https://doi.org/10.17221/19/2025-PSE

Soil lithium affects carrot growth by changing cation concentrations and physiological attributes

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Citation: Bakhat H.F., Rasul K., Farooq A.B.U., Hammad H.M., Afzal S., Abbas S., Shah G.M., Rabbani F., Fahad S., Ondrasek G., Rashid M.I. (2025): Soil lithium affects carrot growth by changing cations concentrations and physiological attributes. Plant Soil Environm., 71: 259–268.

Abstract: Lithium (Li) plays a significant role in human physiology and psychology; however, it is non-essential for plants. The extensive use of Li in industrial processes and battery-powered devices poses a potential global threat to living organisms. This study assessed the impact of varying soil Li concentrations (0, 20, 40, 60, and 80 mg/kg) on carrot (*Daucus carota* L.) plants. Results revealed that Li concentrations exceeding 40 mg/kg soil had detrimental effects on carrot growth. Compared to 0 mg/kg soil, Li concentrations of 60 and 80 mg/kg reduced shoot fresh biomass by 51% and 82%, respectively, and root fresh biomass by 68% and 89%, respectively. Elevated Li levels in the soil also increased hydrogen peroxide (H_2O_2) content in shoots and triggered enhanced activity of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT). Additionally, soil Li disrupted the uptake and translocation of essential nutrients such as potassium (K) and calcium (Ca) from roots to shoots. This study concludes that while low Li levels may elicit a positive response in plants, higher concentrations significantly impair growth and could contribute to the accumulation of Li in the food chain.

Keywords: psychopharmacology; alkali metal toxicity; physiological response; cations accumulation

Lithium (Li) is a naturally occurring element in the earth's crust (0.0017%) with a high chemical activity (Kalinowska et al. 2013). It is the least dense of all the elements, in the solid phase. Lithium compounds, especially carbonate (Li_2CO_3), have been broadly used in psychopharmacology, predominantly for the treatment of manic-depressive psychosis, aggressive behaviour, and unipolar/bipolar disorder in humans (Szklarska and Rzymski 2019). The average human US population consumption of Li has been estimated to range from 650 to 3 100 μg/day. Lithium is an essential micronutrient for humans (Naeem et al. 2021), and its recommended dietary allowance (RDA) is 1.0 mg/day for a 70 kg adult (Schrauzer 2002). Low intake of Li is linked with higher suicide rates, as in Europe, about 800 thousand deaths annually due to suicide and in-

Supported by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia, Project No. GPIP: 1959-188-2024.

https://doi.org/10.17221/19/2025-PSE

adequate Li consumption (WHO 2022). Similarly, data shows that ecologically appropriate Li dosages may have positive health consequences, such as a reduction in suicide rates and levels of violence (Terao 2015, Szklarska and Rzymski 2019). In humans, a lack of this element can cause problems with protein metabolism and reproduction. Further, the presence of Li in drinking water has been shown to have a favourable effect on the circulatory system, avoiding cardiovascular disorders (Kabata-Pendias and Mukherjee 2007) and Alzheimer's disease (Young 2011).

Sources of Li in the environment include natural sources, i.e. parent material and human activities (i.e., rocket propellants and industries like ceramics, glass, nuclear, aluminium production, rechargeable/non-rechargeable batteries, and pharmaceutical), which introduce Li in the water-soil-plant-food continuum. Lithium occurs naturally in soil and water. The element concentration in various components of environments is different; it is 25 mg/kg in soil, 2 ng/m^3 in the atmosphere and 2 mg/L in drinking water (Shahzad et al. 2016). In Australia, Li standards in irrigation water have been set at 2.5 mg/L, and higher levels may cause phytotoxicity (EPR 2005). In the soil, the concentration of Li also varies depending on the type of parent material, rock composition, and redox status (Naeem et al. 2021).

