Exploring the Role of Silicon for Promoting Growth, Physiology and Reducing Pb Uptake in *Spinacia oleracea* Grown on Pb–Polluted Calcareous Sandy Clay Loam Textured Soil

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Abstract - Lead (Pb) is one of the most toxic heavy metals that entered extensively into environment through anthropogenic activities. The Pb became a source of negative impact on plant processes. It causes serious impairment to human health functions. Silicon (Si) can be used to overcome the adverse effects caused by Pb on crop plants. In present study, growth enhancement and the Pb noxiousness at the rate of 1000 and 2000 kg Pb ha^{-1} in spinach (*Spinacia oleracea*) amended by Si at the rate of 400 and 800 kg Si ha^{-1} soil was studied. The results exposed that Pb noxiousness at its higher applied level (2000 kg Pb ha^{-1}) reduced total chlorophyll content, relative water contents, membrane stability index, shoot length, shoot fresh and dry weights in 55 days old spinach seedling. The Si application increased growth and physiological parameters at both applied levels of Pb in soil. Under lower level of Pb contamination (1000 kg Pb ha^{-1}), use of Si (400 and 800 kg Si ha^{-1}) enhanced shoot length (6.8 and 17.1%), shoot fresh weight (6.2 and 11.6%), total chlorophyll contents (2.5 and 15.3%), relative water contents (2.4 and 7.7%) and membrane stability index (7.4 and 14.5%). Whereas by higher level of Pb (2000 kg Pb ha^{-1}), shoot length (12.5 and 17.9%), shoot fresh weight (30.1 and 47.7%), total chlorophyll contents (3.9 and 7.1%), relative water contents (9.7 and 13.3%) and membrane stability index (11.4 and 22.5%) increased by the Si application. The Si application reduced Pb uptake by 78 and 56% under lower level of Pb while Pb uptake reduced by 69 and 52% under higher level of Pb respectively. Thus, Si application can ameliorate Pb toxic effect in spinach under Pb stress. Silicon supplementation enhanced growth and physiological parameters and reduced Pb uptake by spinach grown in Pb poisoned soil.

Keywords - Silicon, Lead uptake, Spinach, Pb Contamination, Calcareous, Sandy Loam Soil.

I. INTRODUCTION

Heavy metal (HM) pollution has been recognized as a widespread hazard. Severe health and environmental hazards are induced by HM contamination due to its noxious nature (Jacob *et al.*, 2018). Environmental pollution is becoming a threatening issue due to adverse effects of heavy metals. It has become of great concern all over the world. Due to rapidly increase in metal industries, inappropriate waste disposal, fertilizer and pesticides, are being discarded in our water, lands and into the atmosphere as inorganic noxious waste (Briffa *et al.*, 2020). Lead (Pb) is categorized an important metal that is of public health concern on account of its carcinogenic nature (Tchounwou *et al.*, 2012). This metal has potential harmful consequence not simply on crops but ultimately on health of human due to its potent cumulative properties and toxicity (Chopra *et al.*, 2009).

Lead can be present in organic and inorganic form. tetra ethyl (organic) is primarily originated in Pb contaminated gasoline whereas inorganic happens in soil, dust, old paints, and additional user products. Lead is noxious in its both forms, though organic complexes are exceptionally noxious to living systems compared to inorganic. After arsenic (As), Pb is the second utmost toxic metal. About 0.002% Pb comprises of earth crust (Kumar *et al.*, 2020). The Pb readily accumulated in soil, sediments, and different parts of the plants. Particle size, pH and cation exchange capacity of soil along with root exudation and physio-chemical properties controls uptake of Pb by plants. Stunted growth, chlorosis and blackening of root systems are toxicity indications in plants which are caused by excessive amount of Pb. Permeability and membrane structure is affected by Pb. Hormonal status is changed by Pb toxicity in plants. Photosynthesis is prohibited by Pb and obstacle mineral nutrition as well as water balance (Sharma and Dubey, 2005).

