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General Information

Overview

"Soil Studies (SoilSt)" is the successor to the "Soil Water Journal (Toprak Su Dergisi)" which has been published since 2012. Based on the experience and strengths of its predecessor, SoilSt has been developed to create a truly international forum for the communication of research in soil science. SoilSt is a refereed academic journal has been published free of charge and open accessed by Soil, Fertilizer and Water Resources Central Research Institute. The journal will be published 2 issues (July & December) starting from 2022. It covers research and requirements of all works within the areas of soil.

Aims and Scope

Soil Studies is an international peer reviewed journal that aims to rapidly publish high-quality, novel research of studies on fertility, management, conservation, and remediation, physics, chemistry, biology, genesis, and geography of soils. In addition, the main purpose of Soil Studies is to reveal the influences of environmental and climate changes on agroecosystems and agricultural production. In this context, Soil Studies publishes international studies address these impact factors through interdisciplinary studies. In the journal, articles on hypothesis-based experimental observation of the interactions of all components of agricultural ecosystems, field trials, greenhouse or laboratory-based studies, economic impact assessments, agricultural technologies, and natural resources management will be accepted within the peer-reviewed process. Topics include, but are not limited to:

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Corresponding Address

Address : Soil, Fertilizer and Water Resources Central Research Institute (Toprak, Gübre ve Su Kaynakları Merkez Araştırma Enstitüsü Müdürlüğü)

Gayret District Fatih Sultan Mehmet Avenue No: 32 P.K:5 Yenimahalle / Ankara 06172 / Turkey

(Gayret Mahallesi Fatih Sultan Mehmet Bulvarı (İstanbul Yolu) No: 32 P.K:5 Yenimahalle / ANKARA 06172 / Türkiye)

Web : http://www.soilstudies.org/

E-mail : info@soilstudies.org

Phone : +90 312 315 65 60 - 64

Fax : +90 312 315 29 31

Editorial Information

Technical Editors

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RESEARCH PAPER

Relationships between some soil properties and bulk density under different land use

Nutullah Özdemir[1], Zeynep Demir[2]*, Elif Bülbül[1]

[1]Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 55200 Samsun, Türkiye
[2]Soil Fertilizer and Water Resources Central Research Institute, Department of Soil Science and Plant Nutrition, 06172 Ankara, Türkiye

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Corresponding Author
Tel.: + 90 312 315 6560
E-mail: zdemir06@yahoo.com

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Abstract

In this study, the changes of some soil physical and chemical properties were investigated under different land use conditions in Turhal, Turkey. Soil samples were collected from 0-20 cm depth from twenty four plots under eight different land uses which are sunflower, wheat, vegetable, orchard, sugar beet, meadow, pasture and alfalfa plants. Some soil properties where these plants are grown and their effects on the bulk density were investigated. The findings show that basic soil properties and practices related to plant management are effective on the bulk density. While the lowest mean bulk density value was determined in meadow (1.00 g cm^{-3}) areas, the highest bulk density value was determined in soils cultivated with sugar beet (1.71 g cm^{-3}). Correlations between the investigated parameters were tested with the use of Pearson's correlation method. Bulk density and some soil parameters used in the evaluation of structural stability and sensitivity to erosion were found significant relationships.

Introduction

Soil is a living, breathing, natural entity composed of solids, liquids, and gases. Bulk density (BD) is defined as the dry weight of soil per unit volume of soil. It is an indicator for soil health and compaction. It affects rooting depth, infiltration, soil porosity, soil microorganism activity, plant nutrient availability and available water capacity. Total volume of surface soil is about 50% solids, soil particles, and soil organic matter (SOM); and about 50% pore space which are filled with air or water. BD is dependent on soil texture, SOM, the density of soil mineral and their packing arrangement. BD is a basic soil property that is effected by the soil properties, tillage climatic conditions and agricultural activities. In an ideal soil, solid components provide root growth medium, attachment and nutrients for plants, while pore spaces provide the air and water needed (Aşkın & Özdemir, 2003). BD which is one of the important indicators of soil quality (Abbott & Manning, 2015) is closely related to environmental quality and biomass production (Makovníková et al., 2017). BD is a dynamic soil property, as it varies in space and time. It is effected by land and crop management practices (Çerçioğlu et al., 2019; Çerçioğlu, 2020), as well as by natural processes such as the climate conditions that influence soil cover, SOM contents, porosity or soil structure (Makovníková et al., 2017). Changes in BD depending on the effectiveness of

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the degrading and forming processes in the soil are closely related to SOM content (Demir et al., 2019; Demir & Işık, 2019, 2020; Demir, 2020) and textural structure (Aşkın & Özdemir, 2003; Makovníková et al., 2017). BD increases with soil depth since subsurface layers are more compacted and have less aggregation, less SOM and less root penetration compared to surface layers, therefore contain less pore space. BD is an important parameter in soil management planning, structural deterioration, soil compaction level and suitability for plant root growth (Dexter, 2004), soil water relationships, and applications related to fertilization, determination of nutrient status and carbon stocks (Ruehlmann & Körschens, 2009; Brahim et al., 2012), and determination of soil porosity (Hillel, 1982; Blake & Hartge, 1986; Aşkın & Özdemir, 2003; Lestariningsih et al., 2013). BD depends on some factors such as consolidation, compaction and amount of soil organic carbon present but it is highly correlated to the organic carbon (Leifeld et al., 2005). Post et al. (1982) reported that SOM and the correlation between BD used frequently to estimate carbon pools. Aşkın & Özdemir (2003) reported the relation of BD with soil particle size distribution and SOM. In many studies, it has been observed that the land use type and changes in use can lead to deterioration in the soil attributes (Arshad & Martin, 2002; Doran, 2002). In this study, the relationships between the BD values and some soil physical and chemical attributes used to investigate in the evaluation of structural stability under different land use types in Turhal district of Tokat province in Turkey.

Materials and Methods

In the study, total 72 soil samples were taken from 0 - 20 cm depth from determined 24 spots (three replications) under 8 different land uses in Turhal, Turkey. Sampling points were selected according to the random sampling method from lands in different uses (90920 ha) included in the entisol soil group. The main products of agricultural production are cereals, tomatoes, sugar beets, sunflowers for oil, fodder crops (vetch, alfalfa, silage corn) and all kinds of fruits and vegetables. The study area is under the influence of a continental-temperate climate. The mean altitude above sea level is 550m. The mean annual temperature and precipitation is 12.9°C and 413.3 mm, respectively (Anonymous, 2020).

Soil particle size distribution was analyzed by hydrometer method (Demiralay, 1993). Modified Walkley-Black method was used to determine soil organic matter (SOM) content (Kacar, 1994). Cation exchange capacity (CEC) were determined as described by Shahid et al. (2018). Scheibler calcimeter was used to determine soil lime contents (Kacar, 1994). A pressure plate apparatus was used to determine soil moisture at field capacity and permanent wilting point (Black, 1965). Soil pH were measured with a pH meter (Bayraklı, 1987) and electrical conductivity were measured with an EC-meter (Kacar, 1994). Consistency limits were analyzed in accordance with the principles specified according to (Demiralay, 1993). Cylinder method was used to determine bulk density (Demiralay, 1993). A wet-sieving apparatus was used to determine aggregate stability (AS) (Demiralay, 1993). Exchangeable Na were determined with ammonia acetate extraction (Sağlam, 1997). Dispersion ratio (DR) values were estimated by the following equation (Equation 1):

Eq. (1) $DR (\%) = (a/b) * 100$

Where, a is the percentage of silt plus clay in suspension, b is the percentage of silt plus clay dispersed with chemical agent (Özdemir, 2013).

Erodibility factor (K) (Wischmeier & Smith, 1978) were estimated by the following equation (Equation 2):

Eq. (2) $K = [(2.1*10^{-4} (M)^{1.14} (12 - a) + 3.25 (b – 2) + 2.5 (c – 3)) 1.292] / 100$

Where, K: erodibility factor; indicates the rate of erosion per unit erosion index from a standard area (22.1 m length and 9% slope continuously in fallow). M is the particle size parameter (% silt + % very fine sand)*(100 - % clay), a is the percentage of organic matter, b is the soil structure type code and c is the permeability class code. Soil samples taken from the specified spots under the different land use conditions were analyzed so that the data basic for estimating erodibility were obtained. In estimating the K factor with this method, silt and very fine sand (0.002-0.1 mm), clay (<0.002 mm), organic matter (%), soil structure and permeability classes are used. Soil structure is determined by using soil profile definitions while the other rates are determined by laboratory analysis.

Percent shrinkage was calculated using the change in the volume of the soil paste stacked in circular molds with an inner diameter of 5mx1cm (Ferry & Olsen, 1975). Correlations between the investigated parameters were tested with the use of Pearson's correlation method by SPSS 19.0.

Results and Discussion

Soil Properties

Some physical and chemical soil properties taken from 24 plots under 8 different land uses are given in Table 1. These soils are in a range varying from coarse to fine in terms of texture, and sand contents vary between 20.2% and 65.5%, silt contents vary between

Table 1. Some physical and chemical properties of soils (n = 72)

Land use types		Sand, %	Silt, %	Clay, %	Texture class	pH, (1:2.5)	EC, dS m^{-1}	$CaCO_3$, %	SOM, %	Exc. Na, %	CEC, me $100g^{-1}$	FC,%	PWC, %	PS	LL,%	PL, %	AS, %	DR, %	K
Wheat	Min.	20.2	36.4	37.5		7.90	0.313	12.3	2.0	4.58	23.5	37.4	17.4	53.4	50.7	28.1	30.2	5.2	0.018
	Max.	25.6	39.6	40.2		7.90	0.504	15.5	3.0	11.20	29.1	49.3	25.5	76.8	61.1	32.2	48.6	8.0	0.022
	Mean	23.6	37.7	38.5	CL	7.91	0.409	14.1	2.6	8.17	27.0	40.8	21.9	63.2	56.1	30.5	41.0	6.9	0.020
Pasture	Min.	28.0	19.3	7.5		7.89	0.178	11.7	0.6	4.24	15.6	17.6	10.7	13.4	32.0	18.8	17.2	5.4	0.012
	Max.	65.5	38.9	33.3		7.90	0.540	21.5	1.4	6.48	24.1	32.0	22.5	49.4	45.7	29.0	48.6	11.0	0.025
	Mean	47.1	28.8	24.0	L	7.89	0.341	17.5	1.0	5.40	19.4	27.1	16.3	36.1	40.7	24.4	36.1	8.3	0.020
Orchards	Min.	27.5	33.6	28.3		7.90	0.340	16.2	1.4	2.24	20.1	31.3	16.2	37.4	28.2	23.1	22.8	5.0	0.015
	Max.	33.7	38.2	37.1		7.91	0.677	23.4	3.2	6.57	33.4	40.4	24.2	63.7	56.0	33.4	43.5	8.1	0.025
	Mean	30.2	36.2	33.5	CL	7.91	0.507	19.1	2.5	4.85	25.8	37.7	20	52.4	43.3	28.9	34.4	6.1	0.020
Sunflower	Min.	44.2	29.8	5.5		7.99	0.282	11.2	2.3	1.68	30.1	21.4	8.11	11.8	12.5	23.2	12.9	5.4	0.016
	Max.	55.4	39.3	24.4		8.01	0.780	24.6	3.4	2.71	38.3	32.0	20.2	40.9	77.3	27.0	62.9	8.9	0.029
	Mean	48.7	31.3	17.4	L	8.00	0.468	18.6	3.0	2.07	35.2	26.5	14.2	28.4	39.4	25.1	38.7	7.8	0.022
Alfalfa	Min.	23.7	42.2	7.4		7.89	0.474	15.3	2.6	1.33	33.0	26.2	7.12	20.5	33.9	20.4	13.9	5.6	0.024
	Max.	47.9	45.1	31.1		8.04	0.596	23.0	3.1	2.31	49.3	40.3	21.3	53.3	47.3	31.2	37.2	15.0	0.036
	Mean	33.4	44.0	22.5	L	7.98	0.540	20.0	2.9	1.74	39.5	31.3	13.9	39.3	40.6	26.2	24.4	9.2	0.028
Vegetable	Min.	45.1	34.7	3.4		7.89	0.332	8.9	1.7	1.41	32.3	17.4	6.26	10.7	18.2	23.0	9.16	6.1	0.029
	Max.	61.6	42.8	13.7		8.06	0.459	12.7	2.8	1.73	42.2	31.1	116	20.4	37.7	33.3	17.6	19.0	0.040
	Mean	54.5	39.2	6.2	SL	7.95	0.388	11.1	2.2	1.59	37.1	23.2	32.2	16.0	28.3	26.8	13.2	13.0	0.034
Sugar beet	Min.	32.8	33.3	11.5		7.89	0.285	16.8	0.5	1.14	40.2	18.1	11.8	23.9	29.6	5.27	9.44	6.4	0.026
	Max.	52.7	40.7	26.6		7.91	0.363	29.9	1.7	2.62	50.7	29.5	19.8	45.3	43.4	27.4	40.3	13.0	0.037
	Mean	44.7	37.8	17.3	L	7.89	0.313	22.2	1.1	1.98	45.3	25.8	14.8	33.8	35.9	21.9	23.2	10.0	0.031
Meadow	Min.	32.8	25.4	30.4		7.89	0.432	23.2	1.8	1.13	43.4	27.3	19.2	44.7	45.4	30.6	46.2	3.0	0.013
	Max.	35.6	34.1	41.1		7.89	0.636	39.5	2.8	3.91	51.2	36.5	20.1	58.1	49.7	31.9	63.0	6.2	0.025
	Mean	33.3	30.6	35.4	CL	7.89	0.518	29.7	2.2	2.33	46.7	33.4	19.8	48.1	47.7	31.4	56.9	4.3	0.019

EC: Electrical conductivity, SOM: Soil organic matter, Exc. Na: Exchangable sodium, CEC: Cation exchange capacity, FC: Field capacity, PWP: Permanent wilting point, PS: Percent shrinkage, LL: Liquid limit, PL: Plastic limit, BD: Bulk density, AS: Aggregate stability, DR: Dispersion, K: Soil erodibility factor, CL: Clay loam, L: Loam, SL: Sandy loam.