Lithium is ubiquitous in soil-plant systems and is taken up by all plants grown in soil medium. Although the element appears not to be required for plant growth and development, plants vary in Li accumulation, and it ranges between 0.2–30 mg/kg depending on plant species, water used for irrigation and soil type (Aral et al. 2008). Plants belonging to the halophytic group, i.e., Asteraceae and Solanaceae families, are Li accumulators, and the species *Brassica napus* L. and *Brassica oleracea* L. may accumulate Li up to 2 590 and 3 091 mg/kg, respectively (Kavanagh et al. 2018, Buendía-Valverde et al. 2024). While such accumulator plants, i.e., *Apocynum venetum* L., store the majority of Li (72%) in the vacuoles as a regulatory mechanism to avoid toxicity (Qiao et al. 2018). Exposure to Li (5 mmol $LiNO_3$) inhibits the germination of tobacco microspores, develops necrosis, and decreases photosynthetic pigment, whereas 50 mmol LiCl elevates lipid peroxidation (Hawrylak-Nowak et al. 2012). Allender et al. (1997) reported that Li exposure higher than 10 mg Li/L in cotton and 16 mg Li/L in maize causes growth retardation and produces chlorosis and necrotic spots. It is evident from the literature that minimum levels of Li have positive effects on plants, as in spinach, it increased the plant biomass from 16% to 97% (Bakhat et al. 2020). Similarly, Li accumulation did not affect the growth and yield of maize (Antonkiewicz et al. 2017). Contrarily, Shahzad et al. (2016) stated that a high level of Li plays an active role in the biochemistry of plants, as huge adaptations were noticed in the transcriptomes and metabolism of *Brassica carinata* A. Braun seedlings when exposed to higher levels of Li (Zonia and Tupý 1995a). Similarly, several studies have shown that plant exposure to higher Li levels reduced seed germination and fresh biomass, hindered root extension and increased leaf chlorosis with reduced pigment contents (Hawrylak-Nowak et al. 2012, Bakhat et al. 2020). In the Li-contaminated soils the induced disease symptoms in corn plants are necrotic spots on leaves, damage to root tips, and chlorosis (Kabata-Pendias and Mukherjee 2007). A higher Li concentration can also disrupt regular *Nicotiana tabacum* L. processes, such as inducing symmetrical mitosis in microspores, disturbing the pollen development and blocking its germination (Zonia and Tupý 1995b). The lethal concentrations of Li induced a hypersensitive-like response in tobacco plants (Naranjo et al. 2003). Further, higher concentrations of Li in the root and shoot resulted in severe yield reduction in lettuce (Kalinowska et al. 2013). Similarly, *Arabidopsis* plants exposure to LiCl caused noticeable curling and chlorosis, primarily affecting the older leaves (Duff et al. 2014). Lithium requirements by humans are primarily met through dietary sources such as grains and vegetables, which account for approximately 66% to over 90% of the total Li intake and are potential contributors to Li consumption. Lithium accumulator plants, such as Rosaceae and Solanaceae families, have the ability to tolerate higher levels of salts (Schrauzer 2002).

The mechanism of the Li being transported to the plants is still unclear. Due to the increasing use and disposal of Li, Li-contaminated croplands are projected to expand in coming years (Bolan et al. 2021, Afzal et al. 2023). Therefore, understanding plant responses to lithium exposure expands our knowledge of plant physiology and helps assess potential ecological risks. Moreover, data concerning the impact of Li on the physiological and biochemical attributes of carrots are scarce. Thus, the present study provides insight into the Li acquisition potential of carrot plants and the contribution of Li to various physiological and biochemical processes at different Li levels.

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MATERIAL AND METHODS

Experimental conditions and sample collection. The study was performed at the research farm of COMSATS University Islamabad (CUI), Vehari Campus, with the soil characteristics of typical hyperthermic fluventic halpocambids. Carrot was selected because (*i*) it has high consumption and greater nutritional value; (*ii*) they historically are used as an indicator of metal toxicity, and (*iii*) for experiment it is short a duration crop. A completely randomised design, having five Li levels, was tested with four replications. A total of 20 pots were prepared for growing carrots. Proper agronomic practices, e.g. watering, fertilisation, hoeing, weeding and earthling up, were followed during the complete experimental period. For the experiment, the soil was collected from random locations at the farm to achieve a uniform mixture, and the soil was sieved using a 4-mm mesh. Pots were filled with soil, each of which was 7 kg. Various physio-chemical properties of the experimental soil are listed in Table 1.

The fertilisers applied during the experiments were phosphorus (P), potassium (K) and nitrogen (N) in ratios of 0.17, 0.21, and 0.21 g/pot, respectively. A full dose of K as sulphate of potash and P as di-ammonium phosphate were applied at the time of sowing, while N as a urea was added in three split doses. Carrot seeds (cv. T29) from Ayub Agriculture Research Institute Faisalabad (AARI) were seeded in twenty pots. Carrot seeds were sown at the humps, formed by raised soil in the pots. After the establishment of seedlings, Li treatments were applied by dissolving the salts in irrigation water.

