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ALLEVIATION OF LEAD INDUCED STRESS IN ARHAR (*Cajanus cajan*) BY APPLICATION OF SOLUBLE SILICA

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Pigeon pea, *Cajanus cajan* [(L.) Millspaugh], is a perennial legume (subfamily Papilionoideae) grown in many regions of India including Madhya Pradesh. It is an important source of protein in human diets used in Dhal and consumed by people daily (Saxena *et al*., 2010). Dried seeds of pigeon pea are used for animal feed (Fu *et al*., 2008). Heavy metal stress is getting attention over the last several years among the abiotic stresses (Bhat *et al*., 2019). Heavy metals get persistently accumulated in the environment because of their stability and the fact that they are not biodegradable in nature. One of the heavy metals lead, even at low concentration in soil becomes toxic for plants. Agricultural soil becomes contaminated due to accumulation of lead through various anthropogenic activities. Cultivation of crops in lead contaminated soil resulted in poor germination, reduced root growth and biomass production (Aslam *et al.*, 2021). Lead toxicity in soil specifically affects seed germination, which is the first step of plant life. Lead accumulation in plants interferes with the formation of spindle fibre and cell wall growth, reducing cell expansion and cell division, resulting in root volume inhibition (Zulfiqar *et al.,* 2019; Kanwal *et al*., 2020). Lead toxicity has been found to affect plant growth by altering the composition and concentration of nutrients and protein conformation such as transporters and regulatory proteins in plant cells (Pirzadah *et al*., 2020).Exposure of ryegrass to 500 μM lead reduced the carotene, chlorophyll a and chlorophyll b levels in plants, and the reduction in photosynthetic rate was 52.9% (Bai *et al*., 2015). Silicon plays a protective role by increasing accumulation of polysilicic acid in plant cells resulting in increased plant tolerance against stress. Silicon treatment alleviated the negative effect of environmental stress by normalizing growth and physiological processes in onion leaves (Rangwala *et al*., 2019). Application of silicon fertilizer in the 180, 360 and 540 mg/kg concentration improved biomass, plant height and chlorophyll pigments in leaves of 3 weeks old Chives plant (Long *et al*., 2018). Si treatments (0, 5, 10, 15, 20, and 25 g/L) significantly increase seed germination, seedling growth, dry weight and chlorophyll content of maize seedlings ($P<0.05$), at an optimal concentration of 15 g/L (Yankun *et al*., 2021). The aim of the present study was to observe the effect of various lead concentrations in soil, viz. 50, 100, and 150 mg/kg, on growth attributes and photosynthetic pigment in Arhar (*Cajanus cajan*) and to find out the concentration of soluble silica (of the various concentrations used, viz. 7.5 mL/litre, 10mL/litre, and 12.5mL/litre) that is most efficient in relieving lead stress.

Field experiments were conducted at village Loharpipliya, district Dewas (M.P.). The geographic location of the fields was latitude and longitude N- 22 54’37.7” – 38.4” E- 075 59’ 33.3” – 34”, respectively. The experiment was laid out in a randomised complete block design having a block size of 1.8 m × 2 m. Arhar crop (Hybrid Arhar dal NTL 724 Nirmal Company) was sown on July 30th, 2018. The experimental design consisted of thirteen treatments, as depicted in Table 2. For creating lead stress, soil was treated with lead nitrate solution after 15 days of sowing. Three concentrations of lead nitrate 50, 100 and 150 mg/kg of soil were used. Dissolving lead nitrate in five liters of water yielded a lead nitrate solution, which was then poured on the soil of the blocks listed in Table 2. Soil of 4 blocks was treated with each concentration of lead nitrate. Three concentrations of soluble silica (7.5, 10, 12.5 mL/litre) were sprayed on plants of 3 blocks of each lead treated blocks after 15 days of lead treatment. Soluble silica as Agribooster® was sprayed four times at intervals of 15 days. Crop grown in untreated soil served as control. Growth parameters, chlorophyll and carotenoid content were studied in triplicate from each treatment. Three plants were chosen at random from each block for plant height, and the height was measured with a measuring tape. Three plants were chosen at random from each block and the number of branches was counted. The spectrophotometric method of Lichtenthalen and Welburn (1983) was used to estimate the chlorophyll and carotenoid content of *Cajanus cajan* leaves. Chlorophyll was extracted from leaves using 80% acetone. The absorbance of the supernatant at 470nm, 646nm, and 663nm, as mentioned below, was used to calculate chlorophyll a, chlorophyll b, and carotenoid.

$$\text{Chlorophyll a } (\mu g/ml) = 12.21(A_{663}) – 2.81 (A_{646})$$

$$\text{Chlorophyll b } (\mu g/ml) = 20.13 (A_{646}) – 5.03 (A_{663})$$

$$\text{Total chlorophyll } (\mu g/ml) = 20.2 (A_{646}) + 8.02 (A_{663})$$

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Table 1. Various concentrations of lead nitrate and soluble silica used in the study.