Table 2. Correlations on some physical and chemical properties of soils (n = 72)

	S	Si	C	SOM	$CaCO_3$	CEC	Exc. Na	FC	PWC	PS	LL	PL	AS	DR	K
BD	0.424**	0.153	-0.500**	-0.627**	-0.402**	-0.253	-0,041	-0.507**	-0.470**	-0.414**	-0.333*	-0.372**	-0.652**	0.526**	0.439**
S		-0.253	-0.892**	-0.607**	-0.180	-0.055	-0.449**	-0.891**	-0.807**	-0.871**	-0.792**	-0.576**	-0.577**	0.676**	0.478**
Si			-0.200	0.036	-0.379**	0.216	-0,119	0.021	-0.140	-0.070	-0.089	-0.134	-0.426**	-0.050	0.662**
C				0.597**	0.364*	-0.045	0.509**	0.891**	0.880**	0.911**	0.842**	0.644**	0.782**	-0.659**	-0.792**
SOM					0.283	0.247	0.169	0.589**	0.538**	0.526**	0.554**	0.442**	0.593**	-0.588**	-0.487**
$CaCO_3$						0.494**	-0.284	0.182	0.199	0.193	0.185	0.387**	0.456**	-0.362*	-0.377**
CEC							-0.680**	-0.038	-0.160	-0.170	-0.144	-0.068	0.072	-0.241	0.207
Exc. Na								0.490**	0.540**	0.622**	0.621**	0.376**	0.319*	-0.102	-0.433**
FC									0.881**	0.888**	0.777**	0.625**	0.586**	-0.685**	-0.631**
PWC										0.867**	0.810**	0.689**	0.664**	-0.575**	-0.767**
PS											0.822**	0.633**	0.663**	-0.508**	-0.641**
LL												0.778**	0.640**	-0.516**	-0.638**
PL													0.431**	-0.398**	-0.492**
AS														-0.598**	-0.741**
DR															0.440**

*Significant at p<0.05. **Significant at p<0.01.

S: Sand, Si: Silt, C: Clay, SOM: Soil organic matter, Exc. Na: Exchangable sodium, CEC: Cation exchange capacity, FC: Field capacity, PWP: Permanent wilting point, PS: Percent shrinkage, LL: Liquid limit, PL: Plastic limit, BD: Bulk density, AS: Aggregate stability, DR: Dispersion rate, K: Soil erodibility factor.

19.3% and 45.1%, and clay contents vary between 3.4% and 41.1%. Rao & Wagenet (1985) stated that variation in basic soil parameters like soil texture is due to the intrinsic (weathering) and anthropogenic (cultivation) factors. The pH values of the soils are between 7.89 and 8.06, with an mean of 7.93. The electrical conductivity (EC) values of the soils vary between 0.178 dS m^{-1} and 0.780 dS m^{-1}, with an mean of 0.436 dS m^{-1}. The pH was found to be slightly alkaline in nature indicative of no salinity problem under different land use types. The EC values of the soils are below 2 dS m^{-1} and the soils are in the salt-free class (Hazelton & Murphy, 2016). The $CaCO_3$ content of the soils is between 8.9% and 39.5%, with an mean value of 19.1%. Generally, the soils have a very calcareous structure in terms of $CaCO_3$ content (Soil Survey Staff, 1993). SOM varied between 0.5% and 3.4%, with a mean value of 2.22%. Soils have organic matter content ranging from very low to high (Hazelton & Murphy, 2016). High concentration of SOM is able to affect soil pH and therefore cation exchange capacity also. SOM is able to explain maximum of the variation in cation exchange capacity, under different land uses and different techniques (Zeraatpishe & Khormali, 2012). The soil CEC varied between 15.6 and 51.2 me $100g^{-1}$. While the lowest mean CEC values (19.4 me $100g^{-1}$) was obtained from the plots under pasture, the highest mean CEC values was obtained from the meadow cover (46.7 me $100g^{-1}$). The mean CEC values are respectively pasture (19.4 me $100g^{-1}$) < orchard (25.8 me $100g^{-1}$) < wheat (27.0 me $100g^{-1}$) < sunflower (35.2 me $100g^{-1}$) < vegetable (37.1 me $100g^{-1}$) < alfalfa < (39.5 me $100g^{-1}$) < sugar beet (45.3 me $100g^{-1}$) < meadow (46.7 me $100g^{-1}$) (Table 1). In present study, the CEC had significant positive correlations with the $CaCO_3$ (0.494^{**}) and significant negative correlations with the exc. Na (-0.680^{**}) (Table 2). Changes in the CEC due to land use changes can be quite considerable. Many soil properties effect the soil exchangeable capacity especially texture, pH, and SOM up to a certain extent. CEC occur near the surface of clay and humus particles, called micelles. Cations from the soil surface can be quite easily exchangeable with the cations from the solution. Exchangeable sites on the soil colloids can be permanent or pH dependant, depending on clay, pH and SOM. Clay particles can possess both permanent and variable charge depending on clay type, while the SOM can possess only variable charge (Wang et al., 2005). In this study, the CEC across all land use types varied due to differences in the amounts of SOM contents. SOM contents in the 1.0 m soil layer varied significantly with respect to land use type and soil depth (Yimer et al., 2007). In this study, the amount of SOM varied between 1.0 - 3.0%. The mean SOM values are respectively pasture (1.0%) < sugar beet (1.1%) < vegetable = meadow (2.2%) orchard (2.5%) < wheat (2.6%) < alfalfa < (2.9%) < sunflower (3.0%) < (Table 1). In this study, soil CEC had a positive correlation with organic matter (0.247) in the entisol soils (Table 2). In addition, this causes loss of soil physical structure by clay swelling, and dispersion because of high Na^{+} concentrations in the soil solution or at the exchange phase (Yu et al., 2010). Divalent cation Ca^{2+} can replace adsorbed Na^{+} in soil colloids, causing flocculation of colloids and enhanging soil structure (Jalali, 2008). Ca^{2+} could improve soil structure by formed cationic bridges between clay particles and SOM (David & Dimitrios, 2002). In addition, Ca^{2+} can inhibit clay dispersion and the associated disruption of aggregates by replacing Na^{+} and Mg^{2+} in clay and aggregates, thereby promoting aggregate stability (Zhang & Norton, 2002). CEC, as an important indicator for soil quality, represents soil's ability to hold positively charged ions (Li et al., 2013). It is the relative capacity of a soil to hold and exchange cations (Saidi, 2012). Parfitt et al. (1994) indicated that dissociation of carboxyl groups increased CEC of soil organic matter. CEC of organic matter was reported as between 100 to 1000 cmol kg^{-1} (Oades, 1989). On the other hand, cation exchange capacity of clay minerals was reported as between 0 (pure kaolinite) and 110 cmol kg^{-1} (smectite) (Dixon & Weed, 1989). Low CEC under different land use types was observed which may be due to presence of low activity clay (kaolinite) as the CEC of soils is immensely affected by the mineralogy of the soil (Bhattacharyya et al., 1994). The variation in cation exchange capacity values along the different land uses can be supported with the results Brevik (2009) and Mukherjee & Zimmerman (2013), as they mentioned pH, soil organic matter and particle size distribution are the main drivers of cation exchange capacity in soils. The exc. Na values of the soils varied between 1.13% and 11.20%. While the lowest mean exc. Na values (1.59%) was obtained from the plots under vegetable, the highest mean exc. Na values was obtained from the wheat (8.17%). The mean exc. Na values are respectively vegetable (1.59%) < alfalfa < (1.74%) < sugar beet (1.98%) < sunflower (2.07%) < meadow (2.33%) < orchard (4.85%) < pasture (5.40%) < wheat (8.17%) (Table 1). Exc. Na values had significant correlations with CEC (-0.680^{**}), sand (-0.449^{**}) and clay (0.509^{**}) (Table 2). The stuations of the relationships obtained may have resulted from soil characteristics (organic matter content, texture), number of soil samples studied and forms with practics of agricultural activity. High cation exchange capacity may indicate high levels of clay, internal drainage and low permeability due to high soil compaction. Low levels of cation exchange capacity may indicate a soil texture ranging from clay-sandy to sandy, with variable grain size and high permeability (Aprile & Lorandi, 2012).

Bulk Density

Relationships between the mean bulk density values and the land use type in the surface soil samples taken from 24 parcels under eight different land uses in Turhal district are given in Figure 1. While the lowest BD values (1.00 g cm^{-3}) was obtained from the plots under meadow cover, the highest bulk density values was obtained from the sugar beet producing areas (1.71 g cm^{-3}). The bulk density values are respectively meadow < orchard < sunflower < wheat < pasture < vegetable < alfalfa < sugar beet (Figure 1). It has been determined that the bulk density values are affected by the basic soil properties and land use. It has been determined that as the land use density increases, the bulk density values also increase. The variation in bulk density can be explained with the differences in organic matter content, cultivation process and biotic activities (Rao et al. 2008). Krull et al. (2003) indicated that medium and fine-textured soils (loamy and clay) had greater organic matter contents than coarse-textured (sandy) soils. Rice (2006) indicated that clay particles sheltered organic matter and prevented decomposition of organic matter. In this study, while the lowest mean soil organic matter contents (1.0%) was obtained from the plots under pasture, the highest mean soil organic matter contents was obtained from the plots under sunflower (3.0%).

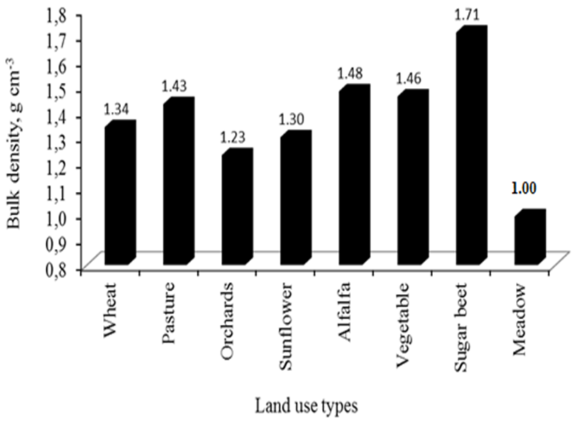

Figure 1. Changes of bulk density (BD) values depending on land use types

It was determined that the plots with weak structure and low organic matter content had higher bulk density values. Tufa et al. (2019) found that the BD were significantly effected by the land use type and basic soil characteristics, with the highest BD in cultivated land (1.37 g cm^{-3}), and the lowest in the fields used as pasture (1.10 g cm^{-3}). They stated that the low bulk density values in the pasture lands was associated with the high clay in these lands and the low density use of the grazing lands. Parlak et al. (2015) investigated the effects of different reclamation practices on soil loss and bulk density values in the grasslands of Gökçeada, Çanakkale. They found that there were significant differences between protected and unprotected plots, and there was no significant difference in aggregate stability.

The relationships between some soil properties are given in Table 2. According to Table 2, clay content (r = -0.500**), soil organic matter (r = -0.627**), $CaCO_3$ (r = -0.402**), field capacity (r = -0.507**), wilting point (r = -0.470**), percent shrinkage (r = -0.414**), plastic limit (r = -0.372**), aggreagte stability (r = -0.652**) values were found to have significant negative correlations at the level of 1% between the bulk density values of the soils. This correlation suggests that the increase in aggregation could lead to an increase in porosity, and thus, a decrease in BD. BD was negatively correlated with SOM, as SOM generally lowers the mean bulk density (Hillel, 1998). Gülser (2006) and Demir & Işık (2019) found that BD gave the negative correlation with SOM. The negative relationship of BD to aggregate stability is reflecting the extent of soil degradation that occurs over time, which in turn has effected factors such as SOM, which contribute directly to the formation of stable soil aggregates. Correlations at the level of 1% were obtained between the values of bulk density and sand content (r = 0.424**), dispersion ratio (r = 0.526**) and soil erodibility factor (r = 0.439**). There was no statistically significant relationship between the values of silt (r = 0.153), CEC (r = -0.253), exchangeable Na (r = -0.041) and bulk density values. The stuations of the relationships obtained may have resulted from soil characteristics (texture, organic matter content), number of soil samples studied and forms with practics of agricultural activity. Mamedov et al. (2002) reported that high exchangeable Na weakens cohesive forces within aggregates and enhances their slaking. However, Ca and Mg have been considered ions maintaining soil structure. Agassi & Bradford (1999) have found that erodibility varies with aggregate stability, soil textures, soil structures, shear strength, soil depth, infiltration capacity, SOM and BD. SOM has the ability to disperse or aggregate the soil, depending on the threshold level of organic matter and the ratio at which it occurs with other aggregating agents. In this study, we reported that the higher the soil organic matter of the soil the less the ability of the soil to disperse. In this study, the soil organic matter content had significant negative correlations with dispersion rate (-0.588**) (Table 2). When the soil disperses, the microaggregates that make up the structural framework of the macroaggregates are disintegrated, hence progressively detached at the weakest point of the aggregate structure (Legout et al., 2005).

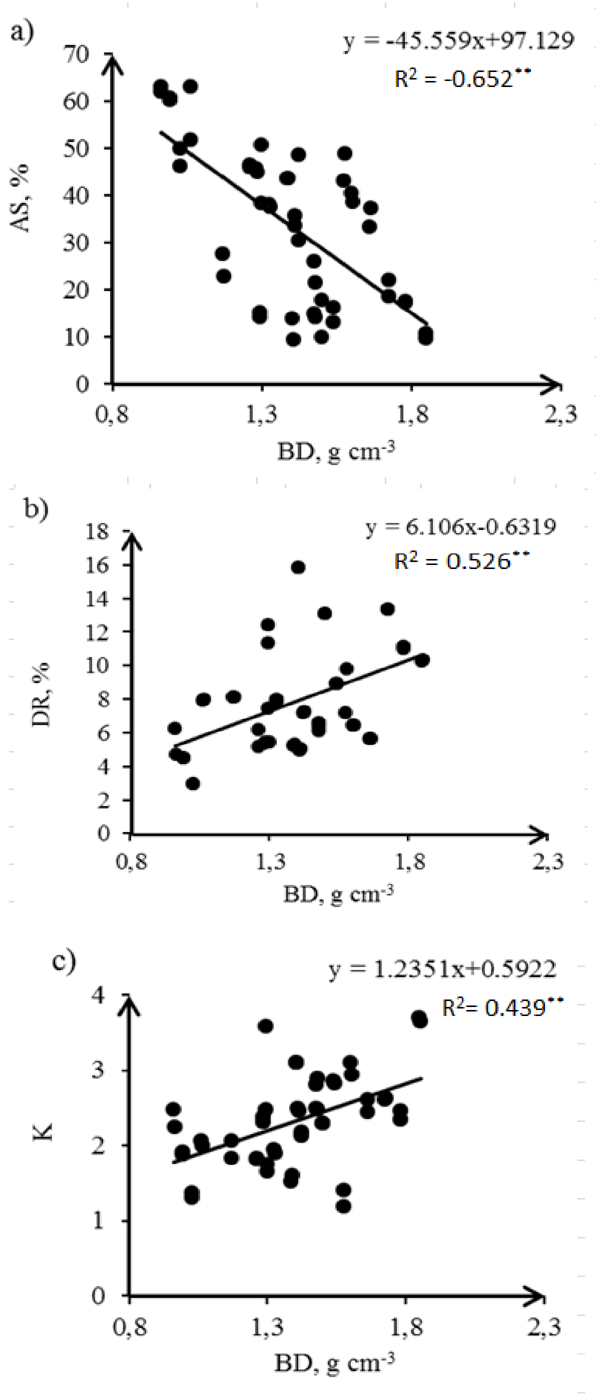

Figure 2. Relationships between bulk density and aggregate stability (a), dispersion rate (b), soil erosion factor (c), (AS: Aggregate stability, BD: Bulk density, DR: Dispersion rate, K: Soil erodibility factor)

Relationships between bulk density and aggregate stability (a), dispersion rate (b), soil erosion factor (c) were given Figure 2. According to these findings, it has been determined that the bulk density values and the parameters used in the evaluation of structural stability were in close relationship (Figure 2). It has been determined that soils with low bulk density values also have low erosion rate values and high aggregate stability values. In other words, it can be stated that low bulk density values also reflect a structure resistant to erosion.