Additionally absorption of Pb in plants may be boost slightly when plants grown up on Pb rich incorporated land (Murtaza *et al.*, 2022). Lead is possibly the prolonged used and finest known noxious environmental chemical, up till now it is used haphazardly. Hematopoietic, muscular, skeleton, cardiovascular, renal, immune, gastrointestinal, nervous and reproductive systems along with developmental processes are adversely affected by Pb (Johnson, 1998). It have been investigated for many years for its toxic effects and its compounds in a variety of systems, existing data regarding its carcinogenic, clastogenic and mutagenic properties are quite conflicting (Gjorgieva Ackova, 2018). Lead is accumulated in seed, root nodules, leaves, stems, and roots of plants. Enhancement of exogenous Pb level, absorption of Pb accumulation increases in plants. Productivity and plant growth affected by Pb and plant species defines magnitude of effects. Primarily toxic effects are at physiological level. Endogenous, environmental and nutritional factors explain response of plants to the metal (Singh *et al.*, 1997; Ghafoor *et al.*, 2023).

To rectify the HM contamination, ex-situ and in-situ remediation methods have been established, including bioremediation, solidification, stabilization, extraction, landfilling and encapsulation (Gao *et al.*, 2018). For the contaminated soil remediation, in situ immobilization has been considered as a cost-effective measure of HM in polluted soils by applying extraneous amendments. Available fractions of heavy metals can be decreased by the application of immobilization amendments. Immobilization amendments can change the redox states of heavy metals and hence, toxicity, mobility and bioavailability of heavy metals decreased efficiently (Li-qun *et al.*, 2009).

Silicon is the second most common element after oxygen found in soil. One of the most important roles of Si is in enhancing the crops growth and yield principally under stress condition. The Si resource mends the structural integrity of yield and also improves potted plant acceptance to disorders and metal toxicity (Ma *et al.*, 2004). For most of the plant species Si is not necessary; however, in the existence of Si both metal toxicity and deficiency stress in plants may effected (Liang *et al.*, 2015). The Si has been regarded as advantageous or valuable, play significant roles in structural, physiological and metabolic processes (Dhiman *et al.*, 2021). Silicon lessens the noxious influence of heavy metals and is dumped in the cell wall of roots, stems, leaves. The Si plays major role in metal decontamination is credited to modification of plant biological connections with the exterior growing medium and cellular mechanisms (Wang *et al.*, 2004). Silicate application can enhance soil pH that result in metal silicate precipitates which reduces the metal phyto availability and declared as exterior mechanism of elevating HM tolerance (Cocker *et al.*, 1998). Silicon influence the dispersal and translocation of toxic metals in numerous portions of plants and under advanced metal stress Si allow plants to persist (Zhang *et al.*, 2008).

Spinach is an important nutritional herb. On the other hand, spinach is a precise collector of heavy metals, specifically Pb (Lamhamdi *et al.*, 2013). Main objective of this experiment are to approximation the use of Si to improve the development of spinach and to diminish the acceptance of Pb by spinach through Si application.

II. MATERIALS AND METHODS

A. *Experimental layout*

A pot experiment was carried out in the wire house (with a glass-covered roof) of the University of Agriculture, Pakistan,. The pre-sowing detailed analysis of soil used for this current study are depicted in Table 1, those were determined following the methods depicted in ICARDA Manual (Estefan et al., 2013).

Table 1: Soil Properties

Sr. No.	Parameters	Values	Units
1	pH_s	7.92	……
2	ECe	2.58	$dS\ m^{-1}$
3	Organic mater	0.69	%
4	Saturation percentage	33.41	%
5	Texture	Sandy clay loam	…….
6	$CaCO_3$	6.11	%

The preserved pots were organized in completely randomized design, with three repetitions each. Soil was unnaturally contaminated with Pb using $Pb\ (NO_3)_2$ salt and Si using calcium silicate as a source. Along with control two levels of Pb at the rate of 1000 and 2000 kg Pb ha^{-1} soil as well as two levels of Si at the rate of 400 and 800 kg Si ha^{-1} were exogenously applied to soil. After contaminating soil with Pb and Si, for homogeneousity in soil, it was allowed to equilibrate for two months, after addition of distilled water at field capacity by alternate wetting and drying. Each pot was filled with 4 kg soil and polythene sheets were used for the lining of the pots.

Seeds were taken from Vegetable Research Institute, Ayub Agricultural Research Institute Faisalabad. Initially 8-10 seeds of *Spinacia oleracea* were sown in soil in each pot. After two weeks of development, three plants were retained in each pot.

The recommended dose of NPK fertilizer at the rate of 100:60:60 kg ha^{-1} as urea, di-ammonium phosphate and potassium sulfate, respectively was used for spinach. The crop was irrigated as needed using distilled water. The spinach crop was collected as it reached maturity, and growth characteristics were noted. Samples of spinach leaves were gathered for further physiological and chemical examinations.