Table 1. Physio-chemical properties of soil used for the experiment

Characteristic	Value	Remark
Sand (%)	35	
Silt (%)	13	soil texture (silt loam)
Clay (%)	52	
Electric conductivity (dS/m)	1.70	normal soil (no salinity)
$pH_{H_2O\text{-extract}}$	8.03	alkaline soil
Organic carbon (%)	0.48	low
Soil available phosphorus (mg/kg soil)	2.01	low
Soil available potassium (mg/kg soil)	196.45	high
Soil soluble lithium (mg/kg soil)	0.33	–
Soil $calcium_{Mehlich\ 3}$ (mg/kg soil)	423	low

Lithium chloride monohydrate ($LiCl \cdot H_2O$) (with 99.9% purity and 60.41 g molecular weight) was used as a source of Li. Five levels of Li treatment: 0 (control), 20, 40, 60 and 80 mg/kg soil were applied. Each treatment has four replications. After 70 days of seed sowing, plants were harvested and proceeded for various physiological parameters, i.e., antioxidant enzyme activities, lipid peroxidation, H_2O_2 contents and chlorophyll contents, while growth parameters, including plant length and biomass, were determined after 140 days. After harvesting, the carrot plants were washed with deionised water in the lab to remove soil/dust particles. These plants were then separated into roots and shoots. The harvested carrot plants were air-dried and oven-dried (71 °C). The weight of the samples was recorded (Anten and Ackerly 2001).

Cations analysis. Dry ashing was used to determine the Li, K, and Ca concentrations according to the technique outlined by Parr et al. (2001). A dried sample of almost 250 mg was ground and placed into crucibles, which were then heated at 400 °C in the muffle furnace for 4 h until the plant material changed to ash, then cooled for 4–6 h at room temperature. The ash was dissolved in a 5 mL solution of 2 mol/L HCl and heated on a hot plate until completely dissolved. The samples were filtered and diluted to a final concentration of 50 mL with distilled water. The samples were kept in airtight bottles until further analysis. The flame photometer (BWB XP 5; BWB Technologies, Newbury, Berkshire, UK) was used to measure the K, Li and Ca concentrations in the samples.

Biochemical analysis. To determine the bio-physiological activities of plants, carrot young leaves were taken and instantly frozen in liquid nitrogen, then kept at 4 °C. For the pigment content analysis, 1 g of carrot root sample was obtained from the frozen sample and was ground with hydro-acetone (80% *v*/*v*). Afterwards, the samples were centrifuged for 10 min at 3 000 rpm, and absorbance was noted at 663.2, 646.8 and 470 nm, using a UV-visible spectrophotometer (Waltham, USA). The concentrations of pigment contents were calculated by following all the calculations described by Lichtenthaler (1987). Pigment contents were mentioned based on the fresh weight of the leaves.

Oxidative stress attributes. We followed the procedure described by Hodges et al. (1999) to determine lipid peroxidation. Using thiobarbituric acid-reactive substances (TBARS), lipid peroxidation was mea-

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sured. For that purpose, 1 g of liquid nitrogen-frozen material was homogenised, then incubated at 95 °C with butyl hydroxytoluene (BHT) and trichloroacetic acid (TCA) both with and without thiobarbituric acid (TBA). The supernatant was tested for absorbance at 532 nm after centrifugation (3 000 rpm, 10 min), and the MDA contents were calculated according to the equations (Hodges et al. 1999).

Hydrogen peroxide (H_2O_2) was determined by using the procedure developed by Islam et al. (2008). The leaf samples of 1 g were homogenised using a trichloroacetic acid solution (0.1%). After that, samples underwent a 20-min centrifugation at 11 000 × *g*. The reaction mixture had 1 mL of potassium iodide (2 mol/L), 1 mL of potassium phosphate buffer (10 mmol), and 1 mL of plant leaf extract and its pH was maintained at 7.0. A UV visible spectrophotometer was used to analyse this mixture at a wavelength of 390 nm, and the concentration of H_2O_2 in the sample was estimated.