Conclusion

In this study, the relationships between some soil properties and bulk density values under different land use types (wheat, pasture, orchard, sunflower, clover, vegetable, sugar beet and meadow) in Turhal district of Tokat province were compared. It has been determined that the bulk density values are affected by basic soil characteristics (texture and organic matter content) and land use type. The lowest BD were determined in the plots under the meadow cover (uncultivated, medium texture), while the highest BD were determined in the sugar beet production areas (frequently processed, coarse textured). Important relationships have been determined between the bulk density values of soils and the parameters used in the evaluation of structural stability and susceptibility to erosion, and it would be beneficial to expand research on these issues.

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Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Author Contribution

NÖ: Conceptualization, investigation, methodology, validation, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization, supervision, statistical analysis, project administration; **ZD:** Conceptualization, validation, data curation, resources, writing—review and editing; **EB:** Investigation, methodology, validation, software, validation, investigation, resources, data curation. All authors have read and agreed to the published version of the manuscript.

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RESEARCH PAPER

Influence of some plant nutrients on sweet cherry cultivars grafted on plum rootstocks (*Prunus cerasifera*) in soil with high pH

Cafer Hakan Yılmaz[1]*, Remzi Uğur [2]

[1]East Mediterranean Transitional Zone Agricultural Research of Institute, Department of Soil and Water Resources, Onikişubat Kahramanmaraş, Türkiye
[2]East Mediterranean Transitional Zone Agricultural Research of Institute, Department of Horticulture, Onikişubat Kahramanmaraş, Türkiye

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Corresponding Author
Tel.: +90 533 561 5351
E-mail:
caferhakan.yilmaz@tarimorman.gov.tr

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Plum rootstock

Abstract

This study was aimed to investigate the ability of the plums belonging to Prunus cerasifera species to be rootstock to some cherry cultivars and to examine the effect of rootstock, variety and their combinations on mineral nutrition. Rootstock-scion relationship and plant nutrient transmission were investigated in Napoleon, Starks Gold and Lambert cherry cultivars grafted on 10 selected rootstocks. As a result of the investigations, it was determined that the selected rootstocks were quite effective in transmitting the macro and micro plant nutrients to the cherry cultivars. It was noteworthy that the foliage phosphorus (0.15-0.52%) and potassium (1.65-4.00%) contents of the rootstocks were high. Although the leaf iron contents of rootstocks (49.11-74.42 mg kg-1) remained relatively below the reference values, deficiency symptoms such as chlorosis were not observed in the leaves. A strong correlation (r = 0.780**) was found between leaf chlorophyll contents and Cu. Translocated graft incompatibility was observed in some rootstocks. In combinations with not good graft compatibility, decreases in the transmission of plant nutrients were determined in general. At the end of the study, the idea that Prunus cerasifera wild plum species can be a good clone rootstock alternative for sweet cherry growing in high pH soils has emerged.

Introduction

According to the data of 2019, the world sweet cherry production is approximately 2.595.812 tons. Within this production amount, Türkiye ranks first in the world with 664,224 tons (FAOSTAT, 2020). As in other countries in the Mediterranean zone, soils in Turkey are also mostly calcareous and have a high pH. For this reason, mostly classical wild cherry (Prunus avium), mahaleb (Prunus mahalep) and a little wild cherry (Prunus cerasus) are used as rootstocks in sweet cherry cultivation in the country. Since Prunus avium rootstocks are not suitable for intensive cultivation due to their excessive strong grew, Prunus mahalep and Prunus cerasus rootstocks have excessive bottom suckering, irregularities in nutrient transmission (Jimenez et al., 2007), drying in high ground water and irrigation applications and Its use remains limited, as it carries a risk of delayed graft incompatibility. In recent

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years, the use of clonal hybrid rootstocks cultivated by different methods brought from outside the country has also increased. However, although some of these rootstocks have received positive results, serious problems occur in some of their adaptation to the high pH soils of the Anatolia. Therefore, it has become more important to carry out rootstock breeding studies in other Prunus species in order to use suitable rootstocks. Among these species, Prunus cerasifera is an important and suitable alternative. However, this species adapts easily to different climatic and soil conditions (Topp et al., 2012). In the breeding programs for the use of Prunus cerasifera as rootstock for stone fruits, it has been determined that this species is quite compatible with the calcareous and high pH soils of the typical Mediterranean region where root rot and chlorosis problems are intense (Moreno, 2004). Failure of a developed rootstock to adapt well to different soil conditions could be difficulties in the transmission of plant nutrients, as well as problems in the graft compatibility rate and post-grafting development.

There are three main factors that affect plant nutrition and the nutritional situations of a plant: Soil, rootstock and environment. Soil texture, pH, salinity, lime, organic matter, cation exchange capacity, available nutrient concentrations, depth and balance are among the soil factors. Species, variety, root structure, age, growth period and other genotypic characteristics of a plant play different important roles on nutrients uptake ability of a plant (Erdal et al., 2008; Marschner, 2012). Factors such as humidity, temperature, precipitation and its characteristics, lighting duration also play a role as environmental factors in soil fertility and plant nutrition. Plant factors are the basic characteristics in determining the degree of influence of these factors. Nutrient uptake capacity of a plant still varies from plant to plant, even if there are different genotypes of that plant species (Clark & Gross, 1986). These variations can be seen even if grown in the same soil and under the same conditions (Küçükyumuk & Erdal 2011; Marschner, 2012; Küçükyumuk et al.2015). In the science of horticulture, rootstock and variety are two important basic factors affecting the performance and survival of a variety against negatory conditions. In order to obtain better plant growth and quality yield, nutrients uptake and transport capacities of plant cultivars and its rootstocks should be taken into consideration (Tsipouridis et al., 1990; Küçükyumuk & Erdal 2011).

In this study, Napoleon, Starks Gold and Lambert sweet cherry cultivars grafted on Prunus cerasifera, the most important goal was to shed light on similar studies to be done after that by revealing how nutrient transfer, rootstock scion match, and sweet cherry cultivars will develop forces. The study was carried out in the greenhouses, laboratories and land of Kahramanmaraş Eastern Mediterranean Transitional Zone Agricultural Research Institute between 2019-2020.

Materials and Methods

This research was carried out between 2019-2020 in the greenhouses and laboratories of Eastern Mediterranean Transition Zone Agricultural Research Institute in Kahramanmaraş province of Türkiye.

Soil Material

In greenhouse studies, the soil obtained with a

Table 1. Some physical and chemical properties and evaluation of the prepared greenhouse soil.

Soil Properties	Greenhouse soil values	Evaluation	By whom
Saturation (%)	67	Clay-Loam (CL)	Ülgen and Yurtsever (1995)
pH	8.45	Moderately alkaline	USDA (1998)
EC (dS m^{-1})	0.94	Slightly saline	FAO (2006)
Lime (%)	4.82	Moderately calcareous	FAO (2006)
Organic matter (%)	7.12	High	Ülgen ve Yurtsever (1995)
Available phosphorus (mg kg^{-1})	58.74	Very high	Rehm et al. (1996)
Available potassium (mg kg^{-1})	566	Very high	Rehm et al. (1996)
Available calcium (mg kg^{-1})	5499	Good	Loue (1968)
Available magnesium (mg kg^{-1})	550	High	Loue (1968)
Available iron (mg kg^{-1})	20.49	Good	Lindsay ve Norvell (1978)
Available manganese (mg kg^{-1})	4.60	Sufficient	Lindsay ve Norvell (1978)
Available copper (mg kg^{-1})	4.69	Sufficient	Lindsay ve Norvell (1978)
Available zinc (mg kg^{-1})	2.97	High	Lindsay ve Norvell (1978)

mixture of garden soil, sand and peat (3:2:1, respectively) and the content of which can be seen in Table 1 was used.

Plant Material

Napoleon, Starks Gold and Lambert sweet cherry cultivars were grafted on 11 rootstocks plants, including 10 selected clonal Prunus cerasifera and 1 control plant Adara (Prunus cerasifera) as material in the study. The number of grafting was set to be 15 from each rootstock-scion combination. In the fall of 2019, chip budding was applied. Those that got the graft incompatibility were eliminated. In the experiment, 6 dm^3 pots with suitable soil mixture were used.

Greenhouse studies

After the application of the graft, the pots where the saplings were located were placed in a fully controlled greenhouse. During the vegetation period, irrigation was applied to all plants by drip irrigation method, on average, once a week according to temperature values. The drip irrigation application was terminated as of the end of October 2020. Sapling growth status in the vegetation period of 2020 was also examined.

Sapling Growth Status

Growing cases and diameters of rootstock and scion were realized with a digital caliper with a sensitivity of 0.01 mm (0.01 mm Gomax GMX1017020) from 5 cm above and below the grafting point. The height of the saplings was measured with the help of strip meters from the graft point (Tekintaş et al., 2006).

Chlorophyll Analysis

The amount of chlorophyll (Chl) per unit area of the leaves that have completed their development in the greenhouse was carried out with the help of SPAD 502 (Minolta Co., Osaka, Japan) chlorophyll measuring device. After adjusting the calibration of the device, the chlorophyll amount of ten leaves taken from each sapling from different ways and from the outward-facing directions was measured in nmol Chl cm2 and the average of all values was found. Chlorophyll measurements were performed monthly from May 2020, when the leaves were fully grown, to the end of October 2020 (Reig et al., 2018a). The obtained values were evaluated in accordance with the experimental plan.

Plant Nutrient Analysis

As reported by Kacar & İnal (2010), leaf samples were collected, dried, ground. Milled samples were wet-digested in a CEM Mars 6 brand microwave oven, and then the total amounts of P, K, Ca, Mg, Fe, Mn, Cu, and Zn of leaves in an Agilent 5100 brand ICP-OES device determined. The accuracy of the results was also checked with certified values of relevant minerals in reference plant materials obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Histological Analysis

These analyzes were performed by Moreno et al. (1993) and Reig et al. (2019) was made according to the recommendations.

Evaluation of Data

The experiment was established in a randomized plot design with three replications, with 5 pots in each plot. The analysis of variance on the data obtained from the study was performed using the Jmp 5.0 statistical program (Sall et al., 2017), and the comparison of the means was performed using the LSD ($P < 0.05$) test.

Results and Discussion

Sapling Growth Status

It was observed that the selected rootstocks significantly affected the growth vigor of sweet cherry cultivars. It was determined that the greatest scion diameter development occurred in all sweet cherry cultivars grafted on KL-38 rootstock, with values varying between 15.00-15.46 mm. It was observed that the smallest scion diameter was in the Lambert variety grafted on KL-60. In general, the diameters of the scion ranged between 11-15 mm in the rootstock scion combinations, and the rootstocks were significantly effective in the development strength of the scion (Table 4). As well as the differences in scion-caliber development, the development status in sapling sizes showed a similar distribution, and it was determined that the same combinations had similar results.

Chlorophyll Contents

It was revealed that leaf chlorophyll contents of sweet cherry cultivars were significantly affected by rootstocks (Table 4). Leaf chlorophyll contents are highest in Lambert/KL-54 (47.44 nmol cm2) and Napoleon/KL-60 (47.13 nmol cm2) combinations, the lowest is in Napoleon/KL-47 (36.08 nmol cm2) and Napoleon/KL-33 (36.02 nmol cm2) combinations were found.

Leaf Nutrient Concentrations

Leaf macro and microelement contents of three sweet cherry cultivars were statistically significantly affected by rootstocks. Leaf phosphorus contents of sweet

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Table 2. Leaf macronutrient contents of Napoleon, Starks Gold and Lambert sweet cherry cultivars grafted on rootstocks.

Variety	Rockstock	P (%)		K (%)		Ca (%)		Mg (%)	
	KL-11	0.25 ±hıj		2.17 ±0.05 ıj		0.76±0.01op		0.25 ±0.01 st	
	KL-15	0.33 ±0.01 de		2.64 ±0.06 efg		1.39±0.01 kl		0.35 ±0.01 op	
	KL-29	0.23 ±0.01 lm		2.29 ± 0.06 hı		2.53±0.03 b		0.50 ±0.01 g	
	KL-30	0.20 ±0.01op		2.31 ±0.06 h		1.00±0.02 m		0.31 ±0.01 q	
	KL-33	0.32 ±0.01 e		4.00 ±0.10 a		1.94±0.02 fg		0.52 ±0.01 ef	
Napoleon	KL-38	0.26 ±0.01 gh	0.31 ±0.09 A	2.72 ±0.07 e	2.88 ±0.54 A	2.58±0.03 b	1.97±0.31 A	0.61 ±0.01 a	0.46 ±0.11 A
	KL-47	0.26 ±0.01 hı		3.45 ±0.08 b		2.03±0.02 e		0.40 ±0.01 kl	
	KL-54	0.45 ±0.01 b		2.98 ±0.07 cd		2.62±0.03 b		0.62 ±0.02 a	
	KL-59	0.52 ±0.01 a		3.49 ±0.09 b		2.62±0.03 b		0.57 ±0.01 b	
	KL-60	0.33 ±0.01 d		2.90 ±0.07 d		2.31±0.03 c		0.51 ±0.01 fg	
	ADARA	0.28 ±0.01fg		2.69 ±0.07 ef		1.85±0.02 g		0.42 ±0.01 ıj	
	KL-11	0.19 ±0.01qr		1.65 ±0.04 o		0.58±0.01 o		0.19 ±0.00 v	
	KL-15	0.25 ±0.01ıjk		2.00 ±0.05 kl		1.06±0.01 m		0.26 ±0.01 rs	
	KL-29	0.18 ±0.00 qr		2.53 ±0.06 g		1.61±0.02 ı		0.34 ±0.01 p	
	KL-30	0.15 ±0.01 s		1.76 ±0.04 no		0.76±0.01 op		0.23 ±0.01 tu	
	KL-33	0.24 ±0.01 kl		3.04 ±0.07 c		1.47±0.02 jk		0.40 ±0.01 lm	
Starks Gold	KL-38	0.20 ±0.01 op	0.23 ±0.06 C	1.84 ± 0.05 mn	2.27 ±0.42 C	3.26±0.03 a	1.52±0.35 C	0.56 ±0.01 bc	0.35 ±0.10 C
	KL-47	0.20 ±0.01 op		2.62 ±0.06 efg		1.54±0.02 ıj		0.31 ±0.01 q	
	KL-54	0.34 ±0.01 d		2.27 ±0.06 hı		1.99±0.02 ef		0.47 ±0.01 h	
	KL-59	0.40 ±0.01 c		2.65 ±0.07 efg		1.99±0.02 ef		0.43 ±0.01 ıj	
	KL-60	0.22 ±0.01 mn		2.56 ±0.06 fg		1.07±0.01 m		0.31 ±0.01 q	
	ADARA	0.21 ±0.01 mn		2.04 ±0.05 k		1.41±0.02 kl		0.32 ±0.01 q	
	KL-11	0.22 ±0.01 m		1.91 ±0.05 lm		0.67±0.01 q		0.22 ±0.01 u	
	KL-15	0.24 ±0.01 ıjkl		1.83 ±0.04 mn		0.77±0.01 o		0.22 ±0.01 u	
	KL-29	0.20 ±0.01 op		2.01 ±0.05 kl		2.23±0.03 d		0.44 ±0.01 ı	
	KL-30	0.18 ±0.01 r		2.03 ±0.05 kl		0.88±0.01 n		0.27 ±0.01 rs	
	KL-33	0.28 ±0.29 f		3.57 ±0.09b		1.73±0.02 h		0.47 ±0.01 h	
Lambert	KL-38	0.23 ±0.01 lm	0.27 ±0.08 B	2.39 ±0.06 h	2.50 ±0.60 B	2.27±0.01 d	1.64±0.30 B	0.53 ±0.01 de	0.38 ±0.10 B
	KL-47	0.29 ±0.01 f		3.56 ±0.09 b		1.91±0.02 fg		0.38 ±0.01mn	
	KL-54	0.40 ±0.01 c		2.62 ±0.06 efg		2.30±0.03 cd		0.54 ±0.01cd	
	KL-59	0.46 ±0.01 b		3.07 ±0.08 c		2.31±0.03 cd		0.50 ±0.01fg	
	KL-60	0.17 ±0.00 r		2.12 ±0.05ıj		1.36±0.02 l		0.30 ±0.01q	
	ADARA	0.24 ±0.01 jkl		2.36 ±0.06 h		1.63±0.02 ı		0.37 ±0.01no	
LSD$_{0.05}$		**0.01****	**0.01****	**0.07****	**0.04****	**0.08****	**0.04****	**0.02****	**0.01****