Table 2. Treatments

Sr. No.	Treatments	Symbol
1.	Control (with no amendments)	T_1
2.	400 kg Si ha^{-1} soil	T_2
3.	800 kg Si ha^{-1} Soil	T_3
4.	1000 kg Pb ha^{-1} soil	T_4
5.	1000 kg Pb ha^{-1} + 400 kg Si ha^{-1} soil	T_5
6.	1000 kg Pb ha^{-1} + 800 kg Si ha^{-1} soil	T_6
7.	2000 kg Pb ha^{-1} soil	T_7
8.	2000 kg Pb ha^{-1} + 400 kg Si ha^{-1} soil	T_8
9.	2000 kg Pb ha^{-1} + 800 kg Si ha^{-1} soil	T_9

B. *Measurements of physiological parameters*

After 45 days of sowing, spinach leaf total chlorophyll contents (TCC) in terms of SPAD-value was determined from leaf tip to leaf base through a hand-held SPAD meter.

For the determination of membrane stability index (MSI) of intact plant, Sairam (1994) method was used. Electrical conductivity C1 was evaluated by using EC (electrical conductivity) meter. Consequently, same samples were heated for ten minutes at 100°C. C2 was recorded. The MSI was computed by using formula:

$$\text{MSI \%} = [1-(C1/C2)] \times 100$$

Relative water content (RWC) was determined by using green leaves with full expansion from top of plant from each treatment. A section of 5.00 cm^2 was taken from each leaf. Fresh weight was recorded and after that leaf section was placed in vial for soaking in water for 4 hours. After that samples were removed from vial and weighted. After turgid weight, samples were placed in oven at constant temperature of 70°C (Ghafoor *et al*., 2023). By using formula RWC was determined as:

$$\text{RWC} = \frac{\text{Fresh weight - dry weight}}{\text{Turgid weight - dry weight}} \times 100$$

C. *Determination of growth parameters*

After harvesting spinach, the growth of spinach crop was measured as shoot and root lengths, fresh and dry weights. Shoot and root length of crop was recorded by using meter rod. Shoot and root length was recorded in cm and this rod was set to the lowest end to the upper end of the plant shoot and root. Portable electrical balance was used for calculating shoot and root fresh weight of spinach. Fresh weight of shoot and root was expressed in grams (g). Shoot and root samples of spinach were firstly sun dried. Sun dried root samples were oven dried at a constant temperature of 65+5°C in oven for 72 hours. Shoot and root dry weight was determined by electrical balance and was expressed in grams (g).

D. *Determination of chemical parameters*

The Pb concentration was measured by wet digestion method. To assess the Pb concentration, the leaf samples were firstly sundried and then oven dried at 75°C in an oven for 4 hours and subsequently ground with electric grinder. 0.5 g of each sample was weighted from powdered samples and taken in a 50 ml conical flask. For the digestion of samples di-acid was used. Nitric acid (HNO_3) and perchloric acid ($HClO_4$) were used as a di-acid at a ratio of 3:1. 15ml of digestion mixture was added to each sample and was kept overnight. Next day, flasks were placed on a hot plate at temperature of 260-300°C for 3 hours digestion. After digestion process, samples were allowed to cool down. By using Whatman's filter paper cool down samples were filtered. Filtrate of samples was poured into 50 ml volumetric flask and then by distilled water volume was made up to mark. Plastic bottles were used for transferring samples and Atomic Absorption Spectrophotometer (AAS) was used for Pb analysis (Mustafa, 2003). Pb uptake in plant shoot was calculated by the following formula (Iqbal *et al*., 2000) as: Pb uptake by shoot (mg pot^{-1}) = Pb concentration in shoot (mg kg^{-1}) × shoot dry weight (g pot^{-1}) / 1000

III. RESULTS

A. *Physiological responses of spinach*

Physiological functions such as TCC (Fig 1a), MSI (Fig 1b) and RWC (Fig 1c) were significantly ($p \leq 0.05$) affected by Pb contamination, Si and their interactive effects. Data showed that Si amendment at 400 kg Si ha^{-1} soil affected TCC, MSI and RWC not severely under Pb spoiled soil. The Si amendments enhanced TCC, MSI and RWC in normal along with Pb soil condition. Under Pb contaminated soil at 1000 and 2000 kg Pb ha^{-1} soil, physiological parameters were substantially reduced with increasing Pb in soil. Applied Si at 800 kg Si ha^{-1} soil was further active in ameliorating damaging effect of Pb on TCC, MSI and RWC of spinach crop.