S. No.	Treatment
1	Control-natural soil without any treatment of the field
2	Lead nitrate (50 mg/kg of soil)
3	Lead nitrate (50 mg/kg of soil)+ Silica (7.5mL/litre)
4	Lead nitrate (50 mg/kg of soil)+ Silica (10 mL/litre)
5	Lead nitrate (50 mg/kg of soil)+ Silica (12.5mL/litre)
6	Lead nitrate (100 mg/kg of soil)
7	Lead nitrate (100 mg/kg of soil)+ Silica (7.5 mL/litre)
8	Lead nitrate (100 mg/kg of soil)+ Silica (10 mL/litre)
9	Lead nitrate (100 mg/kg of soil)+ Silica (12.5mL/litre)
10	Lead nitrate (150 mg/kg of soil)
11	Lead nitrate (150 mg/kg of soil)+ Silica (7.5 mL/litre)
12	Lead nitrate (150 mg/kg of soil)+ Silica (10 mL/litre)
13	Lead nitrate (150 mg/kg of soil)+ Silica (12.5mL/litre)

Table 2. Soil composition before and after treatment

Soil	pH	EC (dS/m)	Organic carbon %	Available Nitrogen (Kg/ha)	Available phosphorus (Kg/ha)	Available Potash (Kg/ha)	Lead (ppm)
Before sowing	7.2	0.54	0.76	280	9.0	260	40
After harvesting							
Control	7.42	0.67	0.65	240	13.6	560	71
Treated with 50 mg lead nitrate / kg of soil	7.1	0.72	0.42	244	8.0	360	115
50 mg lead nitrate /kg of soil+ Silica (7.5ml/litre)	8.20	0.41	0.55	218	9.6	340	127
50 mg lead nitrate /kg of soil + Silica (10 ml/litre)	7.86	0.42	0.60	235	11.2	345	131
50 mg lead nitrate /kg of soil + Silica (12.5ml/litre)	7.94	0.43	0.58	230	11.2	333	129
100 mg lead nitrate /kg of soil	7.28	0.77	0.55	248	9.6	400	101
100 mg lead nitrate /kg of soil +Silica (7.5 ml/litre)	7.94	0.68	0.48	230	13.6	380	120
100 mg lead nitrate /kg of soil + Silica (10 ml/litre)	7.64	0.67	0.45	218	11.2	377	126
100 mg lead nitrate /kg of soil + Silica (12.5ml/litre)	7.70	0.62	0.42	204	9.6	360	128
150 mg lead nitrate /kg of soil	7.20	0.82	0.58	236	8.0	400	96
150 mg lead nitrate /kg of soil + Silica (7.5 ml/litre)	7.84	0.73	0.55	217	13.6	360	116
150 mg lead nitrate /kg of soil + Silica (10 ml/litre)	7.90	0.68	0.50	200	9.6	340	105
150 mg lead nitrate /kg of soil + Silica (12.5ml/litre)	7.79	0.79	0.38	176	8.0	376	112

Carotenoid (µg/ml) = [1000(A_{470})-3.27 (Chl a) - 104 (Chl b)]/229

The p-value was calculated to determine whether there was a significant difference between the values. Values are expressed as a percentage increase or decrease.

A significant decrease ($p<0.05$) was observed in plant height upon lead treatments (100 and 150 mg/kg) after 30 days as compared to control (without any treatment). The decrease in plant height was significant and highly significant ($p<0.01$) at 100 and 150 mg/kg lead treatment as compared to control. Change in plant height was insignificant ($p<0.05$) after 45 days of lead treatments. Plant height was reduced by 2.3 %, 4.7%, and 23.3 % after 30 days and 5.7 %, 3.7 %, and 11.4 % after 45 days of 50, 100, and 150 mg/kg lead treatment, respectively. The treatment of 150 mg/kg of lead resulted in the highest percent reduction. Plant height significantly increased ($p<0.05$) in 50, 100, and 150 mg/kg lead treated plants in all three concentrations of soluble silica, i.e 7.5, 10 and 12.5 mL/litre after first treatment. After the second soluble silica treatment, the height increase was found to be significant in the 50 and 100 mg/kg lead treatments, and highly significant ($p<0.01$) in the 150 mg/kg lead treated plant. A highly significant increase ($p<0.01$) in height was found in 50 and 100 mg/kg and an extremely significant ($p<0.001$) increase was observed in 150 mg/kg lead-treated plants after the third soluble silica treatment. No change in height was found after the fourth treatment with soluble silica. The highest increase in plant height was observed in the 10mL/litre soluble silica treatment. Height increased respectively by 12.2%, 10.0%, 26.4% (first silica treatment), 13.5%, 11.4%, 12.3% (second silica treatment), 16.1%, 18.6%, 9.7% (third silica treatment), 3.9%, 4.6%, 3.5% (fourth silica treatment) in 50, 100, and 150 mg/kg lead treated plants (Table 3). The findings showed that applying soluble silica to plants reduces lead induced stress and thus improves plant

Table 3. Effect of different concentrations of lead and various concentrations of soluble silica on plant height (inches) after soluble silica spray.