Table 3. Leaf micronutrient contents of Napoleon, Starks Gold and Lambert sweet cherry cultivars grafted on rootstocks.

Variety	Rockstock	Fe (mg kg^{-1})		Mn (mg kg^{-1})		Cu (mg kg^{-1})		Zn (mg kg^{-1})	
	KL-11	67.06 ±0.51 e		38.65 ±0.95 ıj		13.97 ±0.10 l		15.58 ±0.38 lm	
	KL-15	72.84 ±0.30 b		37.43 ±0.92 j		10.58 ±0.13 n		24.61 ±0.85 a	
	KL-29	63.87 ±0.31 g		48.35 ±1.18 c		18.73 ±0.46 j		15.42 ±0.38 m	
	KL-30	61.13 ±0.12 lm		34.89 ±0.85 k		1.89 ±0.22 n		10.82 ±0.27 p	
	KL-33	62.27 ±0.28 ıjk		46.66 ±0.24 d		14.34 ±0.11 l		20.74 ±0.51 cd	
Napoleon	KL-38	65.19 ±0.16 f	67.19 ±4.31A	34.54 ±0.85 k	43.77 ±7.66 A	26.43 ±0.65 f	20.83 ±8.13 A	21.70 ±0.53b	18.78 ±4.66 A
	KL-47	62.61 ±0.22 hıj		34.33 ±0.84 k		13.99 ±0.07 l		10.83 ±0.27 p	
	KL-54	69.66 ±0.14 d		54.40 ±0.80 a		30.36 ±0.50 d		23.95 ±0.46 a	
	KL-59	70.29 ±0.21 d		54.92 ±0.56 a		31.87 ±0.53 c		20.19 ±0.37 de	
	KL-60	74.42 ±0.56 a		47.12 ±0.86 cd		30.63 ±0.26 d		21.29 ±0.40 bc	
	ADARA	69.76 ±0.20 d		50.16 ±0.86 b		27.34 ±0.30 e		21.50 ±0.40 bc	
	KL-11	54.72 ±0.17 r		29.37 ±0.72 mno		3.01 ±0.07 q		11.84 ±0.29 o	
	KL-15	53.12 ±0.26 s		28.44 ±0.70 o		14.24 ±0.10 l		21.30 ±0.64 bc	
	KL-29	63.27 ±0.04 gh		32.63 ±0.80 l		18.59 ±0.46 j		16.28 ±0.40 jkl	
	KL-30	55.25 ±0.42 qr		26.51 ±0.65 p		6.76 ±0.17 p		8.22 ±0.20 r	
	KL-33	62.66 ±0.42 hıj		48.26 ±0.61 c		13.30 ±0.08 m		15.76 ±0.39 klm	
Starks Gold	KL-38	62.95 ±0.21 hı	58.15 ±4.62 C	33.46 ±0.94 kl	35.35 ±7.15 B	19.01 ±0.47 j	17.02 ±9.42 C	16.01 ±0.39 jklm	15.54 ±4.20 C
	KL-47	49.11 ±0.32 t		26.09 ±0.64 p		2.22 ±0.06 r		8.23 ±0.20 r	
	KL-54	55.77 ±0.42 pq		40.63 ±0.60 gh		30.47 ±0.38 d		19.40 ±0.35 ef	
	KL-59	62.23 ±0.60 ıjk		39.42 ±0.43 hı		31.62 ±0.41 c		16.54 ±0.28 jk	
	KL-60	58.81 ±0.31 n		42.27 ±0.62 ef		23.59 ±0.21 ı		19.81 ±0.60 ef	
	ADARA	61.81 ±0.24 jk		41.72 ±0.66 efg		24.38 ±0.23 h		17.67 ±0.31 ı	
	KL-11	56.06 ±0.24 p		29.01 ±0.84 no		3.49 ±0.09 q		13.71 ±0.34 n	
	KL-15	55.14 ±0.19 qr		24.06 ±0.59 q		14.11 ±0.10 l		17.67 ±0.43 hı	
	KL-29	52.40 ±0.47 s		42.55 ±1.04 e		16.48 ±0.40k		13.57 ±0.33 n	
	KL-30	63.19 ±0.61 gh		30.70 ±0.75 m		7.82 ±0.19 o		9.52 ±0.23 q	
	KL-33	54.88 ±0.55 r		38.44 ±0.84 ıj		13.87 ±0.09 lm		18.52 ±0.45 g	
Lambert	KL-38	57.97 ±0.33 o	59.31 ±4.95 B	30.40 ±0.74 mn	35.07 ±6.76 B	23.26 ±0.57 ı	18.21 ±10.45 B	19.09 ±0.47 fg	16.18 ±3.86 B
	KL-47	57.88 ±0.33 o		26.02 ±0.64 p		3.01 ±0.07 q		9.62 ±0.24 q	
	KL-54	61.92 ±0.58 jk		40.51 ±0.70 gh		32.92 ±0.44 b		21.68 ±0.41 b	
	KL-59	71.28 ±0.15 c		42.17 ±0.49 ef		34.24 ±0.47 a		18.37 ±0.33 gh	
	KL-60	60.90 ±0.27 m		41.01 ±0.71 fg		25.20 ±0.25 g		16.73 ±0.29 j	
	ADARA	60.78 ±0.12 m		40.94 ±0.76 fg		25.86 ±0.27 f		19.52 ±0.36 ef	
$LSD_{0.05}$		**0.75****	**0.21****	**1.49****	**0.44****	**0.63****	**0.19****	**0.79****	**0.24****

Table 4. Chlorophyll concentrations and seedling growth status of Napoleon, Starks Gold and Lambert sweet cherry cvs. grafted on rootstocks

Variety	**Rockstock**	**Mean Chlorophyll (nmol cm²)**		**Rootstock Diameter (mm)**		**Scion Diameter (mm)**		**Sapling Height (cm)**	
	KL-11	36.81 ±1.45 jk		12.12 ±0.11 ghı		7.69 ±0.31 jkl		67.99 ±2.45 o	
	KL-15	36.77 ±0.64 jk		12.00 ±0.11 g-j		7.61 ±0.30 kl		67.32 ±2.42 o	
	KL-29	41.37 ±1.06 def		11.60 ±0.56 h-k		12.58 ±0.50 b		134.33 ±1.89 cd	
	KL-30	36.45 ±1.44 jk		8.13 ±0.21 n		9.34 ±0.30 ghı		102.19 ±2.80 j	
	KL-33	36.02 ±1.42 k		12.12 ±0.11 ghı		7.69 ±0.31 jkl		68.00 ±2.45 o	
Napoleon	KL-38	37.91 ±0.97 ıjk	40.03 ±4.17	12.76 ±0.70 d-g	11.95 ±2.02 A	15.46 ±0.90 a	10.61 ±2.54	145.66 ±2.49 a	106.88 ±28.43B
	KL-47	36.08 ±1.42 jk		8.12 ±0.17 n		8.93 ±0.50 hıj		96.05 ±4.78 k-n	
	KL-54	46.51 ±1.11 a		14.02 ±0.54 ab		12.81 ±0.56 b		135.66 ±1.70 bcd	
	KL-59	43.16 ±0.56 cde		13.56 ±0.82 a-d		10.97 ±0.98 ef		101.50 ±4.30 jk	
	KL-60	47.13 ±0.95 a		12.91 ±0.46 c-g		12.45 ±0.47 bc		131.00 ±4.32 def	
	ADARA	42.08 ±0.94 c-f		14.14 ±0.58 a		11.19 ±1.13 c-f		126.00 ±2.16 efg	
	KL-11	37.95 ±1.31 ıjk		11.64 ±0.15 h-k		8.99 ±0.21 hıj		93.74 ±1.43 n	
	KL-15	37.92 ±1.31 ıjk		11.52 ±0.15 h-k		8.91 ±0.20 ıj		118.28 ±1.80 ı	
	KL-29	40.96 ±1.04 efg		11.29 ±0.52 ıjkl		12.15 ±0.52 b-e		131.94 ±4.92 d	
	KL-30	37.58 ±1 30 ıjk		8.05 ±0.21 n		9.25 ±0.30 ghı		101.17 ±2.77 jkl	
	KL-33	37.14 ±1.28 ıjk		11.64 ±0.15 h-k		9.00 ±0.21 hı		94.05 ±1.05 n	
Starks Gold	KL-38	39.09 ±1.35 ghı	40.37 ±3.43	12.38 ±0.68 fgh	11.34 ±1.99 B	15.00 ±0.88 a	10.42 ±2.06	138.06 ±3.49 bc	106.79 ±20.34B
	KL-47	37.21 ±1.29 ıjk		8.04 ±0.16 n		8.84 ±0.49 ıjk		95.08 ±3.92 mn	
	KL-54	46.04 ±1.10 ab		13.59 ±0.53 a-d		12.42 ±0.55 bc		131.60 ±de	
	KL-59	42.73 ±0.55 c-f		13.15 ±0.80 b-f		10.64 ±0.95 f		98.45 ±3.20 j-n	
	KL-60	46.18 ±0.93 ab		9.49 ±0.26 m		8.37 ±0.09 ıjkl		72.66 ±1.25 o	
	ADARA	41.66 ±0.92 def		13.99 ±0.57 ab		11.08 ±1.12 def		124.74 ±2.22 gh	
	KL-11	37.18 ±0.65 ıjk		11.15 ±0.24 jkl		10.30 ±0.69 fg		119.48 ±1.22 hı	
	KL-15	37.14 ±1.47 ıjk		11.04 ±0.23 kl		10.20 ±0.68 fgh		118.28 ±1.93 ı	
	KL-29	40.96 ±1.05 efg		11.45 ±0.54 ıjk		12.36 ±0.51 bcd		133.17 ±3.06 cd	
	KL-30	36.81 ±1.45 jk		8.09 ±0.21 n		9.29 ±0.30 ghı		101.68 ±2.78 jk	
	KL-33	36.51 ±0.69 jk		11.16 ±0.24 jkl		10.30 ±0.69 fg		119.50 ±2.86 hı	
Lambert	KL-38	38.29 ±1.51 hıj	40.43 ±4.19	12.57 ±0.69 efg	11.39 ±1.98 B	15.23 ±0.89 a	10.75 ±2.16	140.12 ±2.38 ab	113.41 ±22.02A
	KL-47	36.44 ±1 29 jk		8.08 ±0.16 n		8.88 ±0.49 ıjk		95.57 ±3.96 lmn	
	KL-54	47.44 ±1.13 a		13.80 ±0.53 abc		12.62 ±0.56 b		133.96 ±1.53 cd	
	KL-59	44.03 ±0.57 bc		13.35 ±0.80 a-e		10.81 ±0.97 f		100.31 ±3.85 j-m	
	KL-60	46.64 ±0.94 a		10.53 ±0.10 l		7.09 ±0.02 l		60.00 ±2.94 p	
	ADARA	43.26 ±0.91 cd		14.06 ±0.57 a		11.37 ±1.12 def		125.37 ±2.19 fg	
$LSD_{0.05}$		**2.24****	**N.I.**	**0.89****	**0.27****	**1.27****	**N.I.**	**5.67****	**1.71****

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Table 5. Graft compatibility status among rootstocks and Napoleon, Starks Gold and Lambert sweet cherry cultivars

Variety	Rockstock	Translocated İncompatibility Symptoms	A	B	C	D	E
	KL-11	Ab	—	—	—	—	6
	KL-15	Ab	—	—	—	—	5
	KL-29	N	—	2	12	—	—
	KL-30	N	—	—	—	10	—
	KL-33	Ab	—	—	—	—	9
Napoleon	KL-38	N	—	—	1	14	—
	KL-47	N	—	—	—	8	—
	KL-54	N	14	1	—	—	—
	KL-59	N	15	—	—	—	—
	KL-60	N	13	2	—	—	—
	ADARA	N	15	—	—	—	—
	KL-11	Ab	—	—	—	—	7
	KL-15	Ab	—	—	—	—	5
	KL-29	N	—	—	10	—	—
	KL-30	N	—	—	—	9	—
	KL-33	N	—	—	—	—	8
Starks Gold	KL-38	N	—	—	—	15	—
	KL-47	N	—	—	—	6	—
	KL-54	N	12	3	—	—	—
	KL-59	N	11	4	—	—	—
	KL-60	N	15	—	—	—	—
	ADARA	N	15	—	—	—	—
	KL-11	Ab	—	—	—	—	6
	KL-15	Ab	—	—	—	—	5
	KL-29	N	—	—	11	—	—
	KL-30	N	—	—	—	7	—
	KL-33	Ab	—	—	—	—	8
Lambert	KL-38	N	—	—	3	12	—
	KL-47	N	—	—	—	—	—
	KL-54	N	15	—	—	7	—
	KL-59	N	15	—	—	—	—
	KL-60	N	15	—	—	—	—
	ADARA	N	15	—	—	—	—

cherry cultivars were found the highest in Napoleon/KL-59 and Lambert/KL-59 (0.52% and 0.46%, respectively). The lowest leaf K contents were found in Starks Gold sweet cherry cultivars grafted on KL-30 and KL-11 rootstocks (1.76% and 1.65%, respectively). Ca contents showed a very wide distribution. Starks Gold/KL-38 had the highest Ca content (1.63%), while Starks Gold/KL-11 had the lowest content (0.29%) (Table 2). In rootstock-scion combinations, leaf Fe contents ranged between the highest Napoleon/KL-60 (74.42 mg kg-1) and the lowest Starks Gold/KL-47 (49.11 mg kg-1). The lowest Mn contents were found in Lambert sweet cherry cultivar grafted on KL-47 and KL-15 rootstocks (26.02 mg kg-1 and 24.06 mg kg-1, respectively) (Table 3).