B. Growth responses of spinach

Shoot length (Fig. 2a), shoot fresh weight (Fig. 2b), shoot dry weight (Fig 2c), root length (Fig. 2d), root fresh weight (Fig. 2e), and root dry weight (Fig. 2f) of spinach were significantly influenced by Pb contamination, Si amendments and their interactive effects. Data showed that growth parameters were not severely countered by Si amendment at 400 kg Si ha^{-1} soil. Growth parameters were affected by increasing Pb contamination in soil. In standard as well as Pb poisoned soil growth parameters were enhanced by Si application. The Si application (800 kg Pb ha^{-1} soil) was more effective and indicates positive effects in growth parameters under 1000 and 2000 kg ha^{-1} Pb contamination in soil.

C. Tissue Concentration of Pb

The Pb concentration in the leaves of spinach grown under Pb contaminated soil at 1000 and 2000 kg Pb ha^{-1} soil was illustrated in Fig 3a. The Pb concentration in spinach was considerably influenced by Pb contamination and applied amendments and their interactive effects. There was 67 and 80% increase in leaf Pb concentration as compared to control when spinach was grown in Pb polluted soil having 1000 and 2000 kg Pb ha^{-1} soil respectively. Applied Si at 800 kg Si ha^{-1} soil reduced the Pb concentration in spinach grown in Pb contaminated soil having 1000 and 2000 kg Pb ha^{-1} soil respectively as compared to respective control. Silicon application reduced the Pb concentration in spinach and ameliorated the negative effect of Pb contamination.

D. Concentration of Pb in post spinach soil

The Pb concentration in post spinach soil is presented in Fig. 3b. Spiking of soil with Pb resulted in enhanced extractable Pb in soil. Lead concentration was considerably alarmed by Pb pollution and Si alterations and their interactive outcome. The Pb in soil was enhanced by 45 and 39% at 1000 and 2000 kg Pb ha^{-1} soil as compared to respective control. However, at 1000 and 2000 kg Pb ha^{-1} soil, applied Si at the rate of 400 and 800 kg Si ha^{-1} soil decreased the extractable Pb concentration in soil as compared to respective control. Results revealed that Si application reduced concentration of extractable Pb in soil and ameliorated the negative effects of Pb.

E. Uptake of Pb by spinach

The Pb uptake by leaves of spinach grown under Pb contaminated soil at 1000 and 2000 kg Pb ha^{-1} soil was illustrated in Fig. 3c. The Pb uptake in the leaves of spinach was notably disturbed by Pb pollution and applied amendments and their interactive effects. There was 47 and 60% increase in leaf Pb uptake as compared to control when spinach was grown in Pb polluted soil having 1000 and 2000 kg Pb ha^{-1} soil respectively. Applied Si at the rate of 800 kg Si ha^{-1} soil reduced Pb uptake by spinach leaves grown in Pb contaminated soil having 1000 and 2000 kg Pb ha^{-1} soil respectively as compared to respective control. Silicon application reduced the Pb uptake by spinach and ameliorated negative effect of Pb contamination in soil.

IV. DISCUSSION

Excessive amount of Pb cause stunted growth, chlorosis and blackening of root systems in plants Permeability and membrane structure is affected by Pb. Hormonal status is changed by Pb toxicity in plants. Photosynthesis is prohibited by Pb and setback mineral nutrition and water balance (Sharma and Dubey, 2005). Different techniques are used to overcome the toxicity of Pb in plants. These techniques have beneficial effect and have advantage over other approaches that is effective, good public acceptance, environment friendly and can be utilized as in-situ. For instance in situ chemical immobilization (that constrains the concentration of softened toxins by precipitation or through sorption) that decrease metal solubility and reduce metal transport (Basta and McGowen, 2004).

