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Determination of Morphological and Physiological Changes of Ornamental Cabbage (*Brassica oleracea* var. *acephala*) Against Boron Toxicity in Phytoremediation

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Abstract

Boron toxicity in agricultural lands limits plant breeding as a plant nutrition problem. Some plants are able to tolerate high levels of heavy metals at potentially toxic doses, accumulate them in their bodies and remove them from the soil. In this study, it was aimed to determine the morphological and physiological responses of ornamental cabbage in phytoremediation against boron toxicity. This study was conducted under controlled greenhouse conditions, 4 different boron doses (0, 10, 25, and 50 mg kg^{-1} B) were applied to 2 different soil structures (acidic (S1) and alkaline (S2) soils). Toxicity symptoms were appeared at 50 mg kg^{-1} B. At alkaline soil, plant weights and visual properties of plants were found to be low in quality. Results show that as the boron dose increased, shoot-root fresh and dry weight, stomatal conductance, chlorophyll content, relative water content (RWC) were decreased. Boron accumulation in the shoot, root and whole plant was at 50 mg kg^{-1} B. As a result, it is understood that ornamental cabbage used for decontamination of boron element by phytoremediation method for the first time in this study. We suggest that it has a potential to as hyper-accumulator plant for the remediation of boron-contaminated soil.

1. Introduction

Environmental pollution by heavy metals is severely threatened human and animal health and agricultural production. Heavy metal pollution in the soil has risen as a result of the increase in industrial production, mining activities and pesticide and fertilization activities used in agriculture in recent years.

Boron, which may occur commonly in the soil and/or in groundwater, is the source of high boron concentration in the soil. The amount of boron in the soil may also increase due to fertilization, irrigation and mining site (Nable et al., 1997). Boron toxicity is common in agricultural lands where there are thermal power plants using lignite coal or compost fertilizers are used. In addition, B levels that may be toxic to plants can be found in salty and sodic soils (Gence, 2015).

Heavy metal contaminated soils can be remediated different remediation technologies such as soil replacement, soil isolation, vitrification, encapsulation and bioabsorption (Alaboudi et al., 2018). Phytoremediation techniques provide cleaning and stabilization of contaminated soils and groundwater through hyperaccumulator plants and trees (Paz-Alberto and Sigua, 2013). Hyperaccumulator plants are defined as plant species that can accumulate heavy metals (Ali et al., 2013; Khalid et al., 2017). The most well-known heavy metal collector hyperaccumulator species are belongs to Brassicaceae (Kumar et al., 1995; Gall and Rajakaruna, 2013). The important of *Brassica* species in phytoremediation is derived from extensive above ground biomass production and rapid growth (Mourato et al., 2015).

Boron is fundamental micronutrients for plants. Boron takes the form of boric acid by plants, mostly through range between B levels that cause toxicity and deficiency in plants (Chapman et al., 1997; Goldberg, 1997; Yau and Ryan, 2008; Brdar-Jokanovic, 2020). Soil boron concentrations are proposed to scale 0.0–0.2 ppm as low, 0.21–0.60 ppm medium, 0.61–1.10 ppm high, 1.2–3.0 ppm very high, and >3.0 ppm as toxic (de Abreu et al., 2005; Brdar-Jokanovic, 2020). The most common boron toxicity visible symptom in plants is the existence of burns, which develop as chlorotic and/or necrotic spots, often found on the edges and tips of the mature leaves (Nable et al., 1997; Ramila et al., 2015; Garcia Sanchez et al., 2020). With boron toxicity, root growth is also often inhibited and roots are shortened (Schnurbusch et al., 2010).

Some edible plants under the *Brassica* genus are noted accumulate comparatively large amounts of toxic heavy metals (Mourato et al., 2015.) The aim of study is to evaluate the potential of ornamental cabbage to be hyperaccumulator plant to remediate boron contaminated soils and to be alternative for edible plants.

2. Material and Methods

2.1. Plant materials and treatments

This study was conducted in the greenhouse with automatic temperature and relative humidity control (at 13-17ºC temperature and 40-45% relative humidity) and laboratories of the Department of Horticulture in Ankara University and Soil, Fertilizer and Water Resources Central Research Institute in Ankara, Turkey. Two different soil structure (S1: acidic and S2: alkaline soils) and four different boron doses (0, 10, 25, and 50 mg kg^{-1} B) was used in this experiment. The pH degrees of studied soils were 7.49 and 5.87, respectively. Table 1 shows chemical and physical properties of studied soils. Ornamental cabbage seedlings with 3-4 true leaves were planted in pots 15×17×22 cm (a plant per pot) in October 18, 2017. The pots were irrigated at the level of field capacity with tap water. Boron was applied as boric acid (H_3BO_3, 17.5% B) with irrigation water (25 October). Plants were grown for eight weeks. In order to determine the changes in plants according to soil type and boron concentrations, every week three plants were harvested for observations and measurements. Experiments were carried out randomized plots with a factorial design with 3 replications for soil type and boron dose.

