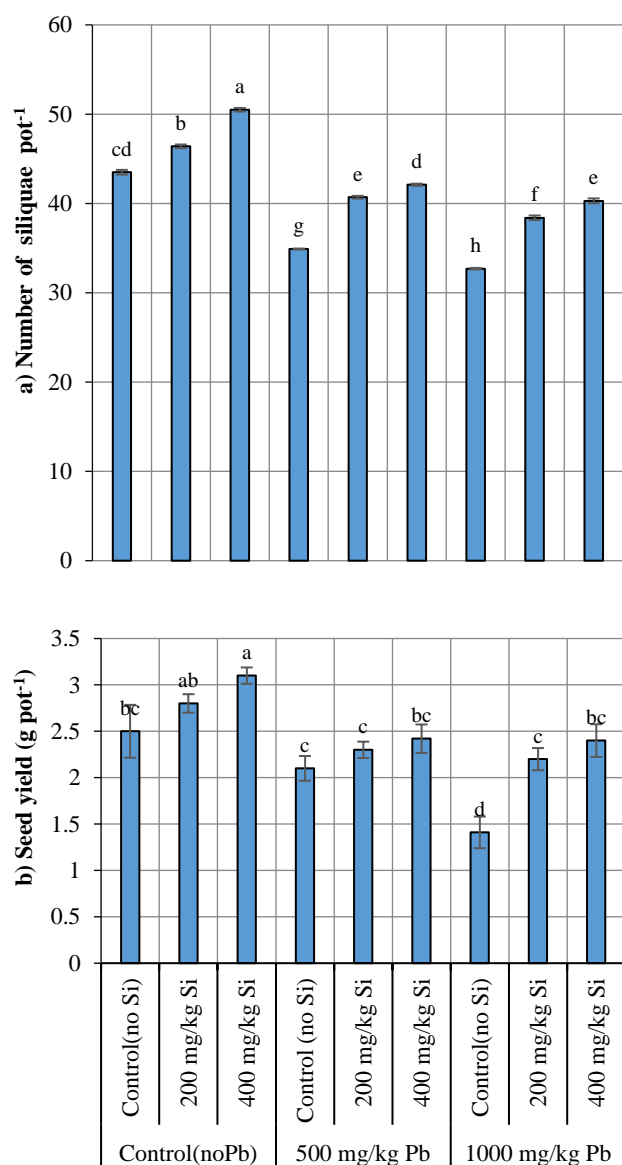


Fig. 3(a, b). *Brassica campestris* yield (a = number of siliques pot⁻¹, b = seed yield) as affected by applied Si in Pb-contaminated soil (Means + SE, $n = 3$). [LSD values for treatments interactions: seed yield, Pb \times Si = 0.57; seed weight, Pb \times Si = 0.46].

Concentration of Pb in shoot, seed, and post-experiment soil: In this pot experiment, the Pb concentration in the seed, shoot and soil (Fig. 4a-c) was significantly ($p \leq 0.05$) affected by Pb contamination and applied Si amendments, and their interactive effect. Pb concentration was increased in seeds and shoot and in post-experiment soil in response to Pb spiking in soil. While the application of Si at 200 and 400 mg kg⁻¹ in soil reduced Pb concentration in the mustard seed and shoot, while in the post-experiment soil, an increase in Pb concentration was found due to Pb spiking as compared to the respective controls. Usage of Si at 200 and 400 mg kg⁻¹ decreased the Pb concentration by 12% and 24% in shoot, 21% and 55% in seeds, while in soil increased 4% and 10.1% in the post-experiment soil, respectively, compared to the respective controls.