To examine the effect of applied Si amendments (400 and 800 kg Si ha^{-1} soil) on growth and Pb concentration of spinach (*Spinacia oleracea*) exposed to different Pb levels (1000 and 2000 kg Pb ha^{-1} soil) a pot trial was established. The Pb induced physiological parameters including TCC, MSI and RWC (Fig. 1a, 1b, 1c) of spinach increased by applied Si amendments. The Pb stress undesirably exaggerated different attributes in plant species (López-Orenes *et al.*, 2018). The Pb concentration had a negative impact on RWC, MSI and TCC (Ashraf and Tang, 2017). The Pb interfered with chloroplasts ultrastructure, block the manufacture of necessary pigments, blocking the electron transport chain and the Calvin cycle, and causing a carbon dioxide deficit by blocking the stomatal pores, all of which had an adverse effect on photosynthesis (Sofy *et al.*, 2020). Relative water content showed hydration state of leaf. Due to Pb stress relative water content was decreased as it decreased the water content (Posmyk *et al.*, 2009).The Si application improved the chlorophyll concentrations, transpiration rate, water relations, and photosynthetic properties (Sattar *et al.*, 2019). Silicon that is formed on the epidermal cells of leaves may act as "windows," letting more light enter the photosynthetic mesophyll tissue and boosting photosynthetic rates (Dorairaj *et al.*, 2020). The Si application played a significant role in reducing chlorophyll degradation. Applied Si lessened the transpirational loss and enhanced relative water content (RWC). Stomatal conductance, net carbon assimilation rates and transpiration rates were increased with Si application (Gong *et al.*, 2003).

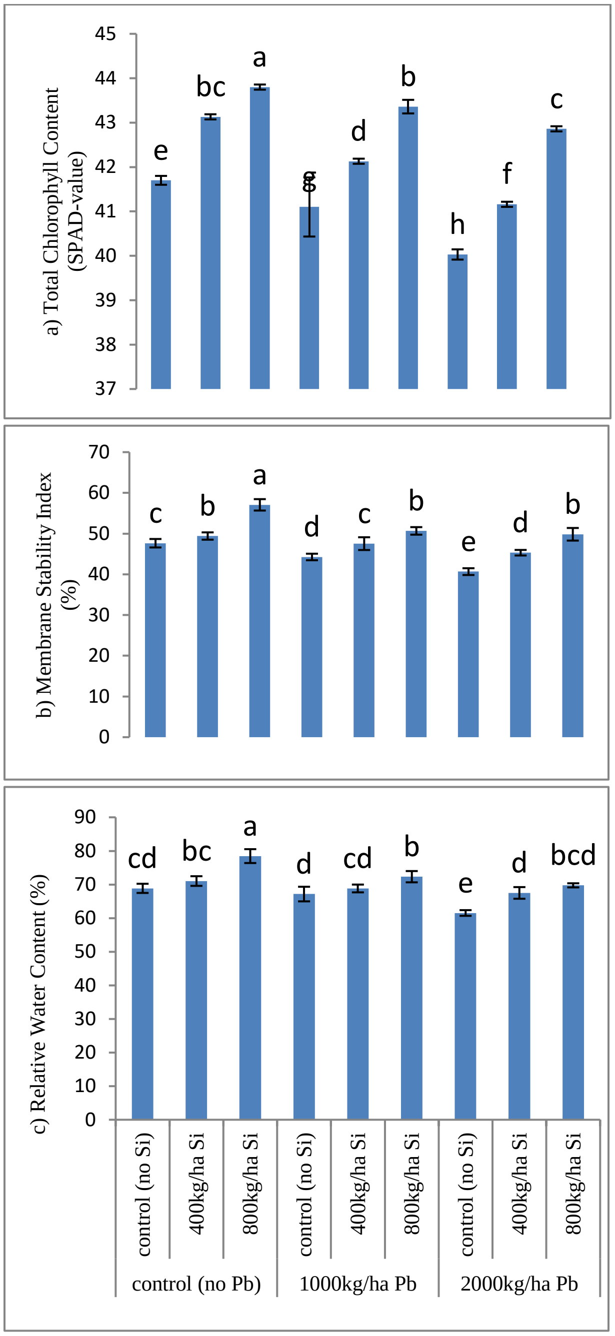

Fig. 1: Physiological responses (a = total chlorophyll contents, b = membrane stability index, c = relative water content) of spinach as affected by Si amendments in Pb poisoned soil (Means ± SE, n = 3). [LSD for total chlorophyll content = 0.39, MSI = 1.75, RWC = 2.63].