2.2. Determination of plant growth characteristics

2.2.1. Plant shoot and root fresh and dry weight measurements

The parts of the plants that are divided into two as root and shoot parts of each plant are weighed on a precision scale in grams on a weekly basis. After their fresh weights measured, the samples were dried in the oven set at 65ºC until they reach constant weight then their dry weight was measured.

2.3. Measurements of physiological properties

2.3.1. Chlorophyll content

Before harvest, the chlorophyll amounts of the plants were measured each week by using Minolta Chlorophyll Meter (SPAD-502). Chlorophyll measurements (SPAD values) were taken as three readings on the 5 leaves of each plant, based on the central part of the leaf.

2.3.2. Stomatal conductance (g_s)

Decagon SC-1 model porometer was used to determine stomatal conductance. It was determined by making measurements on the same leaf randomly determined each week before harvest between 13.00-14.00 p.m.

2.3.3. Relative water content (RWC)

Leaf samples taken before harvest were immediately weighed and their fresh weights (FW) were measured, the samples were kept in pure water for 4 hours and then their turgor weight (TW) was measured. Finally, the leaf samples were dried in an air circulation drying cabinet at 65°C for 24 hours and their dry weight (DW) was measures

Table 1. Chemical and physical properties of studied soils

Proporties	Acidic soil (S1)	Alkaline soil (S2)
Texture class	Clay (C)	Clay Loam (CL)
EC (dS m^{-1})	0.71	0.92
pH	5.87	7.49
$CaCO_3$ (%)	0.90	37.60
Available P (P_2O_5) (kg ha^{-1})	41.0	110.00
Available K (K_2O) (kg ha^{-1})	340.00	780.00
Organic Matter (%)	1.80	1.86
Available Fe (mg kg^{-1})	16.24	15.44
Available Cu (mg kg^{-1})	1.81	2.59
Available Zn (mg kg^{-1})	3.35	2.42
Available Mn (mg kg^{-1})	4.89	16.68
Available B (mg kg^{-1})	0.00	0.00

(Dhanda and Sethi, 1998). The relative water content of the leaves was calculated with the help of the equation below:

RWC (%) = [(FW-DW) / (TW-DW)] (×) 100

2.3.4. Boron analysis in plant

The shoots and roots of the plants to be sampled were washed first with tap water and then with pure water, then placed in a paper bag and dried until they reached a constant weight at 65ºC. Plant shoots and roots were ground finely to pass through a 200 µm sieve for analysis. 0.25 g of shoots and root samples was first digested with nitric acid (HNO_3) in a microwave device, then these samples were transferred to a 50 mL Erlenmeyer flasks and completed with deionized water and filtered through the blue tape filter paper. Total Boron in the plant solution obtained by method of wet decomposition was determined in Shimadzu UV-160 Spectrophotometer according to the vanadomolybdophosphoric yellow color method (Kacar and İnal, 2008). The boron content of the sieves obtained according to the method of wet decomposition was determined in Varian 720-ES ICP-OES (Kacar and İnal, 2008).

2.3.5. Statistical analysis

All data was statistically analysed using the MSTAT-C. The significant differences were compared with LSD test at P< 0.05.

3. Results and Discussion

3.1. The effect of B on plant shoot-root fresh and dry weights

Plant species that form large biomass, grow rapidly, form a wide root system and can be cultivated and harvested easily are considered to be an ideal hyper-accumulator plant species (Paz-Alberto and Sigua, 2013). The biomass of the plant selected for phytoremediation is one of the factors to be considered during the removal of toxic heavy metals (Alaboudia et al., 2018).

Effects of soil type on the fresh and dry weights of shoots and roots under four different B doses are shown in Table 2. The effect of "soil type and B dose" treatments interaction was found statistically significant (P<0.05) in terms of shoot fresh and dry weights, while it was not significant for root fresh and dry weights (P>0.05). The highest shoot fresh weight in plant under boron stress was obtained 'S2×25 mg kg^{-1}B' (85.08±4.30 g $plant^{-1}$) in S2 soil, while the lowest shoot fresh weight was 'S1×50 mg kg^{-1} B' (47.00±5.00 g $plant^{-1}$) in S1 soil. The highest shoot dry weight was determined in the combination of 'S2×10 mg kg^{-1} B' (17.44±1.31 g $plant^{-1}$). The lowest shoot dry weight was found to be at a dose of 50 mg kg^{-1} B' in S1 soil, showing similarity with the lowest shoot fresh weight ('S1×50 mg kg^{-1} B', 8.30±1.13 g $plant^{-1}$) (Table 3).