Enzymatic activities. Fresh leaves of carrot plants were taken to measure the antioxidant enzymes. Plant (leaf) samples were frozen in liquid nitrogen and later used a 0.1 mol/L phosphate buffer solution (pH 7); thereafter, these samples (250 mg) were ground. The leaf extract of ground samples was obtained after centrifugation for 30 min at 4 °C and 15 000 × *g*. After centrifugation, the supernatant was carefully preserved in a refrigerator at –30 °C to assess enzyme activity. The SOD activity was estimated to correspond to a 50% decrease in nitro blue tetrazolium (Dhindsa et al. 1981). The catalase activity (CAT) was assayed according to Aebi et al. (1984) and described as μmol of H_2O_2 stained per min per mg protein. The method of Hemeda and Klein (1990) was followed to estimate the activity of peroxidase (POD) that was presented as μmol guaiacol oxidised per min per mg protein. The method of Nakano and Asada (1981) was used to estimate the activity of ascorbate peroxidase (APX) that was presented as μmol of H_2O_2 degraded per min per mg protein at 290 nm spectrophotometer (PerkinElmer- LAMBDA 25 UV/Vis Spectrophotometers, Waltham, USA).

Statistical analysis. A completely randomised design (CRD) was used to analyse the experimental data. Using the SAS (2004) (SAS/STAT 9.1, Cary, USA) software, experimental data were subjected to variance analysis. Using the least significant difference test, multiple comparisons were made. Data was considered statistically significant for all analyses when the *P*-value was less than 5% ($P < 0.05$).

RESULTS

Lithium accumulation in carrot root and shoot. The effect of varying soil lithium concentrations on Li accumulation in carrot plants is illustrated in Figure 1. As Li levels in the soil increased, the Li concentration in both roots and shoots rose significantly. In carrot shoots, Li concentration increased steadily with higher soil Li levels, reaching a maximum of 440 mg/kg dry weight (DW) at a Li concentration of 80 mg/kg soil. The lowest Li concentration in shoots (60 mg/kg DW) was recorded in the control treatment (0 mg Li/kg soil). Similarly, in carrot roots, the Li concentration increased significantly ($P < 0.05$) with soil Li levels, ranging from 16.20 mg/kg DW in the control to 50.01 mg/kg DW at a Li concentration of 80 mg/kg soil. These results showed that soil Li level is related to Li accumulation in both plant organs, with higher soil Li concentrations leading to substantial increases in root and shoot Li content.

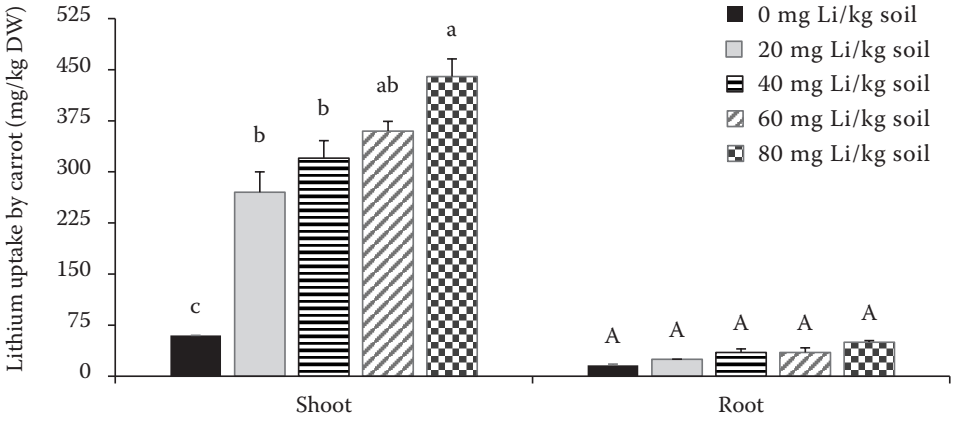

Figure 1. Effect of lithium (Li) application on carrot plants' root and shoot lithium concentration. Carrot plants were given Li treatment for 140 days. The values are the means of the four replicates ± standard error. DW – dry weight

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Effect of lithium on carrot growth. The impact of Li on carrot growth parameters is presented in Table 2. The control treatment exhibited the highest shoot fresh biomass ($P < 0.01$). Low Li levels (20 and 40 mg/kg soil) did not significantly reduce shoot biomass compared to the control. However, higher Li concentrations (60 and 80 mg/kg soil) led to substantial declines in fresh biomass accumulation, with 49% and 83% reductions, respectively (Table 2). These findings indicate that while low levels of Li are not detrimental, high concentrations severely restrict shoot biomass production.