Treatment	1st silica spray Mean±SD	2nd silica spray Mean±SD	3rd silica spray Mean±SD	4th silica spray Mean±SD
Control (without any treatment)	42.00 ±1.50	57.60±1.50	68.60±1.20	81.60±3.05
(50 mg/kg)	41.00±2.60 a^{ns}	54.30±4.00 a^{ns}	66.30±0.50 a^{ns}	78.60±2.50 a^{ns}
50 mg/kg+7.5 mL/litre	45.00±1.00 a* b*	61.30±1.50 a*b*	73.30±2.08 a*b**	81.70±0.50 $a^{ns}b^{ns}$
50 mg/kg+10 mL/litre	46.00±2.00 a* b*	62.30±1.20 a*b*	74.00±2.00 a**b**	80.30±2.50 $a^{ns}b^{ns}$
50 mg/kg+12.5 mL/litre	45.00±1.00 a* b*	61.0±1.70 a*b*	75.3±2.00 a**b**	81.60±0.50 $a^{ns}b^{ns}$
(100 mg/kg)	40.00±1.70 a* b^{ns}	52.60±3.50 a*b^{ns}	66.00±2.50 $a^{ns}b^{ns}$	79.60±2.00 a^{ns} b^{ns}
100 mg/kg+7.5 mL/litre	43.60±1.10 a^{ns}c*	57.30±1.50 a^{ns}c*	75.60±0.50 a*c**	82.30±2.80 a^{ns} c^{ns}
100 mg/kg+10 mL/litre	44.60±3.20 a^{ns}c*	58.60±1.15 a^{ns}c*	74.30±1.10 a*c**	82.00±1.00 a^{ns} c^{ns}
100mg/kg+12.5 mL/litre	43.00-±1.11 a^{ns} c*	58.30±0.50 a^{ns}c*	78.30±3.20 a*c**	83.30±1.50 $a^{ns}c^{*}$
(150 mg/kg)	34.00±4.00 a* b* c*	51.00±1.00 a** b^{ns} c^{ns}	65.60±0.50 a^{ns} b^{ns} c^{ns}	77.30±2.08 a^{ns} b^{ns} c^{ns}
150 mg/kg+7.5 mL/litre	42.30±1.30 a^{ns}d*	55.60±1.10 a^{ns} d**	72.60±1.50 a*d***	78.60±0.50 a^{ns} d^{ns}
150 mg/kg+10 mL/litre	43.00±0.01 a^{ns}d*	57.30±0.50 a^{ns} d**	74.00±1.00 a*d***	80.00±2.00 a^{ns} d^{ns}
150mg/kg+12.5 mL/litre	42.30±1.10 a^{ns}d*	56.30±1.50 a^{ns} d**	74.30±1.50 a*d***	80.00±2.00 a^{ns} d^{ns}

(a)- as compared to control *- Significant change
(b)- as compared to 50mg/kg **- highly significant change
(c)- as compared to 100mg/kg ***- extremely significant change
(d)- as compared to 150mg/kg ns - not significant

growth. According the findings of this study, increasing lead concentration in soil has a negative impact on plant height. After 15 and 30 days of lead treatment, the morphology and growth of *Abutilon indicum* L. were found to be negatively affected by a 25 M lead nitrate concentration (Shahoo *et al.,* 2015). The buildup of lead in the shoot part of the plant may cause a decline in plant height by interrupting cell division in the shoot region (Shrivastava *et al.,* 2018). Lead binds to nucleic acids, causing chromatin to aggregate and condense, hindering replication and transcription, and ultimately affecting cell proliferation and plant development (Johnson, 1998). A high level of lead in the soil has a negative impact on the biosynthesis of the plant growth hormone auxin (Neto *et al.,* 2017). Toxic levels of lead in soil may be to blame for nutrient metabolic disturbances that slow plant growth (Gopal and Rizvi, 2008). Plants treated with lead grew taller after being sprayed with soluble silica. Soluble silica reduces the availability of heavy metals like lead to plants by raising the pH of the soil. After silicon application in contaminated soil, reduced uptake of lead in banana has been reported (Li *et al.,* 2012). Silicate dissolves in aqueous solution and produces gelatinous metasilicic acid, which can absorb heavy metals or cause metal deposition on their surfaces in silicon rich soil (Gu *et al.,* 2011), both of which reduce metal availability for uptake. Soluble silica could form complexes with heavy metals and induce them to precipitate. According to Zang *et al.* (2013), silicon application in Cr contaminated soil significantly reduced the amount of chromium by enhancing the precipitation of organic matter bound Chromium fraction.

After 30 and 45 days of lead treatment, there was a significant reduction ($p<0.05$) in the number of branches in plants treated with 100 and 150 mg/kg lead compared to control plants (no treatment). After 45 days of lead treatment, no change in branch count was observed. When compared to untreated plants, the number of branches in *Cajanus cajan* plants decreased by 7.0%,

Table 4. Effect of different concentrations of lead and various concentration of soluble silica on the number of branches after soluble silica spray.