The effect of B doses on both shoot fresh weight and shoot dry weight showed itself as of the 5th week and its effect increased as the stress period increased. Similar findings were obtained in both root fresh weight and root dry weight (Figure 1). It was also determined that the plants grown in S2 soil had lower root fresh and dry weight than the plants grown in S1 soil. In agreement with conducted study by Eraslan et al. (2007) toxicity symptoms were seen on the tips and edges of the old leaves of the plants in this study. It has been reported that the most severe boron toxicity symptoms occurred 50 mg kg^{-1} B in tomato and pepper plants (Eraslan

Table 2. ANOVA for shoot - root fresh and dry weights, stomatal conductance, chlorophyll content, relative water content

Source of variation	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Stomatal conductance	Chlorophyll (SPAD value)	Relative water content
Boron dose (BD)	**	**	**	**	**	**	**
Soil type (ST)	**	**	NS	NS	**	**	NS
BD x ST	*	*	NS	NS	**	NS	*
CV (%)	5.37	12.82	8.55	19.51	5.99	10.09	5.33

CV: Coefficient of variation; **: P<0.01 is significant at probability level; *: P<0.05 is significant at probability level; NS: Not significant

Table 3. The effect of 'Soil type × B dose' interaction on the fresh and dry weights of shoot, root fresh and dry weights, stomatal conductance, chlorophyll and relative water content

Soil type	Boron dose (mg kg^{-1})	Shoot fresh weight (g $plant^{-1}$)	Shoot dry weight (g $plant^{-1}$)	Root fresh weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	Stomatal conductance (mmol $m^{-2}s^{-1}$)	Chlorophyll (SPAD value)	Relative water content (%)
S1	Control	77.17±5.01 c*	17.15±3.07 a	27.58±2.50	8.38±1.43	113.18±11.51 b	25.00±2.95	75.33± 3.30 a
	10	59.33±1.53 d	11.55±0.88 bc	25.62±1.01	4.81±1.16	107.22±5.33 bc	17.34±1.90	69.57±1.51 ac
	25	60.67±1.53 d	10.10±1.01 cd	19.17±2.02	4.76±1.00	94.02±2.00 de	15.10±3.00	72.66±5.51 ab
	50	47.00±5.00 e	8.30±1.13 d	13.00±1.00	2.99±0.92	84.00±8.00 e	15.07±0.90	60.08±4.00 e
S2	Control	91.00±6.00 a	17.61±1.86 a	26.30±2.46	7.66±0.86	203.23±7.01 a	30.55±2.06	75.48±1.39 a
	10	81.17±1.52 bc	17.44±1.31 a	24.33±1.53	5.21±1.00	119.08±4.00 b	24.37±1.52	67.01±4.00 bd
	25	85.08± 4.30 ab	14.20±2.03 b	20.33±2.52	4.25±0.67	107.24±8.60 bc	23.18±3.02	63.20±5.01 de
	50	59.00±2.00 d	9.01±1.00 cd	16.51±0.50	3.16±0.81	98.19±4.01 cd	24.04±1.00	65.18±2.12 ce
LSD (%5)		6.51	2.93	-	-	11.99	-	6.33

*: Means with the same letter within column are significantly different (P<0.05). S1: Acidic soil, S2: Alkaline soil