Histological Analyses

In the study, as a result of the longitudinal microscopic observations made in the combinations from which tissue samples were taken, cambial continuity was ensured at the junction point of rootstock and scion in the sweet cherry cultivars grafted on 4 rootstocks, including the control plant, there was no bending in the graft axis, and no thickening or deformation was observed in the phloem of the rootstock and scion. In the dyeing process with potassium iodide, homogeneous staining occurred in rootstock and scion regions (Figure 1). It was decided to classify these combinations as category A. In sweet cherry cultivars grafted on KL-11, KL-15 and KL-33 rootstocks, growth disorders and smaller than normal leaves occurred. In the cross-section analyses, thickening and deformations were observed in the phloem, and necrotic areas were determined also at the graft junction point. In the external examination of the wood tissue, thick black lines were observed along the graft junction line. Some saplings were also seen drying in the middle of the growing period. As a result of these observations, it was concluded that there is a translocated graft incompatibility with the cultivars grafted on these rootstocks (Table 5).

Rootstocks, statistically significantly affected the growth strength, leaf nutrients and chlorophyll contents of Napoleon, Starks Gold and Lambert sweet cherry cultivars grafted on them (P < 0.05). It was determined that three selected cultivars showed strong growth with Adara (Prunus cerasifera) rootstock (Table 4). It has been stated that rootstocks that show strong growth can perform well in soils poor, water-scarce, calcareous and with high pH (Gainza-Cortés et al., 2015).

Leaf chlorophyll contents are generally seen to be at medium and high levels (Table 4). It is thought that there is no problem in leaf chlorophyll contents due to the fact that rootstocks belonging to this species adapt well to soils with high lime and pH and areas with high ground water. Jimenes et al. (2007) measured the leaf chlorophyll contents of Stark Hardy Giant sweet cherry variety grafted on Adara (Prunus cerasifera) rootstock and reported that the leaves did not turn yellow in the following years and the yield was high.

Rootstocks and cultivars individually or combinations of these significantly affected leaf nutrient concentrations. Although some rootstock x cultivar combinations were in the same statistical group in terms of leaf nutrient amounts, some of the

Table 6. Correlation status of leaf nutrient contents of rootstocks

	P (%)	K (%)	Ca (%)	Mg (%)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Klorofil (nmol Chl cm^2)
P (%)									
K (%)	0.574**								
Ca (%)	0.514**	0.439**							
Mg (%)	0.621**	0.551**	0.942**						
Fe (ppm)	-0.143**	0.352**	-0.398**	-0.458**					
Mn (ppm	-0,560**	0.437**	0.495**	0.592**	-0.616**				
Cu (ppm)	0.626**	0.201	0.604**	0.654**	-0.525**	-0.657**			
Zn (ppm)	-0.565**	0.256*	0.390**	0.521**	0.506**	0.563**	0.684**		
Klorofil (nmol Chl cm^2)	0.433**	0.006	0.376**	0.373**	0.310**	0.567**	0.780**	0.475**	

**: *P < 0.01*, significant at the 1% level
*: *P < 0.05*, significant at the 5% level

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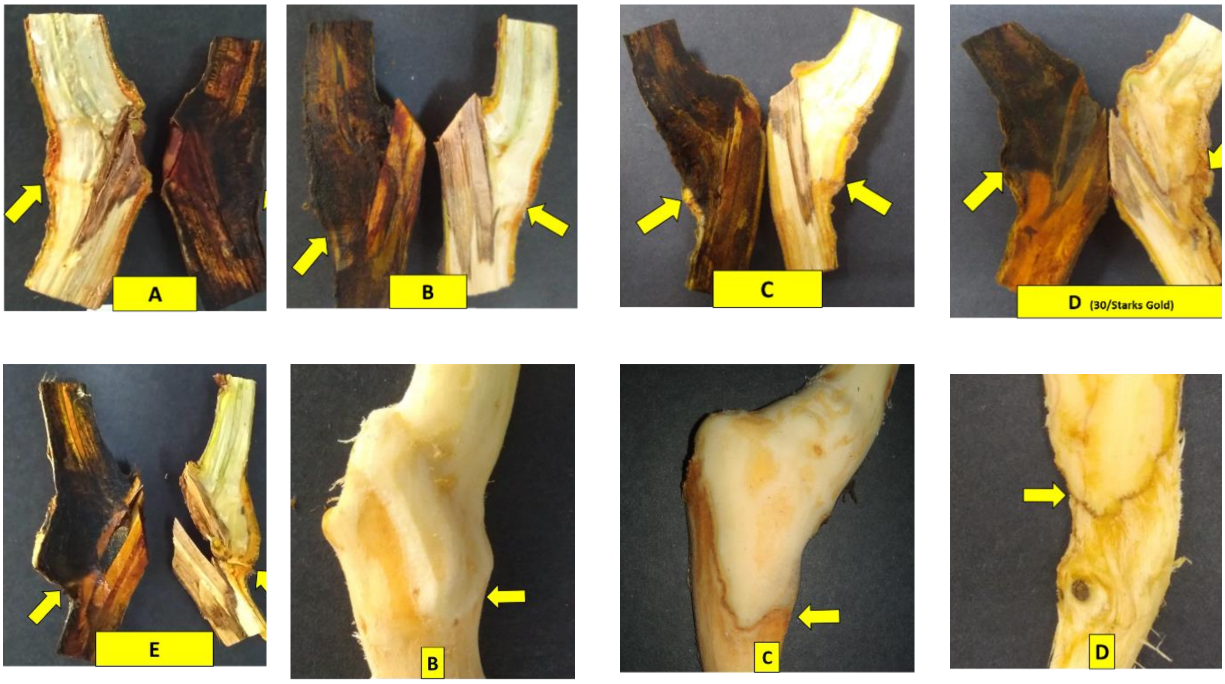

Figure 1. Images of different categories (A, B, C, D and E) from samples taken from the graft junction, accord

combinations contained quite different nutrient amounts. When looking at the individual effects of rootstocks and cultivars on nutrient concentrations of cherries, it is seen that they give variable responses to the different nutrients. The best rootstock x cultivar effect was observed in the rootstocks containing the most P (0.31±0.09 A), K (2.88±0.54 A), Ca (1.97±0.31 A) and Mg (0.46±0.11 A) in the Napoleon sweet cherry cultivar. It was determined that the most productive of these rootstocks were KL-59 in terms of P, KL-33 in terms of K, KL-59, KL-54 and KL-29 in terms of Ca and KL-54 and KL-38 in terms of Mg. The weakest rootstock x cultivar effect was observed in Starks Gold sweet cherry cultivar, in rootstocks containing P (0.23±0.06 C), K (2.27±0.42 C), Ca (1.52±0.35 C) and Mg (0.35±0.10 C).

Also among these rootstocks, it was determined that the most unproductive ones were KL-30 in terms of P and KL-11 in terms of K, Ca and Mg (Table 2). In microelements, the best rootstock x variety effect was observed in the rootstocks containing the most Fe (67.19 ±4.31 A), Mn (43.77 ±7.66 A), Cu (20.83 ±8.13 A) and Zn (18.78 ±4.66 A) in the Napoleon sweet cherry cultivar again. KL-60 (74.42 ±0.56 a) for Fe, KL-59 (54.92 ±0.56 a) and KL-54 (54.40 ±0.80 a) for Mn, KL-59 (31.87 ±0.53 c) for Cu, KL-15 (24.61 ±0.85 a) and KL-54 (23.95 ±0.46 a) for Zn were determined to be the most productive rootstocks.

The weakest rootstock x cultivar effect was observed in Starks Gold sweet cherry cultivar, in rootstocks containing Fe (58.15 ±4.62 C), Cu (17.02 ±9.42 C), and Zn (15.54 ±4.20 C). In terms of microelements, it was determined that the most inefficient rootstocks were KL-47 for Fe, KL-30 and KL-47 for Mn, KL-47 for Cu, and KL-30 and KL-47 for Zn (Table 3). It was determined that leaf Fe contents of three sweet cherry cultivars grafted on rootstocks were within the proficiency reference values determined by Leece and Van Den Ende (1975), but not at the desired level. Zn concentrations were also below the sufficiency level in some rootstocks. This is due to the fact that the P level is quite high. Because iron is in an antagonistic relationship with elements such as P (Aktaş & Ateş, 1998). It has also been reported that Mn can also negatively affect the uptake of Fe by rootstocks (Mengel & Kirkby, 2001). However, no obvious signs of chlorosis were observed.

Similar variable findings were also obtained in cultivars. Differences in leaf nutrient contents among cultivars and rootstocks can be attributed to the connatural nutrient uptake capacity of cultivars and rootstocks and their translocation in plants (Meland, 2010; Mestre et al., 2015). Clark & Gross (1986) reported that even plants grown under the same conditions can take in different amounts of nutrients. Again, the structure of the root system, density, surface area, cation exchange capacities, etc. affects the nutrient absorption capacity of the plant (Marschner, 2012). The diversity of root secretions and their properties depending on the rootstocks, and acidification and chelating properties of the rhizosphere region, might explain the presence of different amounts of nutrients in plants (Dakora & Phillips, 2002; Marschner, 2012). In addition, the resistance of a rootstock or cultivar against to biotic and abiotic stresses may provide an advantage for the plant to absorb more nutrients from the soil (Fazio et al., 2015). Another reason why the nutrient contents is different in the study may be differences in the plant size of the cultivars. Nutrient request and absorb usually increase with plant size and biomass (Mugasha et al., 2013; Peng et al., 2019). Again, the physiological requirement of the cultivars may also have had a significant impact on the nutrient demand.

Although there were significant differences between rootstock and cultivar and their combinations for some nutrient contents in leaves, we could not prominently see rootstock or cultivar or their combination on sweet cherry nutrient concentration. The reason may be the capacity of the greenhouse soil used in the experiment to provide nutrients. Because the nutrient concentrations of the soil were sufficient (Table 1). Almost all of the nutrients in the leaves were within the sufficiency limits (Jones et al., 1991) (Tables 2 and 3). This situation may have prevented the effect of rootstock x cultivar combinations on nutrient uptake capacity.

Positive and significant relationships were determined between plant nutrients and chlorophyll contents in all cultivars. The highest correlation value between chlorophyll and plant nutrient contents was found in Cu (r= 0.780**) (Table 6). Jimenes et al. (2007) also reported this important and strong correlation between chlorophyll content and Cu.

Rootstock-scion incompatibility between the selected and control rootstocks with sweet cherry cultivars gave different results (Table 5; Picture 1). While translocated incompatibility symptoms were found in some rootstocks, localized incompatibility symptoms were found in the majority (Reig et al., 2018b). While a sharp indication of incompatibility was observed in cultivars grafted on some rootstocks, Adara (P. cerasifera) and some selected rootstocks were observed to form a compatible combination with the variety (Figure 1). It can be said that there is no growth in the level of stunting in cherries grafted on rootstocks, but there is a moderate growth when compared to control rootstocks (Table 4).

Conclusion

It can be considered that it is necessary to work with nutrient-poor soils in order to reach definite conclusions about which rootstock and cultivar or their combinations are effective on the mineral nutrition of plant. Prunus cerasifera is a plum species that is closely related to Prunus avium or Prunus cerasus, which is good ability to be produced vegetatively, and can form healthy individuals in saturated with water and calcareous soils. It has been also concluded that this species has the potential to be used as an alternative to P. mahalep rootstock, especially in calcareous and with high pH soils where intensive sweet cherry cultivation cannot be done. This species, which spreads over a wide geography, will make a significant contribution to sweet cherry cultivation with larger capacity rootstock selection breeding programs.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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RESEARCH PAPER

Heavy metal (Cr, Cu, Ni, Pb, and Zn) contents of endemic *Salvia halophila* plants around Lake Tuz

Gökçen Baysal Furtana[1]*, Aynur Demir[2], Mehtap Tekşen[3], Adeleh Rashidi[4], Rukiye Tıpırdamaz[5]

[1]Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, Türkiye
[2]Department of Environmental Technology, Aksaray University, 68100 Aksaray, Türkiye
[3]Department of Biology, Faculty of Science and Letters, Aksaray University, 68100 Aksaray, Türkiye
[4]Department of Environmental Engineering, Faculty of Engineering, Hacettepe University, 06800 Ankara, Türkiye
[5]Department of Biology, Faculty of Science, Hacettepe University, 06800 Ankara, Türkiye

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Corresponding Author
Tel.: +90 312 202 1203
E-mail: gbaysal@gazi.edu.tr

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Abstract

Heavy metals occur naturally in ecosystems at varying concentrations. However, heavy metal sources that have emerged in present-day mainly due to human influence, i.e. industrial activities, agricultural waste, pesticides, use of fossil fuels and traffic, have included a part of heavy metals in the ecosystem. Lake Tuz, together with the entire lake surroundings, water beds and important steppe areas, was declared Turkey's Specially Protected Area (SPA) in 2001. Our aim in this investigation was to determine the levels of heavy metals such as Chrome (Cr), Copper (Cu), Nickel (Ni), Lead (Pb) and Zinc (Zn) in endemic *Salvia halophila* grown in different areas of Lake Tuz. The results of the heavy metal contents analyzed at the plant were compared with the international standard levels of heavy metals. The consequences displayed that differing extents of heavy metals are accumulated in *S. halophila*. The results obtained differed in accordance with the collection time and localities. When the outcomes are appraised, it is achievable to say that Pb is higher than the standard values. The findings of this investigation are the first reported results for this endemic *S. halophila* species that grows naturally at Lake Tuz and are important as they are newly discovered results.