Similar patterns were observed for shoot-dry biomass. The highest shoot dry weight (9.64 g/pot) was recorded in the control treatment, while the lowest (1.25 g/pot) was observed at 80 mg Li/kg soil, representing an 87% reduction (Table 2). This underscores the strong inhibitory effect of elevated Li levels on shoot dry matter accumulation.

At lower Li concentrations (20 mg/kg soil), Li application did not positively affect root biomass compared to the control. However, as Li levels in the soil rose, root biomass decreased significantly. At 60 and 80 mg/kg soil, reductions of 30% and 89%, respectively, were observed, highlighting the negative impact of higher Li levels. Root dry biomass followed a similar trend, with 14.41, 62.11, and 89.0% reductions in root biomass occurring at Li concentrations of 40, 60, and 80 mg/kg soil, respectively. These results reveal an inverse relation between Li concentration in the soil and root biomass. Li had a concentration-dependent effect on root length. At lower Li levels (20 and 40 mg/kg soil), root length increased by 29.37% and 40.72%, respectively, compared to the control. However, higher Li concentrations (60 and 80 mg/kg soil) significantly reduced root length by 6.5% and 49.35%, respectively. The maximum root length (18.59 cm) was observed at 40 mg/kg soil, while the shortest (6.69 cm) was recorded at 80 mg/kg soil (Table 2).

Effect of lithium on potassium and calcium concentrations in carrot plant. The impact of Li on nutrient uptake is summarised in Table 3. Li application did not significantly affect K concentration in carrot shoots. However, in roots, K levels decreased significantly ($P < 0.05$) with increasing soil Li concentrations. Reductions in root potassium content were 26.15% and 34.12% at 60 and 80 mg/kg Li concentrations in the soil, respectively, compared to the control (Table 3). These results indicate that Li interferes with root K uptake at higher soil concentrations.

Li significantly ($P < 0.05$) reduced Ca concentration in carrot shoots. The higher Li levels (60 and 80 mg/kg soil) significantly reduced Ca content in shoot tissues. At 60 and 80 mg/kg of Li in the soil, the respective Ca content in carrot shoot tissues was decreased by 14.57% and 25.64% compared to the control (Table 3). However, Li application did not significantly affect Ca concentrations in carrot roots. These findings suggest that Li selectively disrupts nutrient uptake, with more pronounced effects on shoot Ca levels.

Effect of lithium-ion on lipid peroxidation and H_2O_2 production. The impact of Li concentration on lipid peroxidation and the content of malondialdehyde (MDA), which is an indicator of lipid oxidation in carrot plant roots, is presented in Figure 2A.

Soil Li application showed no significant changes in MDA content in plant leaves, as depicted in Figure 2A.

The root of the carrot plant did not show a significant ($P \geq 0.05$) change in H_2O_2 production under Li application (Figure 2B). H_2O_2 production in carrot roots did not vary significantly across treatments. At a Li concentration of 60 mg/kg soil, a significant reduction in H_2O_2 production was noted compared to other treatments, suggesting the activation of detoxification mechanisms at higher Li levels (Figure 2B).

Effect of lithium on plant pigment concentrations. Figure 3 presents the effects of Li on pigment contents. There was no significant increase or reduc-

Table 2. Effect of various lithium (Li) concentrations on carrot biomass accumulation and root length

Li treatment (mg/kg soil)	Shoot fresh	Shoot dry	Root fresh	Root dry	Root length (cm)
	biomass (g/pot)				
0	37.98 ± 1.73^{a}	9.64 ± 1.10^{a}	63.48 ± 7.19^{ab}	9.16 ± 0.98^{a}	13.21 ± 0.93^{b}
20	37.47 ± 1.37^{a}	7.57 ± 1.10^{ab}	76.05 ± 2.58^{a}	10.63 ± 1.82^{a}	17.09 ± 0.86^{a}
40	37.03 ± 0.95^{a}	6.77 ± 0.42^{b}	58.95 ± 3.78^{ab}	7.84 ± 0.76^{ab}	18.59 ± 0.77^{a}
60	18.78 ± 2.59^{b}	3.98 ± 0.41^{c}	19.38 ± 1.98^{c}	3.47 ± 0.85^{b}	12.35 ± 0.98^{b}
80	6.65 ± 0.81^{c}	1.29 ± 0.29^{d}	6.89 ± 0.55^{d}	1.00 ± 0.19^{b}	6.69 ± 0.43^{c}