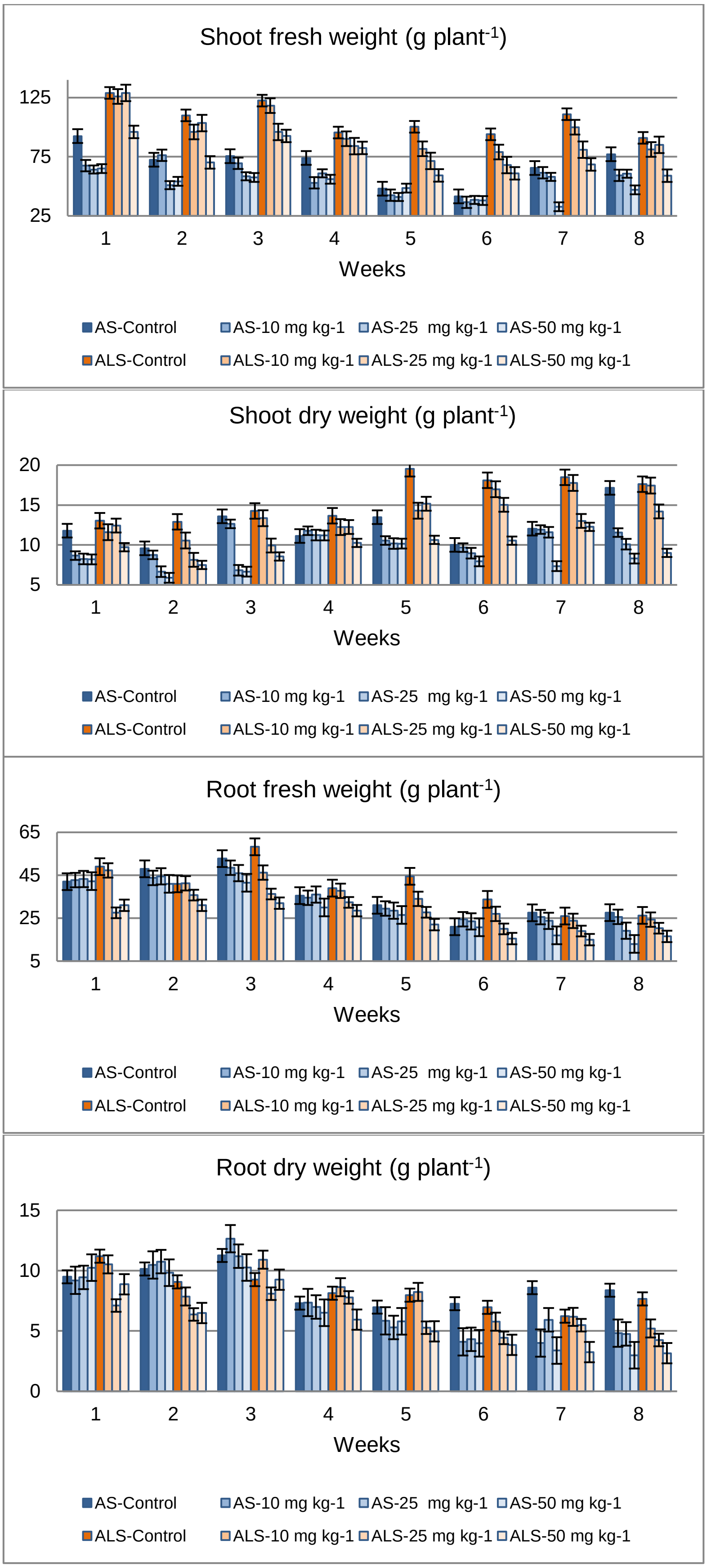
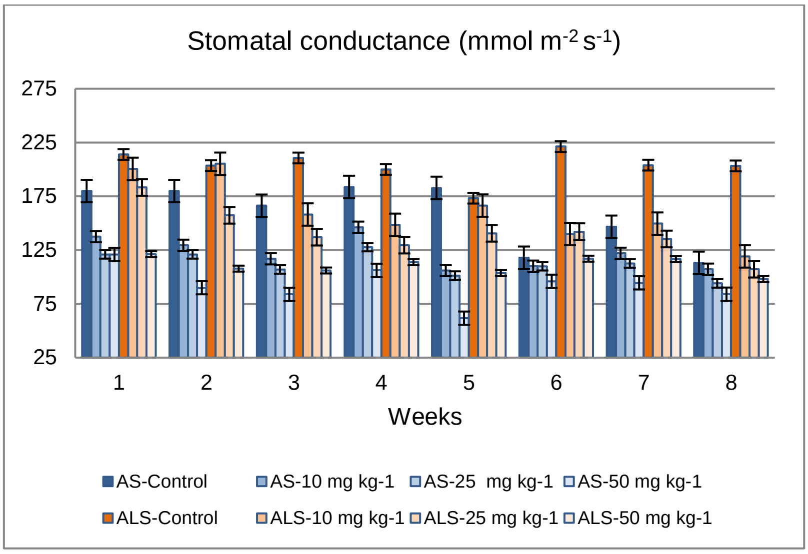
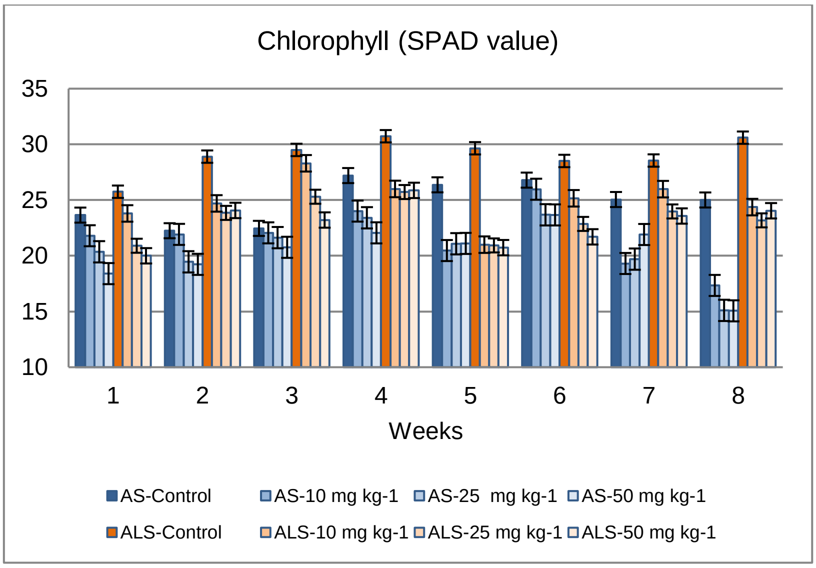