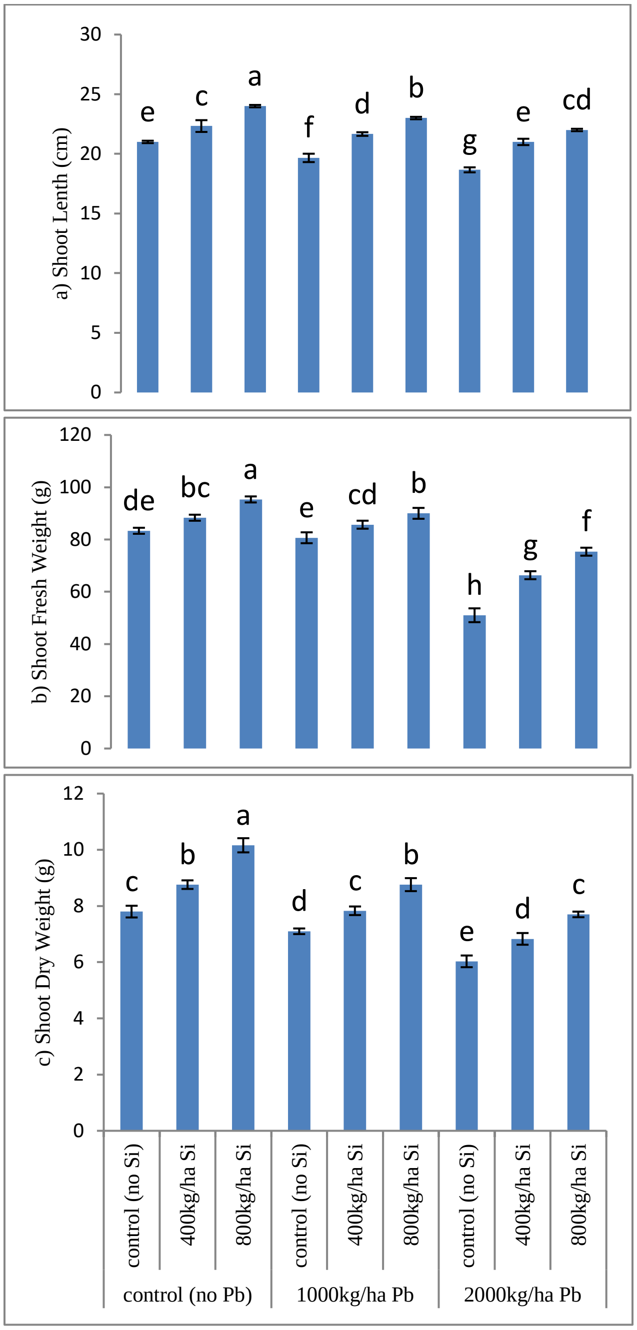

Fig. 2(I): Growth responses (a = shoot length, b = shoot fresh weight, c = shoot dry weight) of spinach shoots as affected by Si amendments in Pb poisoned soil. [LSD for shoot length = 0.43, shoot fresh weight = 3.05, shoot dry weight = 0.30].