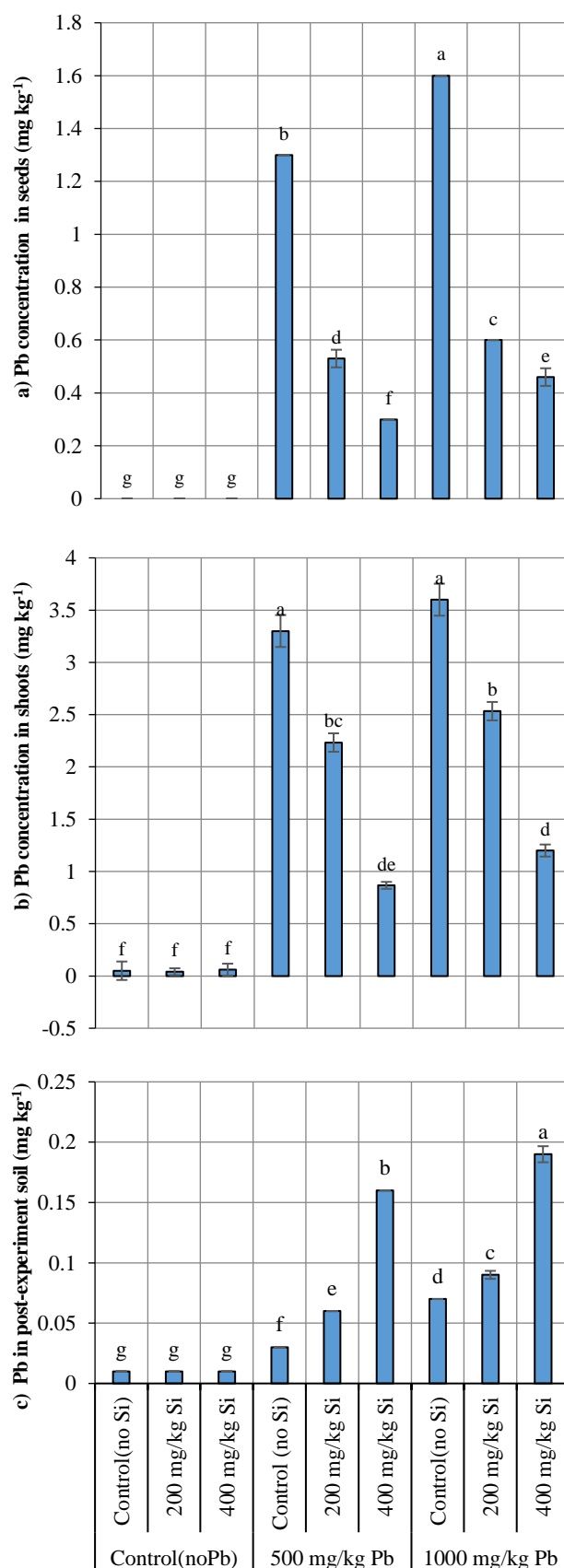


Fig. 4(a, b, c). Pb concentration in seeds, shoot, and in post experiment soil as affected by applied Si in Pb-contaminated soil (Means + SE, $n = 3$). [LSD values for treatments interactions: Pb concentration in seeds, Pb \times Si = 0.05; Pb concentration in shoot, Pb \times Si = 0.35; Pb concentration in soil, Pb \times Si = 0.0072].

Discussion

Lead suppressed growth, physiological, and biochemical attributes of mustard plants. The harmful effects of Pb increased with increasing concentration of Pb in soil. The present study results are conferred like outcomes that described reduction in plant growth and biomass due to exposure of plants to Pb stress (Wu *et al.*, 2013; Nas & Ali, 2018). The reduction in plant growth due to Pb toxicity may be attributed to reduced nutrient uptake and oxidative damage (Mehmood *et al.*, 2018). Similarly, Pb toxicity also inhibits physiological processes in plants (Nas & Ali, 2018; Mehmood *et al.*, 2018).