Introduction

The problem of environmental pollution, which arises with the rise of heavy metals above the natural level, has increased significantly from the beginning of the industrial revolution to the present and continues. Heavy metals either essential or non-essential are naturally present in the ecosystem at trace concentrations. While heavy metals are found in harmless amounts and forms in nature, their amounts have reached harmful concentrations due to increasing industrialization, fossil fuels, road traffic, wrong agricultural techniques and pesticides in the last decades. The presence of heavy metals in the ecosystem is ecologically critical as they can transport to the biosphere in soil and water ecosystems and become toxic to all living organisms at certain concentrations. Also, the responses of plants to high levels of heavy metals in their growth media vary in a species-specific manner. Some plant species can be adversely affected and damaged by relatively smaller concentrations of heavy metals while others can tolerate extremely high concentrations (Srivastava et al., 2017; Srivastava et al., 2020; Nedjimi, 2021).

Heavy metals, which are found in excess in our environment, are an important source of problems for all living environments due to their permanence properties and pose a serious threat to human health (Capparos et al., 2022). As a result of many studies, it has been emphasized that the heavy metal concentration in the soil where the plants used for various purposes (such as food, medicinal and herbal tea) grow, will adversely affect human health (Barthwal et al., 2008; Chary et al., 2008; Capparos et al., 2022).

It was reported that many medicinal plants used in China can uptake harmful metals from the soil and accumulate them in their shoots and/or roots (Meng et al., 2022). The concentrations of Cu, Cd, Hg and Pb in the plants were above the permissible limits; for this reason, it was stated that it would be more accurate to use these plants to be used for medicinal purposes by producing them under controlled conditions instead of collecting them from nature (Meng et al., 2022).

Heavy metal (Pb, Cu, Zn, and Cd) accumulations in different organs of *Mentha piperita* L. *Salvia officinalis* L. and *Salvia sclarea* L. species collected at different growth periods from the industrial area near Plovdiv (Bulgaria) were investigated. The results revealed that the amounts of Pb, Zn, and Cd in the leaves were above the permissible limits. It has also been emphasized that these plants, which have the potential to be used as spice and herbal tea, can pose a great danger to human health if they are collected and used from the region (Angelova et al., 2006). In a different study conducted by the same researcher group, the potential to use *Salvia sclarea* L. collected from this region for the phytoremediation of the soils, where heavy metal pollution is observed, was investigated. According to the results, it has been reported that *S. sclarea* plant is a hyperaccumulator for Pb and an accumulator for Cd and Zn. It was emphasized that this plant has the potential to be used for phytoremediation of these soils, where heavy metal pollution problems are seen (Angelova et al., 2016).

It is known that halophyte plants, which represent 1% of the global plant wealth, can activate more than one tolerance mechanism against salinity. It is also reported that halophytes are better adapted than glycophytes to avoid the harmful effects of heavy metal accumulation. With these features, it has been emphasized by many researchers that halophytes have an important potential for the improvement of problem areas (Flowers & Colmer, 2008; Manousaki & Kalogerakis, 2011a; Manousaki & Kalogerakis, 2011b; Caparros et al., 2022; Peng et al., 2022). Li et al. (2019) reported that halophytes could be used for phytoremediation in both salt-affected and heavy metal-contaminated soils. According to the results of their study with halophyte *Halogeton glomeratus*, it was emphasized that this plant was a promising plant for soils with such problems.

Turkey's Authority for Specially Protected Areas (ASPA) conducts various studies so as to reduce the number of pollutants that may affect special areas for managing and protecting their different natural characteristics. In 2001, Lake Tuz was declared one of the Specially Protected Areas (SPA) of Turkey, including all of the lake surface, surrounding water beds and some of the important neighboring steppic areas. The lake is surrounded by salt marshes are the richest areas in terms of endemism and plant communities with halophyte characteristics (Baysal- Furtana et al., 2013). Although, it is such an important area, it has been facing a pollution problem, especially in the last decades. The main sources of pollution are pesticides, heavy metals, detergents, oil, and waste water, which are carried by drainage systems (Tuğ & Duman, 2010; Demir et al., 2021).

The *Lamiaceae* family is the third-largest family in Turkey. Also, Turkey has about 10% of all *Lamiaceae* members in the World. Salvia is one of the largest genera (945 species) in *Lamiaceae* and has a high endemism ratio (Celep & Dirmenci, 2017). *S. halophila*, a species belonging to this genus, is an endemic perennial herb in the Irano-Turanian phytogeographic region and is spread on the inland salt steppes of central and western Anatolia, Turkey. The essential oils in sage are of great medicinal importance (Gruenwald et al., 2004). For this reason, it is used in the pharmaceutical industry in many parts of the world. The antiseptic and antispasmodic properties of the essential oils increase the medicinal value of the plant. It is used in traditional medicine for colds, wound healing, stomach complaints, rheumatic pains, and liver diseases (Baytop, 1984; Sezik & Yeşilada, 1999). Due to its medicinal importance, its unconscious collection from nature causes great problems for the continuation of generations. *Salvia halophila*, which is distributed in a limited area in our country, is one of the endemic species under threat and is in the endangered (EN) (species with a very high risk of extinction in the wild) category (Kuşaksız, 2019). For this reason, it is very important to protect the biodiversity of endemic and economically important plants such as *S. halophila*.

In this study, we aimed to determine the heavy metals (Chrome [Cr], Copper [Cu], Nickel [Ni], Lead [Pb], and Zinc [Zn]) levels of *S. halophila*, an endemic plant that grows naturally in various parts of Lake Tuz. For this purpose, heavy metal contents in plant samples collected at different times in their natural areas were analyzed and the results were compared with international standard heavy metal levels. It is crucial that the findings of this investigation are the first reported results for this endemic species of Salvia located at Lake Tuz, Turkey.

Materials and Methods

Lake Tuz, which is a tectonic lake, is located in the vicinity of Ankara-Konya-Aksaray provinces of the Central Anatolia Region. It is the second-largest lake in Turkey, situated 905 m above the sea level. Although it has a large area (1500 km^2), it is a very shallow (0.5 to 1 m deep) lake, especially in the dry summer months when water evaporates in large quantities, its depth drops to 30 cm and a thick salt crust forms on its surface. The highest temperatures are recorded in June and September when the rain is minimal. The arid season begins at the end of June, continues for 3–4 months. The area is under the influence of a semiarid cold Mediterranean climate. Seventy-eight per cent of the soils in the basin are characterized as saline and alkaline. Consequently, they behave as suitable habitats for halophytic plants and a few glycophytic ecotypes (Baysal-Furtana et al., 2013).

Figure 1. Localities of *Salvia halophila* marked in Google Earth. The coordinates were determined by Global Positioning System (GPS)

The study areas are located in Eskil district of Aksaray province and Cihanbeyli district of Konya province, which are the natural habitats of *S. halophila* (Figure 2). Plant materials were collected from these areas at month intervals between June-September 2016 in accordance with the vegetation period. The aerial parts (shoots, leaves, flowers) of the three plant samples that best represent the population were regularly collected from designated localities (Eskil (N 38°24´-29´ E 33°26´-30´) and Cihanbeyli (N 38°32´-34´ E 38°06´-08´) at 4 different times (Figure 1). Fresh plant samples were kept in ice-box and immediately transferred in to laboratory. The contaminants such as dust and soil on the plant material were removed by sequential washing with dilute acid, tap water and distilled water. The samples were oven dried at 65°C and grinded to have homogenous alicots.

Then, 1 g of powdered samples were digested with 10 mL of concentrated HNO_3 (Merck, 64%) by means of a the microwave oven (EPASW-846 3051) and passed through a 0.45 µm (Minisart® NML Syringe Filters) filter and the volume was made up to 15 ml by adding distilled water. Finally, the heavy metal concentrations (Cr, Cu, Ni, Pb, Zn) of the digests were determined by Atomic Absorption Spectrophotometer (Perkin Elmer, AAnalyst 800 Atomic Absorption Spectrometer). The AAS were calibrated by a High Purity Standards brand QCS-27 series and an internal standard (10 ppb 209Bi) was used to increase the precision of the measurement that produced a calibration curve with a determination coefficient above 0.99.

Figure 2. General view of Salvia halophila

Taking into account the density and abundance ratios from the saline steppe vegetation, which is the habitat of *S. halophila*, from the areas where the vegetation is homogeneous, 3 representative individuals determined as the representative of the population were collected for analysis. Plant samples for analysis were collected from locations with at least 10 individuals in an area of 25 m^2, paying attention to the distribution, abundance and overlap of the population of the species, so that collection could be made from the same location at all sampling times. It is very difficult to collect numerical data from natural populations, but it may be possible to obtain results representative of the population by studies that can be planned under laboratory conditions with plant materials collected in limited amounts from natural areas. Plant samples were collected from localities at 4 different times. The experiments were set up in a factorial randomized block design. All analyses were carried out with 3 replications. The findings were evaluated with SPSS 23, ANOVA statistical methods. Analysis of variance (ANOVA) was performed to determine significant differences. Means were seperated using Duncan Multiple Range Test at $p < 0.05$.

Results and Discussion

Halophytes, which can easily live and reproduce in saline soils, can be ideal alternative phytormediators for saline soils exposed to heavy metal pollution (Flowers & Colmer, 2008; Manousaki & Kalogerakis, 2011a, 2011b; Wang et al., 2013). Salinity in the soil can also increase heavy metal mobility (Acosta et al., 2011; Manousaki et al., 2008). The resistance of halophytes to heavy metals may be related to the salt tolerance properties of these plants (Wang et al., 2013; Liang et al., 2017) because they use almost the same tolerance mechanisms for the heavy metals (Nedjimi, 2021).

In this study, the heavy metal (Cr, Cu, Ni, Pb and Zn) levels of *S. halophila*, which is an endemic halophytic plant that grows naturally in various parts of Lake Tuz, were determined. The results reveal that heavy metals such as Cr, Cu, Ni, Pb and Zn accumulated in varying amounts in *S. halophila* plants (Table 1). The table also presents recommended optimum levels according to FAO/WHO standards (EC, 2001; Srivastava et al., 2017). When the Table 1 showing the heavy metal concentration of the plant is examined, it can be seen that the accumulated heavy metal amounts varied in accordance with the collection time (as plant's growth period, season) and/or the locality.

According to the observed results, *S. halophila* was seen to accumulate Pb above the permissible limit at both locations. When the plant analyzes collected from the Cihanbeyli are evaluated; it is seen that the investigated metals in the plant are lower than the samples collected from Eskil. The highest concentration of Pb was recorded as 1.440 $\mu g.g^{-1}$ (±0.510) in the sample taken from Eskil in June (Table 1). According to the results, it is achievable to conclude that the amount of Pb determined in the samples collected from Eskil is above the standard values. The metal content of plant changes according to time and vegetation period. (Angelova et al., 2006; Naser et al., 2011; Mensah et al.,2008) accounted for that the concentration of Pb increased consistently during the growth period in lettuce plants. Nevertheless in our study, the highest Pb concentration was recorded in June, and it was found to be below this value in other investigated months. It has been reported by Naser et al. (2011) that the time-dependent changes in metal concentrations in plants differ according to the plant, its species and the type of metal. It was determined that there were significant differences in Pb concentrations of the examined plant depending on time ($p<0.05$) and locality ($p<0.01$) (Table 2).

Diacu et al. (2011) analyzed the cadmium and lead content of Sage- *Salvia officinalis* samples from the Prahova Valley region–Romania. They reported that the potential *S. officinalis* to be used as a detoxifier cannot be ignored due to its heavy metal accumulating capacity. Lead is unstable and can readily decompose into oxides, carbonates, and sulfates, often penetrating into soil through airborne deposition on roadsides (Wuana & Okieimen, 2011). The cause of Pb accumulation was thought to be due to the air pollution, the traffic emissions and domestic waste and energy. Also, Pb accumulation can be attributed to a high concentration in the soil where the plants grow.

The highest mean concentration of Ni was recorded as 0.583 $\mu g.g^{-1}$ (±0.161) in the sample taken from Cihanbeyli in September (Table 1). It has been observed that the accumulated Ni levels varied in accordance with the collection time and the locality. The time-dependent changes in the amount of the Ni in the plant samples collected from Eskil were not significant. However, time-dependent changes in the amount of the Ni in the plant samples collected from Cihanbeyli were significant ($p<0.05$) (Table 2). It has been reported that some plant groups are called "Ni hyperaccumulators" because they have the capacity to tolerate and store more than 1 $\mu g.g^{-1}$ Ni in their bodies (Baker et al., 1994), whereas the permissible limit set by FAO/WHO (Srivastava et al., 2017) in plants was 1.5 $\mu g.g^{-1}$. When the Ni concentrations given in Table 1 are examined, none of the samples examined in our study had a Ni value above 1.5 $\mu g.g^{-1}$.

In various studies conducted around Lake Tuz, it has been reported that especially the Pb and Ni ratios are quite high (Tuğ & Duman, 2010; Demir et al., 2021). In a similar study by Kılıç (2019), the heavy metal accumulation (Ni, Fe, Co, Mn) and the usability of *Calepina irregularis* species that naturally grown in Amasya, as a biomonitor was investigated. The heavy metal content in the roots, stems and leaves of the plant samples was evaluated. The samples were collected from 4 different areas: the city center, close to the highway, suburban and traffic-free (control) areas. According to the data obtained, it has been stated that the accumulation of heavy metals is higher in the leaves and root parts of the plant samples grown around the highway, and in the stems of the plants grown in the suburbs. Kılıç (2019) also stated that Ni and Mn may be found higher in plants, which are close to the highways due to air pollution because of traffic.