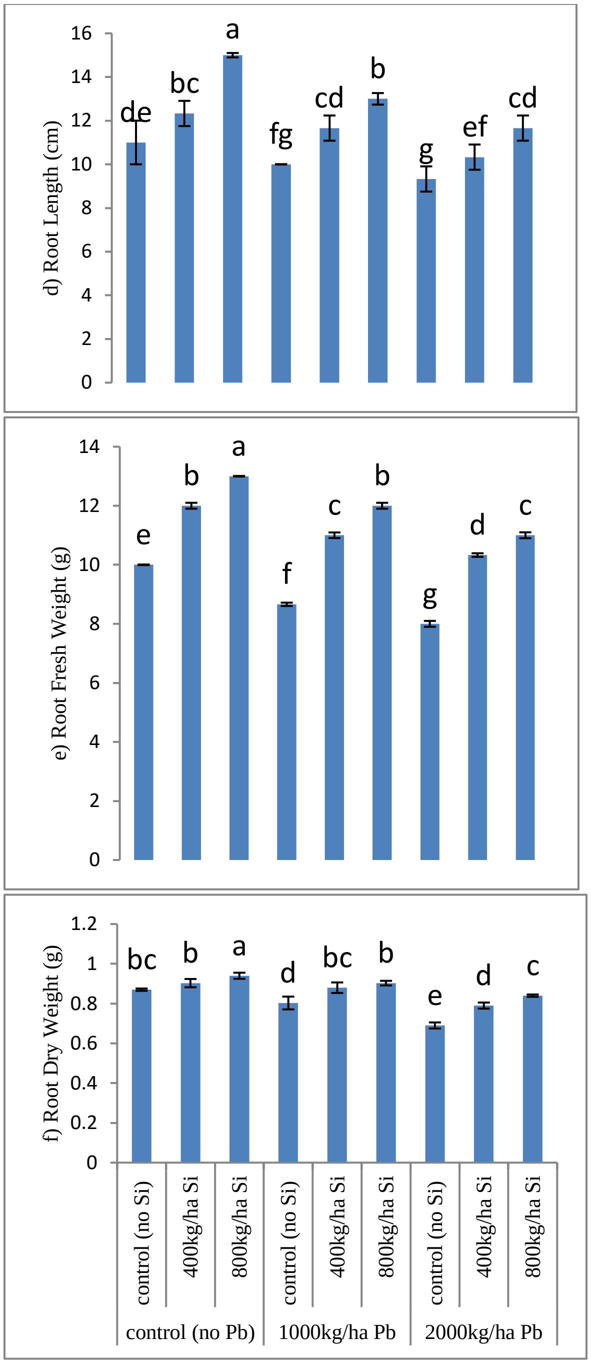

Fig. 2 (II): Growth responses (d = root length, e = root fresh weight, f = root dried up weight) of spinach roots as influenced by Si amendments in Pb poisoned soil. [LSD for root length = 0.80, root fresh weight = 0.14, root dry weight = 0.03].

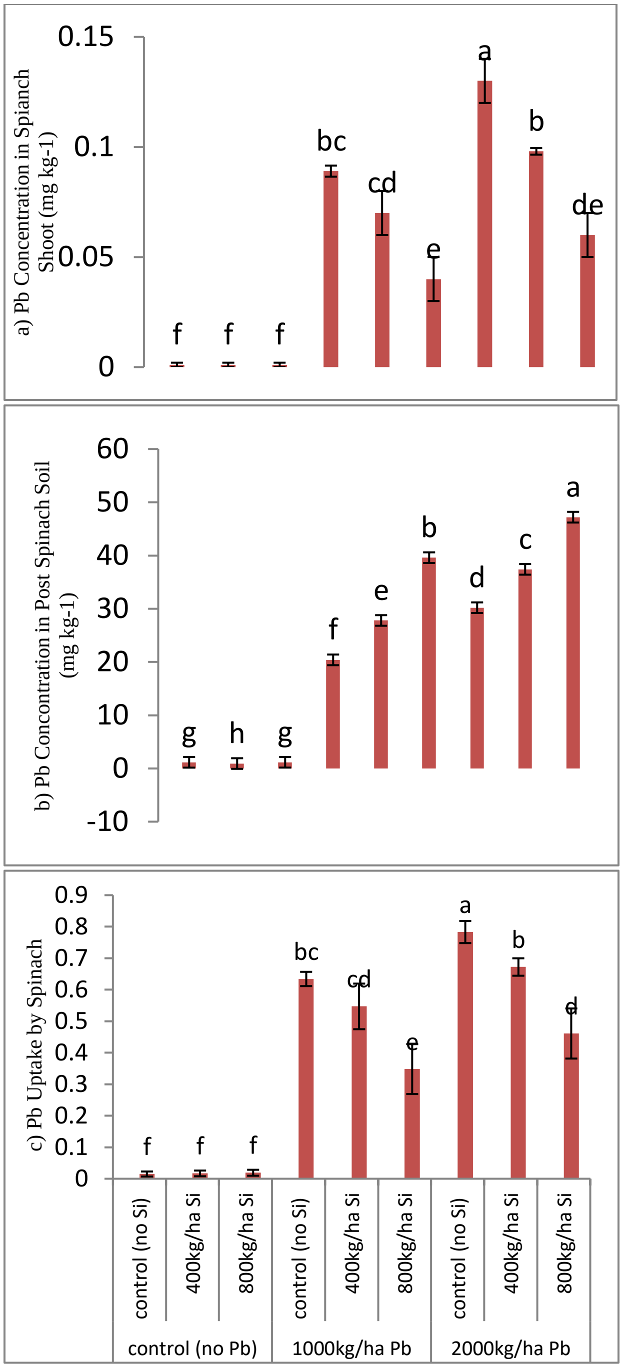

Fig. 3: Chemical parameters (a = Pb concentration in plant shoot, b = Pb concentration in post spinach soil, c = Pb uptake by spinach) of spinach as affected by Si amendments in Pb poisoned soil. [LSD for Pb absorption in spinach shoot = 0.014, Pb intensity in spinach soil = 0.32, Pb uptake by spinach = 0.087].