In the present study, there was a significant reduction in TCC (Fig. 1a), RWC (Fig. 1b) and MSI (Fig. 1c) due to toxicity of Pb in mustard plants. It has been described that exposure of plants to Pb results in decreased root growth, inhibition of chlorophyll biosynthesis and a reduction in photosynthesis and respiration due to inhibition of electron transport mechanisms and enzymes synthesis as well as cell disturbances and chromosomal lesions (Patra *et al.*, 2004; Sharma & Dubey, 2005) resulting in growth inhibition and plant damage (Iqbal *et al.*, 2015). These negative effects on plant's physiological responses with Pb stress might have resulted from reduced synthesis of chlorophyll and plastoquinone, distorted chloroplast ultra-structure, blocked electron transport and CO₂ deficiency due to stomatal closure, all were occurring simultaneously (Sharma & Dubey, 2005; Ashraf & Harris, 2013). Chlorophyll content is frequently determined in crop plants owing to evaluate the effect of abiotic and biotic stresses, as deviations in pigment content are interconnected to visual symptoms of plant disorders and photosynthetic efficiency (Purnama *et al.*, 2015). Inhibition of chlorophyll synthesis is one of the most Pb-sensitive plant physiological features (Iqbal *et al.*, 2017; Li *et al.*, 2012). The RWC, MSI and growth reduces due to reduction in cell wall elasticity caused by Pb toxicity. The increasing concentration of Pb in soil is responsible for decreased growth and physiological parameters, causing toxicity to plants (Alia *et al.*, 2015). Lead exposure decreases radicle emergence and causes major biochemical alternation in brassica plants (Singh *et al.*, 2011). Both rate of photosynthesis and biomass production positively each other. As a result of heavy metal stress, the cell division and photosynthetic rate undergo a significant decline (Dallas & Ho, 2005; Keller *et al.*, 2015). Metal stress causes damage to stomata cells, alterations in electron transport chain and the Calvin cycle enzyme activities (Souza *et al.*, 2005), thereby greatly reducing biomass.

The Si application increased physiological functioning of mustard plants grown under Pb stress (Fig. 1a, b, c). Similar results have been reported by Fatemi *et al.*, 2020 that Si application enhanced chlorophyll content and photosynthetic rate in coriander plants under Pb stress. Si application in stress conditions enhances photosynthetic pigments and rate, linked to enhance photosynthetic and chlorophyll enzyme activities (Feng *et al.*, 2010). The Si increased the RuBisCo enzyme activity and chlorophyll content leading to enhanced photosynthesis (Liu *et al.*, 2013). Silicon application ameliorated the negative effects of Pb toxicity and growth inhibition of mustard plants by

lowering the accumulation of Pb in mustard plants. The Si application improved plant growth by decreasing the uptake of Pb in wheat (Huang *et al.*, 2019). In addition, the application of Si enhanced the nutrient contents of plant tissue by facilitating the uptake and translocation of nutrients leading to better plant growth and yield (Chen *et al.*, 2018).

In addition, increased solubility of Pb in soil will result in enhanced bioavailability and hence increased accumulation of Pb by crop plants. Such a high magnitude of Pb in present results attributed to exogenous application of Pb decreased soil pH which further increased Pb bioavailability in soil, therefore causing increased Pb accretion in the edible parts of tested crop (Li *et al.*, 2007; Iqbal *et al.*, 2015). In the present study silicon treatment resulted in a decreased Pb accumulation in plants and the concentration of Pb in shoot and seeds was decreased with the application of Si in soil having Pb stress (Fig. 4a, b, c). It has been reported that application of Si results in a rise in pH leading to reduced Pb availability (Rizwan *et al.*, 2018; Xiao *et al.*, 2021). Silicon forms Si-Pb complex and thereby precipitates Pb directly in the root (Adrees *et al.*, 2015; Xiao *et al.*, 2021). Silicon reduced Pb translocation from root to shoot by effectively immobilizing Pb in plant (Xiao *et al.*, 2021). Therefore, Si can create a Pb-silicate complex, which lowers Pb mobility and toxicity resulting in enhanced plant tolerance to Pb (Shah *et al.*, 2021). Silicon prevents Pb from reaching the cytoplasm and also enhances cell wall extensibility and encourages root elongation (Asati *et al.*, 2016; Hattori *et al.*, 2003).

Conclusion

In the present research work, Si was utilized to reduce Pb concentration in mustard plants and improve its growth and yield when grown in Pb contaminated soil. Lead toxicity caused a reduction in plant growth and development. However, it was discovered that addition of Si to the growth medium reduced the negative effects of Pb toxicity by improving growth and key physiological characteristics. The application of Si at 400 mg kg⁻¹ significantly improved plant growth and yield and reduced Pb concentration in plant foliage tissues. It is therefore concluded that Si seems to boost metal tolerance in crop plants. Thus, *Brassica campestris* proved to be more preferable for farmers and can be used in further cultivation programs to increase its production and Pb tolerance with the application of Si. However, the present results need to be confirmed in field trials so as to work out the feasibility and economical profitability of Si supplementation.

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