The highest mean concentration of Cr was recorded as 0.487 $\mu g.g^{-1}$ (±0.075) in the sample taken from Eskil in August (Table 1). When the results of the

Table 1. The concentrations (µg g^{-1}) of heavy metals (Cu, Cr, Ni, Pb and Zn) analyzed in *Salvia halophila*

Location	Date		Pb	Ni	Cr	Cu	Zn
Eskil	June	Range	0.930-1.440	0.160-0.300	0.214-0.380	0.420-0.453	0.560-1.550
		Mean	1.440	0.230	0.297	0.437	1.055
		Std. Dev.	0.510	0.070	0.083	0.017	0.495
	July	Range	0.480-0.870	0.220-0.600	0.105-0.230	0.230-0.430	0.660-1.220
		Mean	0.623	0.420	0.143	0.300	0.980
		Std. Dev.	0.215	0.121	0.075	0.113	0.288
	August	Range	0.880-0.940	0.105-0.660	0.210-0.770	0.330-1.100	1.060-1.600
		Mean	0.913	0.420	0.487	0.697	1.320
		Std. Dev.	0.215	0.191	0.075	0.113	0.288
	September	Range	0.740-1.500	0.100-0.520	0.250-0.660	0.120-1.200	1.030-2.020
		Mean	1.180	0.340	0.413	0.550	1.370
		Std. Dev.	0.215	0.191	0.075	0.113	0.288
Cihanbeyli	June	Range	0.105-0.700	0.105-0.140	0.311-0.455	0.370-0.530	0.140-0.950
		Mean	0.403	0.123	0.359	0.450	0.545
		Std. Dev.	0.198	0.018	0.083	0.080	0.205
	July	Range	0.130-0.162	0.150-0.340	0.110-0.270	0.228-0.700	0.490-0.950
		Mean	0.144	0.230	0.210	0.433	0.696
		Std. Dev.	0.016	0.098	0.087	0.142	0.194
	August	Range	0.370-0.660	0.200-0.300	0.120-0.350	0.330-0.880	0.660-0.980
		Mean	0.523	0.260	0.200	0.543	0.803
		Std. Dev.	0.146	0.053	0.095	0.195	0.103
	September	Range	0.200-0.320	0.400-0.700	0.100-0.550	0.150-0.410	0.940-1.060
		Mean	0.253	0.583	0.260	0.257	0.980
		Std. Dev.	0.061	0.161	0.152	0.096	0.069
	Optimum Value		0.3	1.5	1.5	10	50

*Optimum reference value declared by FAO/WHO (EC, 2001; Srivastava et al., 2017)

Table 2. ANOVA for variation of concentrations (µg g^{-1}) of heavy metals (Cu, Cr, Ni, Pb and Zn) analyzed in *Salvia halophila* by locality and/or time

Source of variance	df	Pb	Ni	Cr	Cu	Zn
Replication	2	ns	ns	ns	ns	ns
Locality (L)	1	**	ns	ns	ns	**
Time (T)	3	*	*	ns	ns	ns
L x T	3	ns	ns	ns	ns	ns
Error	12	0.082	0.023	0.028	0.085	0.130

ns: nonsignificant, ** and *, significant in 1% and 5% area (n=3) analysis were evaluated, it was determined that there were differences in the amount of Cr according to the collection time and the locality (Table 1). While time-dependent changes in the content of the Cr in the plant samples collected from Cihanbeyli were not significant, the differences between the samples collected from Eskil in July and those collected in August and September were significant. According to Allen (1989), the chromium level, which is toxic to plants, is 0.5 µg.g^{-1}, while according to Markert (1994), this limit has been reported as 1.5 µg.g^{-1}. In our study, the mean Cr concentration was generally below the limit level.

The highest mean concentration of Cu was recorded as 0.697 µg.g^{-1} (±0.113) in the sample taken from Eskil in August (Table 1). Although the amount of accumulated Cu varied according to the time (as plant's growth period, season) and location that the plants were collected, these differences were not significant (Table 2). The permissible limit set by FAO/WHO (Srivastava et al., 2017) in plants was 10 µg.g^{-1}. According to the results of the analysis we performed of the samples we collected, it was determined that the amount of Cu was below the limit values.

The highest mean concentration of Zn was recorded as 1.370 µg.g^{-1} (±0.288) in the sample taken from Eskil in September (Table 1). It has been observed that the accumulated Zn levels varied in accordance with the collection time and the locality (Table 1). It was determined that the difference depending on the locality was significant ($p<0.01$).

Due to a study conducted with *Celtis australis* L. in 40 different localities of Istanbul, it was determined that there was a direct correlation between the accumulation of investigated heavy metals (Pb, Cd, Cu and Zn), the traffic density and proximity to the roadside (Ozturk et al., 2017).

According to the study results of Demir et al. (2021), the main sources of heavy metal pollution observed around Lake Tuz are domestic and industrial wastes, sewage and septic wastes, volcanic dust and gases, agricultural activities, pesticide use, use of artificial fertilizers and traffic. They reported that Ni and Pb pollution from heavy metals examined in soil samples analyzed from Eskil and Cihanbeyli was higher than the others. Researchers attribute the reason for this pollution in the region to the fact that it is close to the main drainage channel carrying the industrial waste coming from Konya, that the surrounding districts are garbage collection areas, and that there is an open and irregular sewer system in parallel. Along with all these situations, it is thought that the location of both regions in the volcanic area is effective in heavy metal accumulation (Demir et al., 2021). It has been reported that the volcanic activity, dust particles blown by the wind and desert dust reaching the area are also important heavy metal sources (Nagajyoti et al., 2010; Srivastava et al., 2017).

In accordance with the results of the analysis, it was observed that the heaviest Ni, Cr, Cu and Zn accumulations were in August and September. This situation may be due to the high temperature and evaporation of the research area during these months. In the areas around Lake Tuz, it has been reported by Tuğ & Duman (2010) that the heavy metals and minerals that have become mobile rebound to the upper layers from deeper levels of the soil by evaporation with increasing temperature in summer. For this reason, an increase in heavy metal concentrations in the soil is observed during the summer.

Conclusion

The data we obtained from this study are critical as they are the first results presented to the scientific world for this endemic *Salvia halophila* species that grows naturally in Lake Tuz. The fact that our results can be used in similar studies in the future will both increase the economic and ecological value of the plant and determine its potential for use phytoremediation. Additionally, it is vital because it can provide the use of plants determined to have phytoremediation properties with such studies as a biomonitor that will allow monitoring of short-term changes in environmental pollution in the near future.

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Author Contributions

GBF: carried out the field study, collected the plant samples and prepared them for analysis, wrote manuscript (review and editing); **AD**: carried out conceptualization and statistical analysis; **MT**; carried out the field study, collected the plant samples; **AR:** carried out the analysis of the investigated heavy metals; **RT**: carried out conceptualization.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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RESEARCH PAPER

Short-term residual effect of municipal sewage sludge on the soil properties and potato yield

Mehmet Keçeci[1]*, Mustafa Usul[1], İbrahim Güçdemir[1], Veli Uygur[2]

[1]Soil, Fertilizer and Water Resources Central Research Institute, Ankara, Türkiye
[2]Department of Soil Science and Plant Nutrition, Agricultural Faculty, Isparta University of Applied Sciences, Isparta, Türkiye

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Corresponding Author
Tel.: + 90 312 315 6560
E-mail: mkececi@yahoo.com

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Abstract

Sewage sludge (SS) is one of the significant wastes of modern city lifestyle with environmental consequences. This study was conducted to determine the effect of the municipal sewage sludge on the potato plant's yield and nutrient elements and heavy metals content in a clay loam textured calcareous soil. The field treatment (corn-wheat-potato rotation) was implemented as fixed randomized blocks with 3 replications. The treatments were: control (without fertilizer), optimum fertilizer (OCF), 20, 40, 80, 100, and 120 ton ha^{-1} SS incorporation. The third-year results regarding the potato cultivation were presented. Results indicated that 4 ton ha^{-1} SS treatment resulted in a greater yield than the optimum fertilizer. The sewage sludge influenced the mineral nutrient composition of the leaves and the roots and the heavy metal concentrations in the plants were below the Turkish legal threshold values. Excessive application of SS had an inverse effect on the yield and reduced the essential nutrient concentrations of the leaves of the potato plants. This suggested that the residual effects of SS in the third year were considerable for either heavy metal concentrations or plant nutrients in the soil. Therefore, it can be concluded that a site-specific determination of the SS application rate is required to avoid potential deleterious effects of SS.

Introduction

The waste water treatment plants are significant infrastructure units for ensuring the public health in the modern city lifestyle and the number of the treatment plants is growing fast in the last decades in Turkey. Thus, sewage sludge which is the end product of these plants still has environmentally healthy and economically feasible disposal problems in the world and Turkey due to its harmful ingredients such as heavy metals (Tiruneh et al., 2014; Andreoli et al., 2007) and pathogenic and disease-causing microorganisms and other toxic compounds (Latare et al., 2014; Kotowska et al., 2012; Clarke et al., 2011). Application of sewage sludge to ag-lands, which may be a sought-after alternative for incineration method, is economical way of disposal as well as it has beneficial impacts on the soil ecosystem with some reservation (Snyman et al., 2000). For this reason, The European Union encourages the dilution of this potential environmental pollutant in the soil ecosystem to solve the problem (Kominko et al., 2018) by recycling-back it into the soil because the thermal methods are economically non-sustainable, limitation about CO_2 emissions, and accelerated nutrient cycling of organic compounds and nitrogen (Mathews & Tan, 2016). Alternatively, the SS biochar has being used for profiting its plant nutrient ingredients and for the recent years (de Figueiredo et al., 2020, 2021; Chagas et al., 2021; Fachini et al., 2021). The shortage in fertiliser resources and increasing global population leading food and fibre demand can result in alternative nutrient element sources such as SS with its current production which can meet as much as 20% of the phosphorus requirement of crop plants (http://p-rex.eu).

The recycling rate or agricultural use of SS was highly country dependent: it is 37% in the EU (EC, 2010), over 90% in Norway (Xu, 2014), about 60% in France, 57% in Belgium, as well as in Spain, UK, and Italy (EC, 2010; Wang, 2008). Whereas the regulations in Denmark, Germany, Sweden, and the Netherland are very strict about agricultural usage due to its contaminants. The characteristics and possible contaminants of SS are highly plant-dependent which is very much influenced by type and level of industrialisation, the lifestyle of the people in the hinterland of the plant, treatment technology, time of the year, etc. (Praspaliauskas & Pedisius, 2017). This nature of SS is the most significant restriction of it to safe and common usage in the ag-lands. However, their inorganic chemistry for any specific treatment plant would be similar and relatively low degree of variation at a specific period under certain treatment technology (Sharma et al., 2017; Suanon et al., 2017). Therefore, their chemistry and composition determine the potential usage of the sludge or disposal prerequisites (Praspaliauskas & Pedisius, 2017). The sewage sludge collected from non-industrial hinterlands can be recycled back to the agricultural lands more safely in terms of heavy metal contaminants and can be treated as valuable sources of plant nutrients and soil conditioner (Balanica et al., 2018; Sharma et al., 2017).

Due to high organic matter content, biosolids can improve/alter soil physico-chemical properties for example aggregate stability, bulk density, porosity, and water retention characteristics (Mujdeci et al., 2017; Wang et al., 2008) that affect nutrient balance in the soil (Brazauskiene et al., 2008; Wang et al., 2008). The incorporation of SS at differing rates promoted the growth performance parameters of broad bean (Eid et al., 2018), sugarcane (Nogueira et al., 2013), corn (Mahmoud et al., 2021), cowpea (Lopes et al., 2020) without exceeding the permitted heavy metal concentrations in soils. The sewage sludge applied at high rates increases the total heavy metal concentration in soils, however, the mobile fractions are more susceptible in coarse-textured than clay soils (Brazauskiene et al., 2008). Sewage sludge application followed by green manuring increased aboveground biomass and grain yield of corn in a mudflat soil where sludge amendment rates were detrimental for Cd and Ni concentrations in maize grain (Bai et al., 2017). This report suggested the sludge can be a remediation agent or initial fertility driver and/or soil conditioner for mudflat salt-soils or possibly salt-affected soils.

The main objectives of this study were: i) potential safely use of the biosolids, which were generated by the Wast Water Treatment Plant of İnegöl Organized Industrial Zone and which are brought into a state not harmful to the environment, in the agriculture within the context of agricultural use, ii) the determination of the fertilizer value and bio-soil regulating properties of the SS and their metallic and microbiological pollution factors, and iii) the investigation of the likely effects of the biosolids on the receiving environments.

Material and Method

Material

The domestic and urban SS or biosolids from the Treatment Plant of Inegöl Organized Industrial Zone, Bursa, Turkey was used as the organic material. Some chemical properties of the SS used in the experimental field are given in Table 1.

Table 1. Some chemical properties of sludge waste used in the field treatment

Parameters	Results	Parameters	Results
Moisture (%)	32.1	Total Fe (mg kg^{-1})	5523
Total nitrogen (%)	5.26	Total Ni (mg kg^{-1})	132
Organic matter (%)	57.9	Total Cr (mg kg^{-1})	227
Total potassium (%)	0.61	Total Pb (mg kg^{-1})	32.9
Total phosphorus (%)	1.42	Total Cd (mg kg^{-1})	2.00
pH (1/5)	6.9	Total Zn (mg kg^{-1})	225
EC (1/5) dS m^{-1}	4.15	Total Cu (mg kg^{-1})	360
		Total Mn (mg kg^{-1})	221

The data revealed that biosolids can be regarded as valuable nitrogen, phosphorus, and organic carbon source. The heavy metal concentrations of the biosolid are well in the safe range according to Turkish legislation for 'The Solid Waste Control Regulations'. This means the material can supply credible amounts of micronutrients towards treating micronutrient deficiency in calcareous soils, as the experimental soil. The pH of the biosolid has a slightly acidic nature and its salinity can be tolerated at typical application rates due to the richness of the SS in terms of ionic plant nutrients.

Method

The field trial was conducted in a fixed completely randomised block arrangement with three replications for three consecutive years. The treatments were: the control without any treatment (C), optimal chemical fertilisation (OCF, 120 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}), 20, 40, 80, 100, and 120 Mg ha^{-1} SS applications. The experiment was set up in a farmer's field in Yenikent, Inegol, Turkey. The SS was only incorporated in the first year, then its residual effects were followed. The chemical fertilizations were practiced for each growing season in the cropping sequence. The parcel sizes were 3 x 5 m. The SS was evenly distributed on each plot and then immediately incorporated into 0-15 cm depth by utilizing a rototiller. Then, the corn-wheat-potatoes rotation had being practiced for three consecutive years. Here, the third-year results regarding the potato cultivation were presented. Niğde Potato: Native potato produced from Nahita potato seed is a potato type widely produced in the Niğde region. Nahita potato, which aims to expand to the countries of the world from the lands of Turkey, is typical with high yield potential. Its texture and chemical composition are suitable for frying and other consumption types. Niğde potato is a potato planted in the spring and grown at low temperatures. The in-row and between-row spaces were 35 cm and 70 cm, respectively.

Soil Sampling and Analysis

Before experimental set-up, composite soil samples from the experimental field were taken from 0-20 cm and 20-40 cm depths. A plot-based sampling was made after the harvest from both depths. Collected samples were transported to the laboratory in plastic bags and immediately air-dried and passed through a 2 mm mesh sieve for characterization (Kacar, 2012). The physical and chemical characterisation of the soil samples were performed by the following common procedures for calcareous soils given by Kacar (2012): total soluble salts (TSS) and pH were measured in saturation paste, calcium carbonate equivalent (CCE) was determined by a manometric method using Scheibler calcimeter, organic matter (%) was determined by wet oxidation method of modified Walkley-Black with $K_2Cr_2O_7$, plant available phosphorus was extracted by 0.5 M $NaHCO_3$ at pH 8.5 and colorimetrically determined, plant available potassium was extracted by molar ammonium acetate and determined by a flame photometer, plant available cationic microelements (Fe, Cu, Zn, and Mn) were extracted by 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA at pH = 7.3 and the element concentrations of the extracts were determined by ICP-OES. For total element concentration determination, soil samples were wet-ashed (HNO_3 + HCl mixture, 3:1 V/V) and the digests were analysed for Ni, Cd, Pb, and Cr by AAS whereas total phosphorus (P) was colorimetrically measured by using a vanado-molybdate phosphoric yellow colour reagent.