Figure 1. Weekly change of the effect of B applications on shoot-root fresh and dry weight, stomatal conductance, chlorophyll and RWC of ornamental cabbage in two different soil types. (AS: Acidic soil, ALS: Alkaline soil. P<0.05 is significant at probability level).





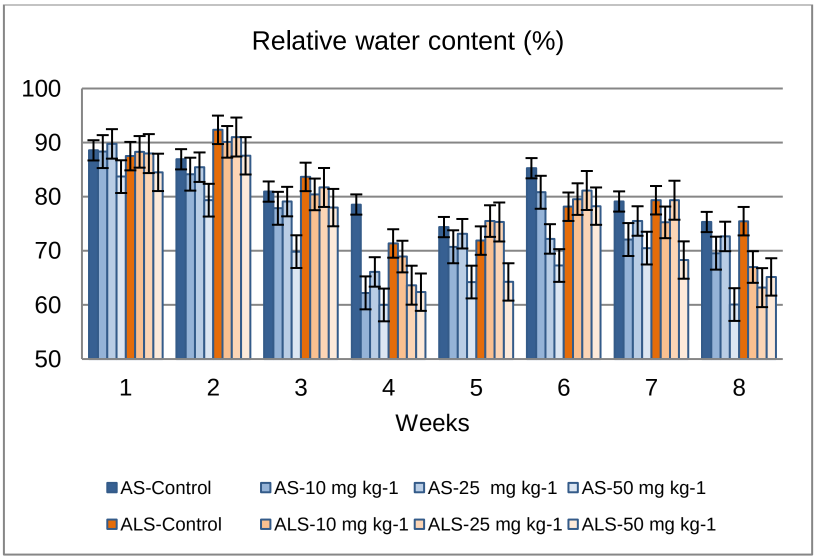


Figure 1 (continued). Weekly change of the effect of B applications on shoot-root fresh and dry weight, stomatal conductance, chlorophyll and RWC of ornamental cabbage in two different soil types. (AS: Acidic soil, ALS: Alkaline soil. P<0.05 is significant at probability level).

et al., 2007). The occurrence of boron toxicity symptoms at 25 mg kg^{-1} B doses in our study is consistent with the study conducted by Akıncı (2006).

In this study, shoot-root fresh and dry weight showed an alteration according to boron dose and soil type during boron stress and decreased compared to control plants. A similar result has been reported in corn (Güneş et al., 2000a), tomato (Güneş et al., 2000b), potato (Ayvaz, 2009), purslane plants (Samet and Çakılı, 2016; 2019) for shoot fresh weight, in pepper (Akıncı, 2006; Eraslan et al., 2007), corn (Palta and Gezgin, 2011), canola (Koohkan and Maftoun, 2016; purslane plants (Samet and Çakılı, 2016; 2019) and buckwheat plant (Yazıcı and Korkmaz, 2020) for shoot dry weight.

Since boron regulates lignin biosynthesis through the formation of stable phenolic acid borate compounds, it has been reported that it limits excess B lignin biosynthesis (Marschner, 1995). Boron toxicity also causes negative physiological effects such as decreased cell division, shoot and root growth (Nable et al., 1997; Liu and Yang, 2000; Reid et al., 2004).

It is reported in different studies in the literature that high levels of boron applications lead to a decrease in root weight. Ayvaz (2009) reported that there was a decrease in root fresh weight in parallel with increasing B doses on potato plant. Similar findings were also obtained in the root fresh and dry weights of barley varieties and barley grass (Keskin, 2010), *Vicia sativa* plant (Karaömerlioğlu, 2011), green bean genotypes (Akoğlu, 2013). Due to the application of B, toxic effects were observed on the leaves. This situation can be explained by the fact that the B element is carried upward due to transpiration and accumulates in the leaves and the boron accumulated in the leaves shows toxic symptoms at the leaf tips (Kacar and Katkat, 2007).