Treatment	1st silica spray Mean±SD	2nd silica spray Mean±SD	3rd silica spray Mean±SD	4th silica spray Mean±SD
Control (without any treatment)	24.00±1.70	29.00±1.00	34.00±0.50	37.30±2.08
50 mg/kg	22.30±3.20 a^{ns}	28.00±3.00 a^{ns}	33.30±3.00 a^{ns}	33.30±1.50 a^{ns}
50 mg/kg+7.5 mL/litre	28.30±0.10 a*b*	33.00±1.70 a*** b*	40.30±1.70 a** b*	40.30±0.50 a**b**
50 mg/kg+10 mL/litre	31.00±3.00 a*b*	37.60±6.02 a** *b*	41.60±1.20 a** b**	41.60±1.15 a**b**
50 mg/kg+12.5 mL/litre	31.00±5.50 a*b*	33.0±2.0 a** *b*	41.30±2.00 a** b**	41.30±2.08 a**b**
100 mg/kg	22.60±0.50 a^{ns} b^{ns}	27.60±1.10 a*b^{ns}	34.00±1.00 a^{ns} b^{ns}	36.10±1.50 a^{ns} b^{ns}
100 mg/kg+7.5 mL/litre	28.60±2.08 a* c^{**}	36.10±4.40 a*c*	41.60±0.70 a*c*	44.30±0.50 a**c**
100 mg/kg+10 mL/litre	30.30±4.60 a* c^{*}	38.30±5.70 a*c*	43.30±3.20 a**c**	45.30±0.50 a**c**
100mg/kg+12.5 mL/litre	30.30±3.40 a* c^{*}	37.60±3.05 a**c**	43.60±1.30 a**c**	43.60±2.08 a*c*
150 mg/kg	20.60±0.50 a*b*c*	27.30±0.50 a* b^{ns} c^{ns}	34.30± 1.00 a^{ns} b^{ns} c^{ns}	36.60±2.08 a^{ns} b^{ns} c^{ns}
150 mg/kg+7.5 mL/litre	23.00±0.00 a^{ns}d**	29.00±1.70 a^{ns} d*	38.30±0.50 a**d**	40.60±1.15 a* d*(12.7%)
150 mg/kg+10 mL/litre	23.30±0.50 a^{ns}d**	33.30±0.40 a^{ns} d*	38.80±2.80 a***d**	44.30±0.50 a**d**(23.1%)
150mg/kg+12.5 mL/litre	24.00±1.30 a^{ns} d**	33.30±1.50 a^{ns} d*	38.70±1.00 a***d**	47.60±2.08 a**d**(32.2%)

(a)- as compared to control *- Significant change
(b)- as compared to 50mg/kg **- highly significant change
(c)- as compared to 100mg/kg ***- extremely significant change
(d)- as compared to 150mg/kg ns - not significant

5.8%, 14.1%(after 30 days), 3.4%, 4.8%, 6.2% (after 45 days), 2.05%, 2.3%, 2.9% (after 60 days) and 2.6%, 3.4%, 3.4% (after 75 days). After the first soluble silica treatment, the number of branches in plants treated with 50, 100, and 150 mg/kg lead increased significantly ($p<0.05$) in each concentration of soluble silica (Table 4). After the second time silica treatment, 10 mL/litre soluble silica concentration significantly increased ($p<0.05$) the number of branches in 50 mg/kg lead treated plants and highly significantly increased ($p<0.001$) the number of branches in 100 and 150 mg/kg lead treated plants. After 60 days of lead treatment, the number of branches in soluble silica treated plants increased significantly ($p<0.001$). when compared to lead treated plants, the highest increase was observed in 10mL/litre soluble silica treatment, with branches increasing by 39.0%, 34.0%, 61.6% (first silica treatment), 34.2%, 38.7%, 21.9% (second silica treatment), 24.9%, 36.8%, 30.0% (third silica treatment), and 33.1%, 25.4%, 32.2% (fourth silica treatment). The two times application of soluble silica alleviated lead induced stress and improved plant growth. *Cajanus cajan* plant growth and branching were improved with the third silica treatment. Lead is recognized as a protoplasmic poison in general. It is a slow acting toxic substance that builds up in the plant's stem. Lead accumulation impedes cell permeability by reacting with enzymes involved in plant processes, the phosphate group of ADP or ATP, or the replacement of ions, resulting in growth inhibition and toxicity (Hall, 2002). Water availability to all parts of the plants gets restricted by heavy metal stress (Sharma *et al.,* 1996). The interference of led with the metabolic and biochemical processes involved in normal plant growth and development induces decrease in the weight and length of lead treated plants (Verma *et al.,* 2003). Wheat (*Triticum aestivum* L.) was subjected to lead stress, which reduced root and shoot length, fresh and dry weight, and chlorophyll a and b levels (Bhatti *et al.,* 2013). Soluble silica treatment increases the amount of water available to plants from the soil, leading to greater enzymatic activity in growth metabolic processes. Spraying soluble silica four times increased the number

Table 5. Effect of different concentrations of lead and various concentrations of soluble silica on chlorophyll a (μ/mL) soluble silica spray.