Carrot plants were given Li treatment for 140 days. The values are the means of four replicates ± standard error

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Table 3. Effect of various lithium (Li) concentrations on potassium and calcium concentrations in carrot root and shoot tissues

Li treatment (mg/kg soil)	Shoot		Root	
	potassium	calcium	potassium	calcium
	(mg/g DW)			
0	17.01 ± 3.04^{a}	7.41 ± 0.19^{a}	12.16 ± 1.48^{a}	0.86 ± 0.25^{a}
20	18.19 ± 2.17^{a}	6.91 ± 0.36^{ab}	10.41 ± 0.53^{a}	1.00 ± 0.05^{a}
40	15.77 ± 1.17^{a}	6.89 ± 0.32^{ab}	9.90 ± 0.49^{a}	1.05 ± 0.02^{a}
60	15.39 ± 2.58^{a}	6.33 ± 0.53^{bc}	8.98 ± 3.25^{b}	1.01 ± 0.06^{a}
80	15.01 ± 0.78^{a}	5.51 ± 0.14^{c}	8.01 ± 1.05^{b}	0.93 ± 0.12^{a}

Carrot plants were given Li treatment for 140 days. The values are the means of the four replicates ± standard error. DW – dry weight

tion in chlorophyll *a* content. However, chlorophyll *b* showed some changes in response to Li in soil. A minimum chl-*b* was observed at a Li concentration of 20 mg/kg soil compared to all other treatments. Carotenoid concentrations remained unaffected by Li application, showing no significant changes across treatments.

Effect of lithium-ion on antioxidative enzyme activity. The activity of antioxidative enzymes in response to Li application is presented in Table 4. The superoxide dismutase (SOD) activity increased gradually with higher Li levels. The maximum activity (32% increase) was observed at 80 mg/kg soil, while moderate increases (17%) were noted at a Li concentration of 60 mg/kg soil. Lower Li concentrations (20 and 40 mg/kg soil) showed no significant effect on SOD activity (Table 4).

The activity of catalase (CAT) was significantly ($P < 0.05$) changed with varying Li doses in the soil. Compared to the control, increased CAT activity ($P < 0.05$) of 24% was observed at 20 mg/kg Li concentration in the soil. However, the Li application did not significantly affect the ascorbate peroxidase (APX) and POD activity.

DISCUSSION

Earlier studies demonstrated that Li significantly decreases plant growth due to the toxic nature of Li-ions, which can trigger the development of necrotic regions (Naranjo et al. 2003). The findings of this study indicated that fresh and dry biomass of shoots and roots of carrot plants were severely affected by higher levels of Li. Carrot shoot and root biomass were significantly reduced by increasing the Li levels in the soil. In context to the negative effects of Li in plants, Magalhães and Wilcox (1990) concluded that Li stress decreases tissues water retention leading to reduced fresh and dry biomass of roots and leaves of radish (Vlasyuk et al. 1979, Tanveer and Wang 2020).