3.2. The effects of B on stomatal conductance, chlorophyll, and relative water content (RWC)

In our study, the interaction of 'soil type x B dose' was found to be important in terms of stomatal conductance and relative water content ($P<0.05$), while the interaction of 'soil type × B dose' was not statistically significant ($P>0.05$) in terms of chlorophyll content. The highest stomatal conductance was measured in leaves of plants grown in S2 soil. The combination of 'S2 × 10 mg kg^{-1} B' has been determined to have both the highest stomatal conductivity (119.08± 4.00 mmol m^{-2} s^{-1}). Especially, when the stomatal conductance of plants grown in S1 soil and S2 soil were compared, it was determined that plants growing in S1 soil had lower stomatal conductance than S2 soil. The RWC of the plants applied with B stress decreased compared to control in both soil types. The highest RWC was determined as 'S1×25 mg kg^{-1} B' (72.66% ± 5.51) (Table 3).

It was determined that B doses decreased stomatal conductivities in both soil types compared to the control starting from the first week. Especially as the dose B increased (25 and 50 mg kg^{-1} B) stomatal conductance decreased compared to the control. The decreases in chlorophyll values differed depending on the duration, severity and soil type of the stress. In the early weeks, losses in chlorophyll values were observed in both soil types with the increase in dose. These losses started to become evident in the following weeks and continued towards the last weeks. It was determined that the relative humidity contents of the plants were close to the control in the early weeks of the stress, and they showed a significant decrease in S1 soil compared to the control in parallel with the increase in the B dose at the 4th, 5th and 6th weeks. In terms of relative moisture content, it was determined that ornamental cabbage plants under boron stress preserved their turgority better in S2 soil than S1 soil, and this was noticeable at 25 mg kg^{-1} B level at 5th, 6th and 7th weeks. Towards the last weeks, the decrease in relative moisture content in plants, especially when 50 mg kg^{-1} dose B was applied, continued (Figure 1).

Stomatal conductance is determined by measuring the carbon dioxide entering or water vapour exiting from the stomata of the leaves per unit time (Erdal, 2016). Closure of the stomata occurs as a result of the direct interaction of toxic metals with guard cells and the initial effects of metal toxicity on the root and stem. Root-derived ABA or ABA-derived signals in metal-stressed plants may play a role in stomatal movement. As a result of the exposure of plants to toxic metal concentrations such as Cd, Co, Ni, Pb and Zn, it has been reported in many studies that stomatal conductivity decreases or causes stomatal resistance to increase (Rucinska-Sobkowiak, 2016). In a study on the response of Clementine mandarins inoculated on two different rootstocks to B toxicity, it was determined that B application reduces stomatal conductance (Papadakis et al., 2004a). In a study investigating the effect of boron toxicity on stomata behaviour in grapevine plants, it was found that stomata resistance was increased in the leaves of plants treated with excess B at 20 and 30 mg kg^{-1} B levels (Güneş et al., 2006). Simon et al. (2013) reported that stomatal conductivity decreases with increasing B concentrations in *Jatropha curcas* plant. When the studies conducted were examined, the results obtained that increasing B doses decrease stomatal conductance were found to be consistent with our findings that stomatal conductivity decreased with the prolongation of the stress period with increasing B doses. It was reported that B application reduced the chlorophyll content in Clementine mandarins (Papadakis et al., 2004a), mung bean (Hasnain et al., 2011), *Brassica juncea* (Varshney et al., 2015), canola (Koohkan and Maftoun, 2016), purslane plants (Samet and Çakılı, 2016).

Boron toxicity, which is one of the abiotic stress factors for plants, causes various morphological and physiological changes in plants. Under these conditions, it has been reported that the RWC of the leaves in the plants and decreases in the rate of photosynthesis occurs with the decrease of the leaf water potential (Lawlor, 2002; Akoğlu, 2013). Chlorosis formation and decreases in relative water content are among the known signs of boron toxicity (Ramila et al., 2016). In a study conducted by Keskin (2010), it was stated that the relative moisture content in the leaves of Tokak and Hamidiye variety wheat decreased with increasing B applications, and this decrease occurred in the highest dose of B, 500 mg kg^{-1} B application. Depending on the sampling time, differences in the relative water content values of the *Puccinellia distens* plant were revealed, while the relative water content values of the plant increased in the 30^{th} day sampling, it was stated that the 60^{th} day sampling decreased. In green bean genotypes, different concentrations of B were applied for 10 and 20 days and it was found that leaf proportional water content decreased in parallel with increasing B concentration. It has been determined by Akoğlu (2013) that in green bean genotypes, different concentrations of B were applied for 10 and 20 days, and that the leaf proportional water content values generally decreased in parallel with the increasing B concentration. Although there was no significant difference between the 2.0, 4.5, and 7.0 mg L^{-1} B applications, the relative water content was significantly reduced with B applications (Simon et al., 2013). In a study conducted on different boron doses in wild wheat (*Triticum boeticum* L.), it was reported that the relative water content of B application decreased with the increase in boron stress time and boron (500 mg kg^{-1} B) dose (Uygan, 2014).