Treatment	1st silica spray Mean±SD	2nd silica spray Mean±SD	3rd silica spray Mean±SD	4th silica spray Mean±SD
Control (without any treatment)	2.50±0.10	2.64±0.3	2.50±0.30	2.16 ± 0.30
50 mg/kg	2.30±0.50 a^{*}	2.50±0.30 a^{ns}	2.31±0.10 a^{ns}	1.70±0.50 a^{ns}
50 mg/kg+7.5 mL/litre	2.40±0.30 $a^{ns} b^{ns}$	2.40±0.10 $a^{ns}b^{ns}$	2.70 ±0.10 $a^{ns}b^{*}$	2.30 ±0.20 $a^{ns}b^{ns}$
50 mg/kg+10 mL/litre	2.70±0.10 $a^{ns} b^{*}$	3.20±0.10 $a^{*}b^{*}$	3.00±0.30 $a^{ns}b^{*}$	2.40±0.20 $a^{ns}b^{*}$
50 mg/kg+12.5 mL/litre	2.70±0.20 $a^{ns} b^{*}$	3.70 ±0.70 $a^{*}b^{*}$	2.70±0.30 $a^{ns}b^{*}$	1.80±0.10 $a^{ns}b^{ns}$
100 mg/kg	2.02±0.10 $a^{**}b^{*}$	2.10±0.10 $a^{*}b^{*}$	2.30±0.10 $a^{ns}b^{ns}$	1.60± 0.40 $a^{ns}b^{ns}$
100 mg/kg+7.5 mL/litre	2.30±0.20 $a^{ns} c^{ns}$	2.60±0.12 $a^{ns}c^{*}$	2.71±0.07 $a^{ns}c^{*}$	1.90±0.40 $a^{ns}c^{ns}$
100 mg/kg+10 mL/litre	2.60±0.20 $a^{ns} c^{*}$	3.40±0.10 $a^{**}c^{***}$	2.70±0.10 $a^{ns}c^{*}$	2.70±0.20 $a^{*}c^{*}$
100mg/kg+12.5 mL/litre	2.70±0.10 $a^{*}c^{***}$	2.70±0.10 $a^{ns}c^{**}$	2.80±0.10 $a^{ns}c^{*}$	2.30±0.30 $a^{*}c^{*}$
150 mg/kg	1.80±0.10 $a^{**}b^{**}c^{*}$	1.90±0.10 $a^{*}b^{*}c^{ns}$	2.30±0.20 $a^{ns}b^{ns}c^{ns}$	1.60±0.20 $a^{*}b^{ns}c^{ns}$
150 mg/kg+7.5 mL/litre	2.30±0.20 $a^{ns} d^{*}$	2.40±0.30 $a^{*}d^{***}$	2.70±0.30 $a^{*}d^{*}$	2.20±0.10 $a^{ns}d^{*}$
150 mg/kg+10 mL/litre	2.30±0.20 $a^{ns} d^{*}$	2.60±0.10 $a^{ns}d^{***}$	2.90±0.80 $a^{*}d^{*}$	2.50±0.40 $a^{ns}d^{*}$
150mg/kg+12.5 mL/litre	2.30±0.20 $a^{ns} d^{*}$	2.40±0.30 $a^{*}d^{***}$	2.90±0.50 $a^{*}d^{*}$	2.30±0.30 $a^{ns}d^{*}$

(a)- as compared to control *- Significant change
(b)- as compared to 50mg/kg **- highly significant change
(c)- as compared to 100mg/kg ***- extremely significant change
(d)- as compared to 150mg/kg ns - not significant

of branches in *Cajanus cajan* plants.

After 30 days of treatment with lead nitrate, the decrease in leaf chlorophyll a content was significant ($p<0.05$) in 50 mg/kg lead treated plants, highly significant ($p<0.001$) in 100 mg/kg lead treated plants, extremely significant ($p<0.001$) in 150 mg/kg lead treated plants as compared to control. Chlorophyll a was significantly ($p<0.05$) reduced after 45 days of lead treatment at 100 and 150 mg/kg lead treatments. In the 50, 100, and 150 mg/kg lead treatment, the percent decrease in chlorophyll a was 8.0%, 19.2%, 28.0% (30 days), and 3.8%, 20.4%, 26.9% (45 days, respectively). After 45 days, lead had no effect on chlorophyll a. With the first and second soluble silica treatments, significant ($p<0.05$) increase in chlorophyll a was found in 50 mg/kg lead treated plant leaves at all silica concentrations, whereas extremely significant ($p<0.001$) raises in 10mL/litre and 12.5 mL/litre have been found in 100 and 150 mg/kg lead treated leaves after first and second soluble silica treatments. After third treatment of soluble silica in lead stressed leaves, chlorophyll a content significantly increased ($p<0.05$) in all concentrations of soluble silica. Highest percent increase in chlorophyll a in *Cajanus cajan* leaves was 17.3%, 33.6%, 27.2% (first silica treatment), 48.0%, 28.5%, 28.9% (second silica treatment), 30.4%, 21.7%, 26.0% (third silica treatment) and 58.8%, 68.7%, 56.0% (fourth silica treatment) in 50, 100, and 150 mg/kg lead treatment, respectively, after soluble silica treatment (Table 5). Result indicated that 12.5 mL/litre silica treatment for two times was effective in relieving lead stress up to 150 mg/kg.

After 30 days, a decrease in chlorophyll b content in *Cajanus cajan* leaves was found to be significant ($p<0.05$), highly significant ($p<0.01$), and extremely significant ($p<0.001$. after 45 days, chlorophyll b was reduced significantly in 50 and 100 mg/kg lead treated plant leaves, and it was highly significantly in 150 mg/kg lead treated leaves. There was no detrimental influence on the chlorophyll b content of *Cajanus cajan* leaves after 60 and 75 days of lead treatment (Table 6). The

Table 6. Effect of different concentrations of lead and various concentrations of soluble silica on chlorophyll b (µ/mL) after soluble silica spray.