The increasing levels of Pb reduced the growth of spinach by causing toxicity and is responsible for the reduced physiological functions (Sharma *et al.*, 2009). Many plants like *Acalypha indica* (Venkatachalam et al., 2017), coriander (Fatemi *et al.*, 2020) and canola (Shakoor *et al.*, 2014) were exposed to Pb contamination. Their vegetative growth and biomass production was decreased with increasing Pb concentration. Plant height was decreased by the Pb stress and it was also observed from results that shoot and root lengths as well as fresh and dry masses were adversely affected by Pb concentration in soil (Tripathi *et al.*, 2016).

It was observed that under Pb contamination there was significant increase in growth parameters such as shoot length, root length, shoot and root fresh and dry weights (Fig. 2a, 2b, 2c, 2d, 2e, 2f) by applied Si amendments in present study. For the growth of plants under HM stressed environment, applied Si was generally considered as favorable element (Wu *et al.*, 2016). Moreover, Si was verified to have potential to eliminate accumulation of heavy metals and oxidative stress in crops. Noxiousness of heavy metals was amended by Si application (Gao *et al.*, 2018). Silicon stimulated rice plant development, resulting in enhancement of extensibility in cell wall (Strubińska and Hanaka, 2011). Likewise, researchers discovered that Si had positive benefits on a variety of crops, including sugarcane, cotton, and rice (Ma, 2004). It has been reported that cell wall was strengthened by Si and by improving silicification, lignification and suberization that provide mechanical support which reduced the water loss and eventually improved shoot and root length (Sattar *et al.*, 2019). The applied Si increased the plant growth, total chlorophyll contents, photosynthetic parameters and biomass. Nutrition was enhanced indirectly (for example increased infection resistance) or directly by Si amendments under Pb contaminated soil (Bharwana *et al.*, 2013).

The available HM concentration in soil are thought to influence plant metal absorption (Li *et al.*, 2005). The Si can also encapsulate heavy metals in the soil, reducing available metal and therefore declining metal uptake by crops while stimulating plant development (Li *et al.*, 2018). In the risk evaluation indemnification of HM contaminated soil, immobilization proficiency is a principal constituent (Gu *et al.*, 2011). Apoplastic bypass flow has been reduced by the deposition of Si in the root and offers metal binding sites, consequential in less harmful metal and salt absorption as well as translocation from roots to shoots (Gong *et al.*, 2005). In recent study Pb concentration in plant was more when spinach grown under Pb contaminated (Fig. 3a). The Si application significantly reduced the Pb uptake by plant. The sequence of Pb content in plant components was root > shoot > husk > grains. The results indicated that supplementing rice grains and shoots with Si reduced Pb in the grains and shoots (Hussain *et al.*, 2020). The possible function of Si in the reduction of hazardous metals is also described (Saidi *et al.*, 2014). By reducing oxidative stress, Se combined with Si in the foliage decreased the accumulation of Pb (Alyemeni *et al.*, 2018).

Yang *et al.* (2012) described that tolerance to toxic metals by diminishing the translocation and uptake of metals might be increased by Si amendments. The Si reduce HM toxicity in plants primarily by reducing oxidative damage (Emamverdian *et al.*, 2018), heavy metals are isolated to metabolically inactive regions (Adrees *et al.*, 2015) and HM chelation to produce co-precipitation (Hasanuzzaman *et al.*, 2017). It was reported that accumulation of Pb was inhibited in crops stimulated by applied Si (Fig. 3c). Consequently, it was described that Si possibly have the potential to make complexes with Pb or persuade Pb to accumulate in cell walls; therefore Pb toxicity in cotton was reduced (Bharwana *et al.*, 2013). The decreased Pb concentration in wheat shoot, bran, and flour was detected in connection to the high Si in root and shoot of wheat. Precipitates Pb absorbed in plants has been formed in the root and shoot possibly by Si, resulting in greater levels d and Pb in the root and preventing Pb from being transferred to the shoot and then to bran and flour (Huang *et al.*, 2019).

V. CONCLUSION

In the demonstrated study, growth and physiological responses of spinach were enhanced by the application of Si amendment. Applied Si amendment was able to increase the growth of spinach at different levels of Pb contamination in soil. Results established that growth of spinach was severely affected by the presence of Pb contamination in soil. It was determined that growth and physiological functions were enhanced and availability of Pb was reduced by Si amendment. Application of Si amendment with higher concentration reduced Pb accumulation in spinach when developed in Pb poisoned soil.

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