3.3. Boron content

The interaction of 'Soil type × B dose' was statistically significant in differences between the amounts of B accumulated in the shoot, root, and plant (Table 4).

In both soil types, the highest amount of B accumulation in the shoot is seen at 50 mg kg^{-1} B. It was determined that the plants growing in S1 soil accumulated more B than S2 soil and it was also statistically significant. In both soil types, there is no statistical difference between the 25 mg kg^{-1} B dose in terms of boron accumulation in plants. There is a remarkable increase in the amount of boron in the shoot of boron applications compared to the control (Figure 2).

When B accumulation in the root was examined, the applied B doses showed a significant increase compared to the control. The highest B accumulation occurred at 50 mg kg^{-1} B as in the root. There is no statistical difference in the application of 50 mg kg^{-1} B dose in plants growing in S1 and S2 soil structure. Similarly, there is no statistical difference in the B accumulation in the

Table 4. ANOVA for boron content in shoot, root and whole plant

Source of variation	B in shoot	B in root	B in whole plant
B dose (BD)	**	NS	**
Soil type (ST)	**	**	**
BD x TT	**	**	**
CV (%)	3.51	5.24	3.65

CV: Coefficient of variation; **: P<0.01 is significant at probability level; *: P<0.05 is significant at probability level; NS: Not significant.

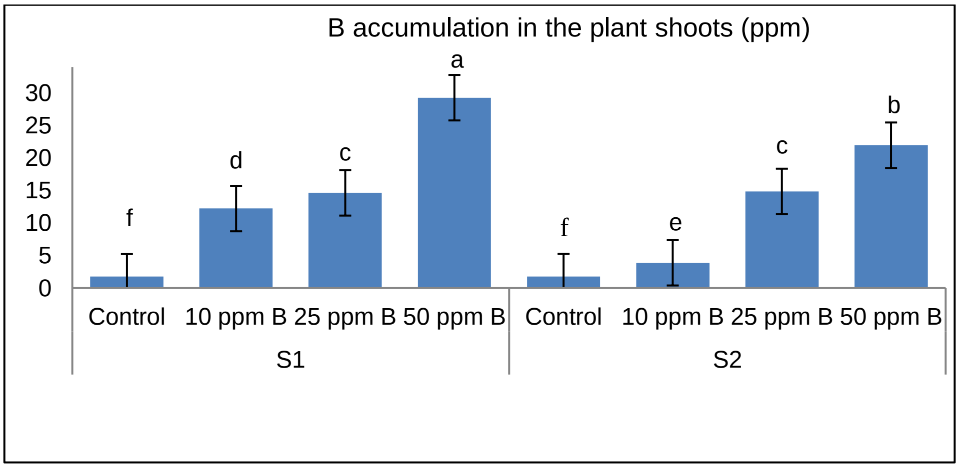


Figure 2. Boron accumulation in the plant shoot in two different soil types (S1: Acidic soil, S2: Alkaline soil. P<0.05 is significant at probability level).

root in the application of 10 mg kg^{-1} B dose in both soil structures. In the application of 25 mg kg^{-1} B dose, there was more B accumulation in S1 soil than in S2 soil and the difference between them was found statistically significant (Figure 3).

The soil type with the highest B accumulation in the whole plant in ornamental cabbage was S1 soil. The highest increase was 50 mg kg^{-1} B, consistent with B accumulation in the shoot and root. Boron accumulation in whole plant was followed by 25 mg kg^{-1} B and 10 mg kg^{-1} B, respectively. When the B doses of both soil types were examined separately, a statistical difference was found between them. It is seen that there is an increase in the boron doses applied to the plant compared to the control (Figure 4).

In terms of soil type, the highest boron accumulation occurred in S1 soil compared to S2 soil. B uptake in plants decreases in parallel with the increase in soil pH and excessive calcification. When the pH of the soil is 6.3-6.5, the B uptake is at the highest level. In addition, due to the ability of boron to be adsorbed by clay minerals, soils with high clay content accumulate more B than sandy soils (Kacar and Katkat, 2007). The pH of the S2 soil was 7.49 and the boron accumulation in the plant was lower than the S1 soil because it showed a calcareous soil feature. However, the fact that ornamental cabbage is a landscape plant and other morphological features were evaluated, the plants growing in S2 soil developed large corolla, making S2 soil stand out compared to S1 soil.