Treatment	1st silica spray Mean±SD	2nd silica spray Mean±SD	3rd silica spray Mean±SD	4th silica spray Mean±SD
Control (without any treatment)	2.40±0.08	1.60± 0.07	1.40±0.20	1.70± 0.40
50 mg/kg	2.10±0.05 a*	1.40±0.05 a*	1.30±0.10 a*	1.20±0.09 a^{ns}
50 mg/kg+7.5 mL/litre	2.40±0.20 a^{ns} b^{ns}	1.40±0.04 a* b^{ns}	1.60±0.04 a^{ns} b**	1.70± 0.10 a^{ns}b*
50 mg/kg+10 mL/litre	2.60±0.07 a*b**	2.40±0.20 a**b**	1.80±0.10 a*b**	1.70± 0.10 a^{ns}b*
50 mg/kg+12.5 mL/litre	2.60±0.40 a* b*	2.43±0.18 a**b**	1.70±0.10 a*b**	1.60±0.20 $a^{ns}b^{ns}$
100 mg/kg	1.80±0.30 a**b^{ns}	1.30±0.20 a* b^{ns}	1.32±0.18 a^{ns} b^{ns}	1.10±0.08 a^{ns}b*
100 mg/kg+7.5 mL/litre	2.20±0.20 a^{ns} c*	1.90±0.09 a* c**	1.50±0.10 a^{ns}c*	1.50±0.20 a*c*
100 mg/kg+10 mL/litre	2.30±0.20 a^{ns} c*	2.30±0.13 a** c**	1.70±0.13 a*c**	2.00±0.60 a^{ns}c*
100mg/kg+12.5 mL/litre	2.20±0.10 a^{ns}c*	2.50±0.13 a**c**	1.80±0.80 a*c**	1.80±0.20 a^{ns}c*
150 mg/kg	1.70±0.10 a***b** c^{ns}	1.20±0.03 a**b* c^{ns}	1.32±0.20 $a^{ns}b^{ns}$ c^{ns}	0.80±0.20 a*b*c*
150 mg/kg+7.5 mL/litre	2.30±0.20 a^{ns} d*	1.40±0.04 a^{ns} d^{ns}	2.10±0.26 a*d**	1.50±0.56 a^{ns}d*
150 mg/kg+10 mL/litre	2.60±0.20 a^{ns} d*	1.53±0.50 a^{ns}d*	2.50±0.40 a**d**	1.20±0.60 a^{ns} d^{ns}
150mg/kg+12.5 mL/litre	2.50±0.20 a^{ns} d*	1.60±0.20 a^{ns}d*	2.30±0.20 a**d**	1.40±0.20 a^{ns}d*

(a)- as compared to control *- Significant change
(b)- as compared to 50mg/kg **- highly significant change
(c)- as compared to 100mg/kg ***- extremely significant change
(d)- as compared to 150mg/kg ns - not significant

percent decline in 50, 100, and 150 mg/kg lead treated *Cajanus cajan* leaves was 2.5%, 25.0%, 29.1% (30 days), 12.5%, 18.7%, 25.0% (45 days), 7.1%, 7.1%, 5.7% (60 days, 4.1%, 3.5% (75 days). After the first soluble silica treatment, the increase in chlorophyll b was highly significant ($p<0.01$) in 50 mg/kg and significant ($p<0.05$) in 100 and 150 mg/kg lead treated leaves. After a second treatment of soluble silica, the content of chlorophyll b increased significantly in 50 and 100 mg/kg lead treated leaves, and it increased significantly in 150 mg/kg lead treated leaves. In 50, 100, and 150 mg/kg lead stressed leaves, the third treatment of soluble silica significantly increased the chlorophyll b content, while the fourth treatment did not. In 50, 100, and 150 mg/kg lead treated leaves, the highest percent increase in chlorophyll b was found at 23.0%, 27.7%, 52.9% (after first silica treatment), 73.0%, 92.0%, 33.0% (after second silica treatment), 41.6%, 81.1%, 87.5% (after fourth silica treatment).

After 30 and 45 days, there was a significant decrease in total chlorophyll in 50, 100, and 150 mg/kg lead stress leaves. Total chlorophyll was reduced by 17.0%, 18.8%, and 20.5% (30 days) and 6.0%, 7.5%, and 9.0% (45 days) in the 50, 100, and 150 mg/kg lead treatments. After 60 and 75 days of lead treatment, there was no perceptible change in total chlorophyll (Table 7). In 50, 100, and 150 mg/kg lead treated leaves, all three concentrations of the first and second, soluble silica treatments significantly ($p<0.05$) increased total chlorophyll. In the 10 mL/litre and 12.5 mL/litre third silica treatments, there was a significant increase in total chlorophyll. After the fourth treatment of silica, there was a highly significant ($p<0.01$) increase in 50 and 100 mg/kg lead treated leaves. In 50, 100, and 150 mg/kg lead treated leaves, the highest percent decrease was 27.8%, 10.5%, 20.4% (first silica treatment), 6.3%, 5.9%, 14.8% (second silica treatment), 4.4%, 14.0%, 42.1% (third silica treatment), 77.7%, 77.7%, 5.5% (forth silica treatment). According to the current findings, as heavy metal lead concentrations increased, the content of

Table 7. Effect of different concentrations of lead and various concentrations of soluble silica on total chlorophyll (µ/mL) after soluble silica spray.