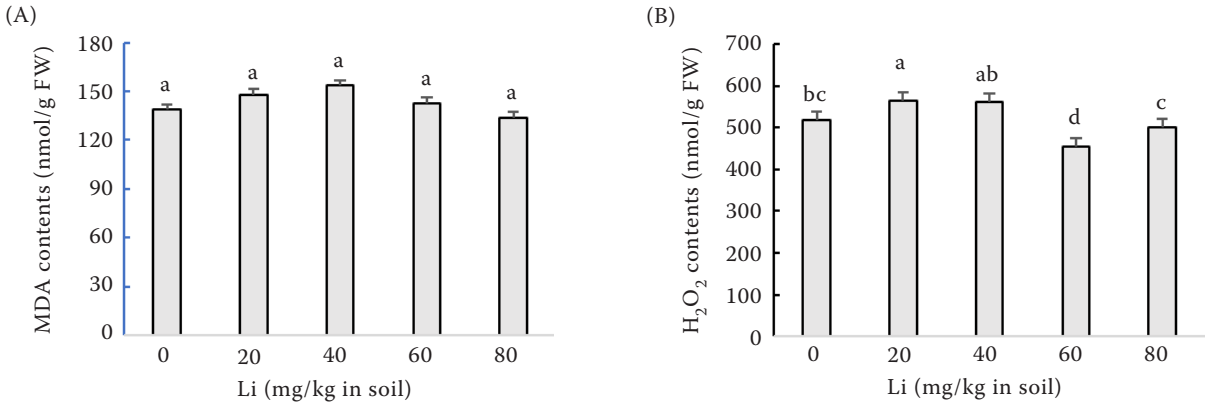

Figure 2. Effect of lithium (Li) application on (A) malondialdehyde (MDA) and (B) hydrogen peroxide (H_2O_2) contents in carrot. The values are the mean of the four replicates. Error bars indicate means ± standard error. FW – fresh weight

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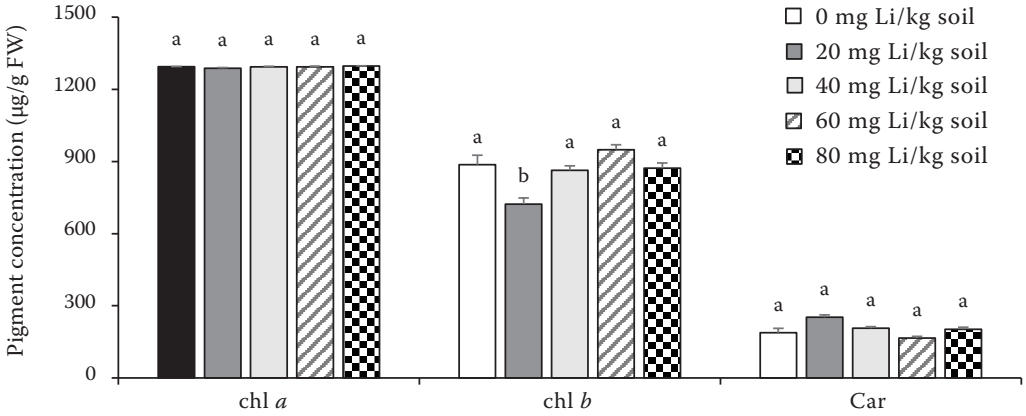

Figure 3. Effect of lithium (Li) application on pigment contents on carrot. The values are the mean of the four replicates. Error bars indicate ± standard error. Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; Car – carotenoids; FW – fresh weight

Similarly, Duff et al. (2014) and Kent (1941) also concluded that Li stress reduces the water contents and dry biomass in *Arabidopsis* and wheat. However, Vlasyuk et al. (1979) proposed that low Li concentrations increased water retention in carrot plants. They suggested that lower concentrations of Li increased water uptake, and higher doses of Li exerted osmotic effects. It has been reported in a study that Li compounds (LiCl, LiOH or $LiCO_3$) did not differ considerably in their phytotoxicity to lettuce, and Li concentration of 50 mg/L causes a major decline in the fresh weight of plants. For maize and sunflower plants, the same Li concentration in the nutrient solution was toxic (Hawrylak-Nowak et al. 2012). The radish root cortical cells have increased with plant exposure to Li (Hassan 1954). The decrease in water retention ability hinders plant growth during changing soil conditions, as water is critical to maintaining cell shape and cell structure. However, in our experiment, we observed that Li-induced changes were not related to changes in water contents (data not shown).

In our experimental condition, potassium (K) concentration in the leaves and roots of carrots was decreased with an increase in Li concentration in the soil. In various studies, the authors have concluded that Li can replace the Na and K ions due to competition between the ions for uptake (Codina et al. 1983). In spinach, the application of Li in soil has been observed to disrupt cation homeostasis. Specifically, as the doses of Li in the soil increased, there was a corresponding decrease in the K concentrations in spinach (Bakhat et al. 2020). Calcium concentration in leaves was decreased with soil Li application. The possible reason behind this Ca reduction in plants with higher Li levels may be competition between Li and Ca uptake. Further, calcium is an immobile element, so its concentration in the shoot was significantly affected by Li in the soil. Similar results were found by Magalhães and Wilcox (1990) and Bakhat et al. (2020), who showed that Li decreases calcium concentration in radish and spinach plants. It has been proposed that Li in soil solution reduces calcium uptake and calcium translocation from root to shoot (Epstein 1960). Lithium can also change the various Ca-dependent signalling pathways, as proposed by

Table 4. Effect of various lithium (Li) concentrations on catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD) and superoxide dismutase (SOD) activity (U mg/protein) in carrot