In the study on cotton varieties, it was reported by Harite (2008) that there are significant increases in shoot and root B contents with B applications compared to control. In another study by Keskin (2010), it was reported that the B accumulation in both the shoot and the root increased as the B dose increased. Examining the B accumulation capacities in *Medicago sativa* and *Vicia sativa* plants, it was notified that the roots, shoots and leaves of the plants showed resistance to the highest dose of 50 ppm and that B accumulation was the highest at this B dose (Karaömerlioğlu, 2011). In a study investigating the B tolerance of corn varieties, it was determined that the dose at

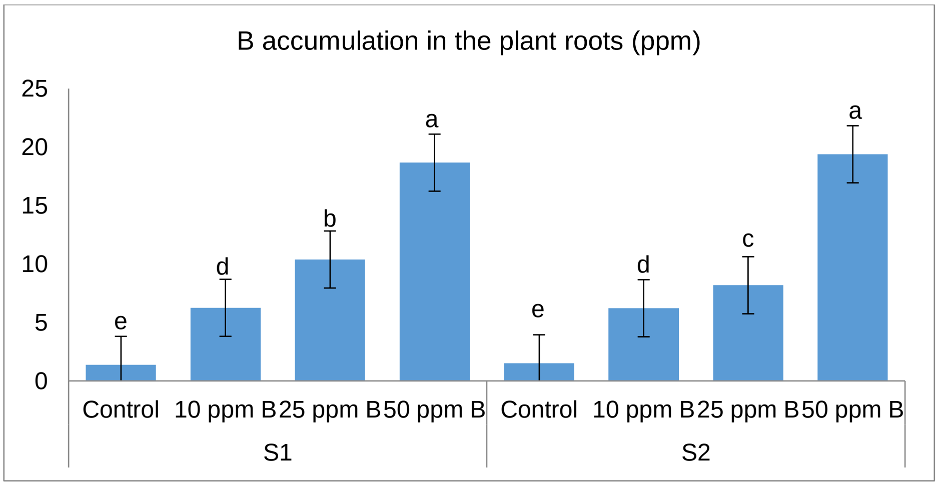


Figure 3. Boron accumulation in the plant root in two different soil types (S1: Acidic soil, S2: Alkaline soil. P<0.05 is significant at probability level).

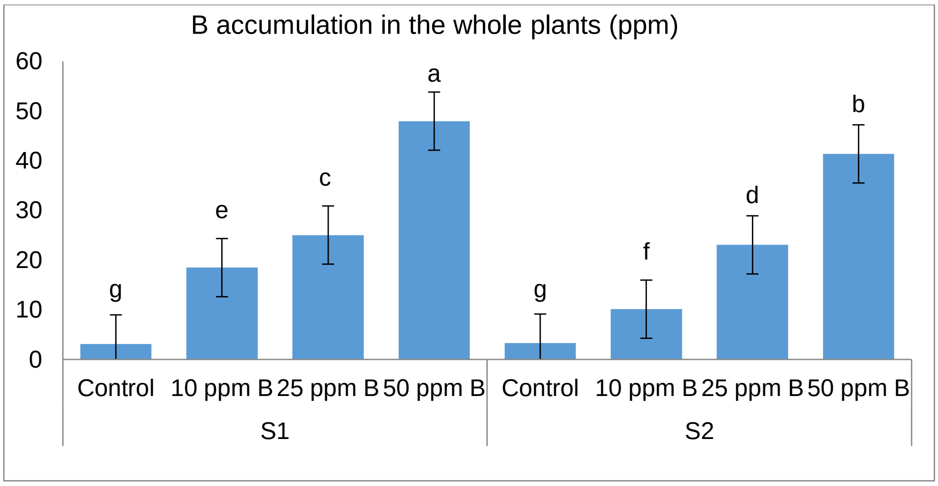


Figure 4. Boron accumulation in the whole plant in two different soil types (S1: Acidic soil, S2: Alkaline soil. P<0.05 is significant at probability level).

which boron toxicity symptoms occur was 40 mg kg^{-1} for all varieties and the highest B uptake occurred at this dose (Palta and Gezgin, 2011). Kookhan and Maftoun (2016) reported that 40 mg kg^{-1} B soil application in canola plant significantly increases the B concentration in shoots. In studies conducted in potatoes and green beans, B accumulation increased in parallel with the increase in B dose in both leaf and root (Akoğlu, 2013). Metwally et al. (2018) informed that canola plant shoots include a higher concentration of B than roots. The amount of boron removed by the shoot in the buckwheat plant increased with the increasing boron applications (Yazıcı and Korkmaz, 2020). Our results showed that boron was mostly accumulated in the shoot of plant, thus supporting previous studies.

4. Conclusion

This study was carried out to identify the ability of ornamental cabbage as hyper-accumulator plant potential and removal of boron from contaminated soil. The obtained results showed that ornamental cabbage has the ability to accumulate B in shoots and roots. Increasing B doses reduced stomatal conductance and relative water content and caused losses in chlorophyll content. As a result, it has been revealed that the ornamental cabbage plant is a potentially usable plant for the removal of boron from the soil by phytoremediation. The use of ornamental cabbage only for ornamental and landscaping purposes seems promising for this plant to come to the forefront in environmental remediation studies.

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