Treatment	1st silica spray Mean±SD	2nd silica spray Mean±SD	3rd silica spray Mean±SD	4th silica spray Mean±SD
Control (without any treatment)	1.17±0.07	1.96±0.01	1.60±0.40	2.10±0.10
50 mg/kg	0.97±0.03 a*	1.88±0.01 a*	1.56±0.01 a^{ns}	1.80±0.10 a^{ns}
50 mg/kg+7.5 mL/litre	1.16±0.05 a^{ns} b*	1.94±0.04 a^{ns}b*	1.61±0.07 a^{ns} b^{ns}	2.40±0.20 a^{ns} b^{ns}
50 mg/kg+10 mL/litre	1.17±0.07 a^{ns} b*	2.00±0.08 a^{ns}b*	1.64±0.04 a^{ns} b*	3.20±0.20 a**b**
50 mg/kg+12.5 mL/litre	1.10±0.80 a^{ns} b*	1.96±0.04 a^{ns}b*	1.63±0.05 a^{ns} b*	2.90±0.30 a**b**
100 mg/kg	0.95±0.02 a**b^{ns}	1.85±0.04 a* c^{ns}	1.50±0.07 a* b^{ns}	1.80±0.20 a^{ns}
100 mg/kg+7.5 mL/litre	1.04±0.01 a*c*	1.92±0.12 a^{ns} b*	1.61±0.01 $a^{ns}c^{ns}$	2.80±0.40 a*c*
100 mg/kg+10 mL/litre	1.05±0.06 a*c*	1.96±0.02 a^{ns} c*	1.71±0.05 a* c*	3.20±0.40 a**c**
100mg/kg+12.5 mL/litre	1.03±0.06 a*c*	1.93±0.02 a^{ns} c*	1.70±0.80 a^{ns} c*	2.80±0.20 a**c**
150 mg/kg	0.93 ±0.06 a*b^{ns} c^{ns}	1.82±0.06 a* b^{ns}	1.45±0.04 a*b* c^{ns}	1.80±0.20 a^{ns} b^{ns} c^{ns}
150 mg/kg+7.5 mL/litre	1.06±0.06 a^{ns} d*	1.92±0.03 a^{ns}d*	1.59±0.30 a^{ns} d^{ns}	1.80±0.30 a^{ns} d^{ns}
150 mg/kg+10 mL/litre	1.12±0.06 a^{ns} d*	2.09±0.10 a^{ns}d*	1.80±0.10 a*d*	1.80±0.30 a^{ns} d^{ns}
150mg/kg+12.5 mL/litre	1.07 ±0.70 a^{ns} d*	2.03±0.10 a^{ns}d*	1.60±0.10 a*d*	1.90±0.20 a^{ns} d^{ns}

(a)- as compared to control   *- Significant change
(b)- as compared to 50mg/kg   **- highly significant change
(c)- as compared to 100mg/kg   ***- extremely significant change
(d)- as compared to 150mg/kg   ns - not significant

photosynthetic pigments chlorophyll a and b in *Cajanus cajan* leaves decreased significantly when compared to lead untreated plants. Chlorophyll is a necessary component of photosynthesis in plants. The complete process of photosynthesis requires chlorophyll a, b, and other accessory pigments. Chlorophyll a is the primary pigment responsible for converting light energy into chemical energy. By interfering with the uptake of essential elements like Mg and Fe, lead inhibits chlorophyll synthesis (Burzyski 1987). It harms the photosynthetic apparatus because of its affinity for protein N and S ligands (Ahmed *et al.,* 1993). The presence of lead alters the lipid composition of thylakoid membranes, resulting in distorted chloroplast structure (Stefanov *et al.,* 1995). Under heavy metal stress, *Atriplex halimus* exhibited a similar decrease in chlorophyll content (Brahim *et al.,* 2011). Emamverdian *et al.* (2015) found that heavy metal stress causes the destruction of chlorophyll pigments and the instability of the pigment protein complex, resulting in a reduction in leaf chlorophyll content. Sinha *et al.* (2012) reported that the chlorophyll content of the leaves of *Brassica juncea* was considerably lowered after they were exposed to cadmium and lead contaminated soil. According to Chakraborty *et al.* (2015), decreased chlorophyll content in tomato plants associated with heavy metal stress is capable of inhibiting key enzymes in chlorophyll biosyntheses, such as protochlorophyllide reductase and aminolevulinic acid synthesis. Our findings indicate that treatment with soluble silica increases chlorophyll content in lead treated and silica treated plant leaves when compared to untreated plant leaves. Soluble silica could help to maintain the integrity of the chloroplast membrane while also protecting the chloroplast from heavy metal stress. The pH of the soil can be changed from acidic to neutral by spraying soluble silica on it, allowing essential nutrients to be absorbed and reducing lead uptake. In cadmium stressed rice seedlings, Wang *et al.* (2015) discovered that foliar application of nano silicon increases magnesium, iron, and zinc nutrition.

Table 8. Effect of different concentrations of lead and various concentrations of soluble silica on carotenoid content (μ/mL) after soluble silica spray.