Li treatment (mg/kg soil)	CAT	APX	POD	SOD
	(U/mg protein)			
0	13.71 ± 1.16^{ab}	7.72 ± 1.90^{a}	26.19 ± 1.08^{a}	44.12 ± 1.18^{c}
20	17.08 ± 0.97^{a}	8.14 ± 2.48^{a}	30.45 ± 1.52^{a}	46.55 ± 1.48^{bc}
40	12.55 ± 0.86^{b}	8.65 ± 1.25^{a}	27.60 ± 1.37^{a}	48.19 ± 3.98^{bc}
60	14.94 ± 0.59^{ab}	9.85 ± 3.07^{a}	27.60 ± 2.70^{a}	54.75 ± 0.75^{ab}
80	16.18 ± 0.42^{ab}	8.95 ± 1.05^{a}	33.22 ± 2.85^{a}	61.78 ± 3.05^{a}

The values are the mean of the four replicates ± standard error

https://doi.org/10.17221/19/2025-PSE

Epstein (1960) and Stevenson et al. (2000) and may change the physiological functioning of the plants.

Our study showed that, in general, Li treatments have no significant effect on the pigment contents. These results are consistent with the previous studies conducted on spinach and quinoa (Bakhat et al. 2020, Afzal et al. 2023). However, some other studies have also observed decreased pigment content in maize (at a Li concentration of 20 mg/kg soil) (Antonkiewicz et al. 2017). It is widely acknowledged that cellular stress leads to the overproduction of reactive oxygen species. At lower Li concentrations of 20 and 40 mg/kg, overproduction of H_2O_2 was observed as compared to the control. However, no significant effect on lipid peroxidation was observed, likely due to the activation of the plant's non-enzymatic antioxidant defence mechanisms (Shahzad et al. 2016, 2017) as previously reported in spinach, maize and sunflower. In contrast, H_2O_2-induced lipid peroxidation was observed in the roots of maize and the leaves of sunflower plants (Mulkey 2005) following increased exposure to Li, particularly at a concentration of 50 mg/kg (Hawrylak-Nowak et al. 2012). The activation of antioxidant enzymes in plants to fight against stress is a key mechanism of plant self-defence (Shahid et al. 2014, Pinto et al. 2016). Lithium application triggers the antioxidant response in plants, and it has resulted in changes in the activity of SOD and CAT activities. That supports the prominent role of Li in carrots as a nutrient. However, further information is still needed to understand how the alteration of antioxidants under Li stress helps the plant maintain its physiological functioning without symptomatic changes in plants except biomass reduction.

Overall, carrot plants respond to soil-applied lithium in a manner similar to other alkali metals, with the element's accumulation in plant shoots influenced by its availability and mobility in the soil-plant system. Li did not significantly influence carrot growth or physiological parameters at lower soil concentrations, indicating minimal immediate risks to plant health. However, higher soil Li concentrations (≥ 60 mg/kg soil) severely reduced plant growth, as evidenced by decreased shoot and root biomass, root length, and altered nutrient dynamics. Specifically, our results revealed that high Li levels disrupted the uptake and translocation of essential nutrients like potassium and calcium, compromising overall plant health.

Furthermore, Li exposure induced oxidative stress, as seen in elevated levels of hydrogen peroxide and lipid peroxidation. The increased activity of antioxidative enzymes, including superoxide dismutase and catalase, suggests that plants activated detoxification mechanisms in response to Li stress. At the same time, lower Li levels had negligible effects on chlorophyll and carotenoid content; higher concentrations altered chlorophyll *b* levels, further indicating physiological stress. Hence, this study provides insights into the dual effects of lithium on plant growth, emphasising both its stimulatory effects at low concentrations and its toxic effects at higher levels due to oxidative stress. These findings contribute to a better understanding of lithium's environmental impact and its implications for ecosystem sustainability. Given the potential risks associated with Li accumulation in the soil-plant-human continuum, future research should focus on identifying early bioindicators for Li stress, optimising soil management strategies to mitigate Li toxicity, and assessing its broader environmental and health implications.

Acknowledgement. This research was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia, under Grant No. GPIP: 1959-188-2024. The authors, therefore, acknowledge the DSR for technical and financial support.

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Received: January 14, 2025
Accepted: March 13, 2025
Published online: April 25, 2025