Treatment	1st silica spray Mean±SD	2nd silica spray Mean±SD	3rd silica spray Mean±SD	4th silica spray Mean±SD
Control (without any treatment)	1.22 ±0.10	1.91 ±0.02	1.43±0.20	1.57± 0.07
50 mg/kg	1.18±0.03 a**	1.40±0.05 a*	1.86±0.03 a*	1.23±0.14 a^{ns}
50 mg/kg+7.5 mL/litre	1.60±0.15 a* b**	1.40±0.04 a* b^{ns}	2.40±0.30 a*b*	1.56±0.32 a^{ns} b^{ns}
50 mg/kg+10 mL/litre	1.70±0.10 a**b**	2.40±0.20 a**b**	2.30±0.11 a**b**	1.59±0.17 a^{ns}b*
50 mg/kg+12.5 mL/litre	1.70±0.10 a**b**	2.50±0.18 a**b***	2.26±0.15 a*b**	1.56 ±0.09 a^{ns}b*
100 mg/kg	0.69 ±0.20 a*b*	1.80±0.05 a* b^{ns}	0.62 ±0.20 a*b*	1.62±0.05 a^{ns} b^{ns}
100 mg/kg+7.5 mL/litre	0.72±0.08 a*** c^{ns}	1.83±0.10 a^{ns} c^{ns} 1	0.84 ±0.30 a* c^{ns}	1.70±0.20 a^{ns} c^{ns}
100 mg/kg+10 mL/litre	1.16±0.29 a^{ns}c*	2.38±0.16 a**c**	0.94 ±0.02 a*c*	1.83±0.06 a**c**
100mg/kg+12.5 mL/litre	0.86±0.23 a* c^{ns}	2.40±0.10 a**c**	0.88 ±0.07 a* c^{ns}	1.55 ±0.08 a^{ns} c^{ns}
150 mg/kg	0.70±0.18 a**b* c^{ns}	1.56±0.07 a**b**c**	0.60±0.03 a** b^{ns}c**	1.34 ±0.06 a** b^{ns}c**
150 mg/kg+7.5 mL/litre	1.00±0.10 a*d*	1.86±0.30 a^{ns} d^{ns}	0.95±0.12 a*d**	1.69 ±0.02 a^{ns}d*
150 mg/kg+10 mL/litre	1.03±0.09 a**d*	1.84±0.05 a^{ns}d**	1.10±0.17 a*d**	1.78±0.14 a^{ns}d*
150mg/kg+12.5 mL/litre	1.21±0.09 a^{ns}d*	1.71±0.02 a**d*	0.65±0.20 a** d^{ns}	1.57 ±0.20 a^{ns} d^{ns}

(a)- as compared to control *- Significant change
(b)- as compared to 50mg/kg **- highly significant change
(c)- as compared to 100mg/kg ***- extremely significant change
(d)- as compared to 150mg/kg ns - not significant

After 30 and 45 days of lead treatment, carotenoid content decreased significantly ($p<0.05$) in 50 and 100 mg/kg lead treated leaves, and highly significantly ($p<0.01$) in 150 mg/kg lead treated leaves. The carotenoid content of lead treated leaves showed no adverse effects after 60 and 75 days of lead treatment. The percent decrease in carotenoid content in 50, 100, and 150 mg/kg lead treatment was 3.3%, 7.3%, 18.0% (30 days) and 2.6%, 5.7%, 18.0% (45 days). After 30 days, there was a highly significant ($p<0.01$) increase in carotenoid content in all three concentrations of soluble silica treated *Cajanus cajan* leaves of 50 mg/kg lead treatment, and a significant ($p<0.05$) increase in 100 and 150 mg/kg lead treatment. After 45 days, there was a highly significant ($p<0.01$)increase in 10 and 12.5 mL/litre silica treatment in 50, 100, and 150 mg/kg lead treated leaves. After third silica treatment, carotenoid content significantly increased in 50 and 100 mg/kg lead treatment and highly significantly increased in 150 mg/kg lead treated leaves, while fourth silica treatment had no effect on carotenoid (Table 8). The highest percent increase in carotenoid was 12.7%, 16.8%, 17.2% (first silica treatment), 26.8%, 34.4%, 19.8% (second silica treatment), 16.9%, 22.1%, 28.9% (third silica treatment). In *Cajanus cajan*, 10 mL/litre silica concentration was found to be effective in lowering 100 and 150 mg/kg lead stress.

Plants, algae, fungi and bacteria all contain carotenoids, which are natural tetraterpenoid pigments. They are essential for the biosynthesis of phytohormones such as abscisic acid and strigolactones (Al Balili and Bouwnmeester, 2015). The reduction in chlorophyll and carotenoid content in lead treated plants could be due to lead absorption from soil and translocation through the root and stem into leaves. Singh *et al.* (2012) discovered that at 50 and 100 µM lead and nickel concentrations, the carotenoid and chlorophyll content in Urd seedlings was significantly reduced. In the presence of chromium, both chlorophyll and carotenoid production decrease significantly (Adress *et al.,* 2015). Silica may form complexes with heavy metals, inhibiting their translocation in the stem, resulting in an increase in chlorophyll and carotenoid after treatment with soluble silica. According to Liu *et al.* (2004), silicon forms a complex with aluminium, preventing it from penetrating into root cortex of *Sorghum bicolor*. Wang *et al.* (2000) discovered that boron forms a boron silicate complex in the soil, which reduces boron availability to plants. One possible reason for the chlorophyll and carotenoid content is that the soluble silica treatment raised pH of the soil, which could reduce lead availability. According to Barwana *et al.* (2013), silicon application inhibits lead uptake and translocation in cotton plants.

According to the findings of this study, lead concentrations greater that 100 mg/kg of soil have a negative impact on *Cajanus cajan* growth and branching. In the presence of lead more than 50 mg/kg, the chlorophyll and carotenoid content was reduced. The use of soluble silica detoxes the toxic effects of lead (and possibly other heavy metals) and allows *Cajanus cajan* to resume normal development. For neutralizing lead toxicity, two times treatments with soluble silica are effective. The recovery of growth and chlorophyll content mediated by soluble silica suggests that spraying soluble silica in 10 mL/litre and 12.5 mL/litre concentrations are effective in alleviating lead induced stress in *Cjanus cajan* plants. To optimize soluble silica concentration in other crops, more research is needed.

Authors' contribution

Conceptualization of research work and designing of experiments (SS, BA, GR, VN); Execution of field/ lab experiments and data collection (SS, BA, GR, VN); Analysis of data and interpretation (SS, BA); Preparation of manuscript (SS, BA, GR, VN)

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