Plant sampling

Youngest fully expanded leaf samples at the fourth and fifth on the main stem, counting down from the growth tip were taken from the mid-rows of each plot and immediately delivered to the laboratory in ice boxes. The possible contaminants were removed by successive washings with a dilute acid solution, tap water, and distilled water, respectively. Clean leaf samples were oven-dried to a constant weight at 65°C and homogenised by reducing the particle size below 0.5 mm. Scoops of 0.5 g leaf samples were acid digested (HNO_3:$HClO_4$ mixture, 4:1 V/V). Iron, Cu, Zn, Mn, Ni, Pb, Cd, and Cr concentrations of the digest were measured by AAS, and P concentrations were determined by a spectrophotometer (Kacar and Inal, 2010). Total nitrogen was determined by the conventional Kjeldahl method and steam distillation.

Statistical analysis

The data were subjected to one-way ANOVA after testing the normality of the data set. The mean separation between the treatments was performed by Duncan's multiple comparisons at p≤0.05 confidence level (Yurtsever, 1984).

Results and Discussion

Potatoes yield

There were no visual symptoms of excessively used SS treatments on the potatoes plant in the third growing season. The main effect of treatments on the yield was very significant (p<0.01) and it was possible to detect the optimal SS application rate. Besides 120 t ha^{-1} treatment and the control, the other SS application resulted in a similar yield that was comparable to the optimal chemical fertilization (Figure 1). 20 (18700 kg ha^{-1}), 40 t ha^{-1}, and 80 t ha^{-1} (16830 t ha^{-1}) treatments had an even higher yield. The yield was drastically decreased for the 120 t ha^{-1} SS treatment which was about 50% of the optimal fertilization. The increasing SS incorporation above the 40 t ha^{-1} resulted in a slightly decreasing trend above the optimal fertilization, but an apparent yield loss was observed above 100 t ha^{-1} SS treatment.

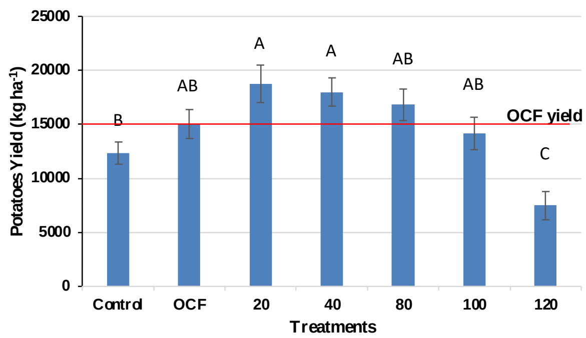

Figure 1. The effects of treatments on the potatoes yield. Different letters on the bar indicate significant difference

Nitrogen, P, and K are of most frequently deficient plant nutrients in agroecosystems. In terms of these nutrients, the SS treatments up to 80 t ha^{-1} can be regarded as a reasonable level of K addition since its concentration in the soil solution is buffered by the cation exchange mechanism, but N and P levels in excessive SS treatments were well above the possible plant uptake by the earlier crops in the rotation (Table 2). However, the experimental soils consist of very low levels of Olsen-P and possibly very high P adsorption capacity due to fine texture and high soil pH (Uygur, 2009), therefore the excessive application of SS-P can be tolerated even in the third year of the application. The available P contents were below 190 kg P_2O_5 ha^{-1} up to 80 t ha^{-1} SS treatments. Above this application rate, it reached up to 495 kg P_2O_5 ha^{-1} which is well above the optimal level (soil-P + Fertilizer should be about 120 kg P_2O_5 ha^{-1} (Gücdemir, 2006), even three years after the application, with possible deleterious effects. Nitrogen is the ultimately deficient nutrient in soils therefore it should be supplied to the plant in every growing season. Organic-N represents 99.8–99.9% of the total-N in SS and the amounts of mineral-N released in soils depended mainly on the decomposition of substances at a different rate in differing soil properties (Bertoncini et al., 2008). The higher soil pH resulted in the higher N mineralization rate from the SS either stabilised or digested. The SS incorporation into soils usually results in increased mineralization rates as readily decomposable organic matter and saprophytic micro-organisms are added (Boeira & Maximiliano, 2009; Tian et al., 2008). As excessive amounts of organic substances incorporated into soil mineralization became rate controlled. If the C: N ratio of the material favour the mineralization processes (i.e. a C: N ratio below 20 as in the current SS) and substantial amounts of N can volatilize. In the third year, relatively recalcitrant organic substances remain in the soil. Therefore, even though the added N (714-4286 kg ha^{-1}) was well above the plant uptake in the current crop sequence (Table 2) limited mineralization of recalcitrant organic substances was able to meet the N requirement of potato crop for 20-40 t ha^{-1} for the optimal yield in the third year. The larger application of SS, however, can supply excess N to the crop which leads to luxurious uptake or even toxic levels of N and possibly some other nutrients (Kacar, 2014).

Table 2. Some plant nutrients and total salt addition to the experimental field at differing treatments

Nutrients	OCF*	20	40	80	100	120
Nitrogen (kg ha^{-1})	360	714	1429	2857	3572	4286
Organic matter (kg ha^{-1})	N/A	7822	15644	31288	39110	46932
Potassium (kg ha^{-1})	N/A	83	166	331	414	497
Phosphorus (kg ha^{-1})	180	193	386	771	964	1157
Soluble salts (kg ha^{-1})**		902	1803	3607	4509	5410

*OCF, optimal chemical fertilization, ** the fertilizers mainly consist of soluble salts.

Soil properties in the third year

In the third year of the study, plot-based soil sampling was performed before the planting and after harvest. The results are provided in Table 3. The SS treatments had an impact on pH, TSS, Olsen-P, NH_4-Ac-extractable K, OM, DTPA-Zn, and total Cd, Cr, Ni, and Pb parameters (Table 3). The pH of the control and OCF plots were rather highly similar around 7.25 whereas the SS incorporated plots were apparently higher around 7.9. The rate-limited nature of the SS can result in relatively larger amounts of ammonia in even the third year after application which can be responsible for the elevated pH (Kacar, 2013). The SS material consisted of significant amounts of readily soluble nutrient elements. The calculated amounts of salt ranged between 902-5410 kg ha^{-1}. Therefore, it is an expected fact that the TTS parameter should increase as a function of application rate. However, even in the highest application rate (0.219%), the TTS was below the critical threshold of 0.35% which is equivalent to 4 dS m^{-1}. Even tough sensitive plants may be inversely affected by an EC above 2 dS m^{-1} (Usta, 1995). 5410 kg of readily soluble salt in 120 t ha^{-1} SS treatment (Table 2) can be equivalent to 2.16% of TSS in the upper layer (0-20 cm) of ordinary soil. However, the reactions of soluble ions with soil solution and solid phases after incorporation of SS into the soil, crop uptake in the preceding growth seasons, and further movements towards deeper layers may render its effect to tolerable levels.

The organic matter content of the soil which had an average of 1.82% in control and OCF plots, reached up to 3.30% in the 120 t ha^{-1} SS treatment. Theoretically, the added organic material in the SS treatments ranged between 7822-46932 kg ha^{-1} which can cause a net increase of 0.313-1.88%. The measured net OM increases, three years after its incorporation, were between 0.29-1.48% which can indicate that the SS was a relatively stable and reliable organic matter source with only 8.3-21.2% loss.

The experimental soil can be classified as K-rich soil. The addition of SS had further increased the portion of plant available K fraction from 1200 kg ha^{-1} to as high as 1530 kg ha^{-1}. Potassium amounting between 83-497 kg K_2O ha^{-1} were added with the SS treatments. The majority of the added K were likely in the readily soluble fraction in the SS and they were adsorbed by the cation exchange processes in the soil (Usta, 1995). Despite some uptake by preceding plants in the crop sequence, the plant availability was increased by a function of SS application rate. The Olsen-P (Olsen et al., 1954) concentration of experimental soil was well below the sufficiency threshold (about 120 kg P_2O_5 ha^{-1}). 193-1157 kg P_2O_5 ha^{-1} were added into the soil by SS treatments (Table 2). Even the smallest application rate was able to meet plant requirements, including the preceding plant in the crop sequence, by considering the fertilizer suggestion (Güçdemir, 2006). On the other hand,

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Table 3. Soil properties three years after the experimental set-up in potatoes growing season

Treatments	ST	CCE (%)	Saturation (%)	pH	TSS (%)	OM (%)	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Fe	Cu	Zn	Mn	Cd	Cr	Ni	Pb
									mg kg^{-1}							
Control	BP	14	64 CL	7.21	0.128	1.85	23	1260	14.5	4.82	0.97	16.6	0.09	0.21	1.06	2.13
	AH	12	65 CL	7.25	0.131	1.90	18	1200	8.22	3.77	0.60	6.32	0.08	0.23	1.09	2.43
OCF	BP	13	60 CL	7.24	0.120	1.67	33	1130	16.5	5.07	1.61	26.3	0.14	0.13	1.14	2.97
	AH	13	58 CL	7.28	0.130	1.87	25	1190	8.68	3.99	0.67	7.37	0.18	0.15	1.12	2.48
20 ton ha^{-1}	BP	14	63 CL	8.00	0.159	2.44	174	1190	17.8	5.84	2.21	27.4	0.16	0.18	1.48	3.05
	AH	12	60 CL	8.02	0.156	2.11	126	1220	11.5	4.12	0.91	8.42	0.21	0.20	1.49	3.28
40 Ton/da	BP	14	64 CL	8.04	0.139	2.17	117	1080	14.8	5.55	0.99	18.2	0.14	0.27	2.80	3.27
	AH	12	62 CL	8.09	0.180	2.51	116	1250	8.77	4.27	1.17	6.38	0.17	0.33	2.85	3.32
80 ton ha^{-1}	BP	14	64 CL	7.95	0.171	2.34	225	1330	10.9	5.54	1.76	25.9	0.24	0.43	3.52	3.35
	AH	12	63 CL	7.85	0.198	2.66	168	1400	9.53	4.88	1.89	7.23	0.38	0.43	3.75	3.69
100 ton ha^{-1}	BP	14	65 CL	7.78	0.182	2.58	470	1140	18.5	6.43	3.73	28.2	0.18	0.39	3.78	2.98
	AH	13	62 CL	7.78	0.224	2.74	260	1330	9.68	5.01	2.10	8.52	0.49	0.50	3.88	3.47
120 ton ha^{-1}	BP	13	65 CL	7.89	0.219	3.30	495	1420	21.8	7.48	5.82	46.7	0.28	0.48	3.81	3.43
	AH	12	64 CL	7.90	0.213	3.18	229	1530	13.2	4.92	2.55	16.1	0.45	0.54	3.91	3.74

T sampling time, BP before planting, AH after harvest, CCE calcium carbonate equivalent, TTS total soluble salts, OM organic matter, OCF optimum chemical fertilization

organic matter, humic substances, and low molecular weight organic acid treatments can facilitate to increase in the availability of indigenous soil-P (Oral & Uygur, 2018; Uygur & Karabatak, 2009). Therefore, adding such substantial amounts of organic matter to a soil rich in P can further increase the availability of P. It reached up to 495 kg P_2O_5 ha^{-1} (Table 3) which may be regarded as an environmentally risky concentration. In general, the P availability was higher before planting than the ones after planting (Table 3) which may be related to differences in the redox potential of soil around the sampling time. Reducing conditions in the spring season can increase the availability of P whereas watertight summer season conditions can promote Ca-related recalcitrant P forms (Mahdi & Uygur, 2018).

The concentrations of DTPA-extractable cationic microelements (Fe, Cu, Mn, and Zn) were given in Table 3. They were above the deficiency threshold, 4.5, 0.21, and 0.5 mg kg^{-1} for Fe, Cu, and Zn, respectively (Lindsay & Norvell, 1978), at both sapling times. However, the concentrations of Mn were above the threshold (14 mg kg^{-1}) in the pre-planting samples whereas after harvest samples were below the sufficiency threshold, besides 120 ton ha^{-1} SS treatment. Micronutrient availabilities were apparently higher at before planting samples. The soil was wetter in the winter-spring period which resulted in more reduced environment that solubilizes oxides of Fe and Mn. These processes increase soil solution concentration and decrease soil pH towards neutrality which was promoted by organic matter addition (Ören et al., 2018). In a relatively drier and hot period, the oxidation condition operates the availability of the elements. During this period recrystallization of amorphous oxides stabilize, co-precipitate, occlude or adsorb the micronutrients with stronger bonding energy that results in lower extractability and bioavailability. Despite huge amounts of SS application, the micronutrient bioavailability did not exceed the critical toxicity levels for the DTPA-extractable concentrations which were 19, 118, and 3.4 mg kg^{-1} for Cu, Zn, and Cd in clayey soils, and 25 and 271 mg kg^{-1} for Cu and Zn respectively in the clay loam soils (Gedikoğlu et al., 1998). The application rate has notably elevated the availability above 100 t ha^{-1} SS incorporations due to micronutrient contents of SS and increased amounts of organic matter addition which can chelate with micronutrients along with other heavy metals.

Total concentrations of nonessential heavy metals (Cd, Cr, Ni, and Pb) in the soils were to increase as a function of SS application rate. However, they were well below the threshold values in Turkish legislation (Anonymous, 2001). Therefore, the currently used treatments resulting in comparable plant performances to OCF treatment can be regarded as environmentally safe.

Nutrient composition of leaves

The effects of treatments on the nutrient element composition of potato leaves were given in Table 4. The sufficiency limits for the nutrients at the beginning of the flowering period growth stage (Reuter et al., 1986) and the toxicity limits for some elements (Sener et al., 1994) are also provided. Besides N concentration all of the nutrients were within the sufficiency limits or just around these limits. Iron concentrations were exceeding the upper limit of the sufficiency threshold

Table 4. Elemental composition of potato leaves

Treatments	N	P	K	Fe	Cu	Zn	Mn	Pb	Cd	Cr	Ni
		%					mg kg^{-1}				
Control	1.91	0.23	3.17	942	23.5	28.0	69.6	0.58	0.16	0.45	1.79
OCF	2.31	0.27	4.42	1043	23.57	25.6	56.1	0.32	0.38	0.53	2.69
20 ton ha^{-1}	2.04	0.25	3.74	975	20.80	18.8	58.0	0.43	0.35	0.41	3.36
40 ton ha^{-1}	2.10	0.24	4.06	851	17.14	28.1	57.5	0.65	0.44	0.52	3.49
80 ton ha^{-1}	2.53	0.22	3.41	855	10.98	19.5	56.2	0.69	0.30	0.61	3.96
100 ton ha^{-1}	2.57	0.29	3.18	988	19.83	24.6	59.1	0.76	0.32	0.69	4.50
120 ton ha^{-1}	2.25	0.19	4.00	997	21.68	23.2	65.2	0.87	0.38	0.73	4.48
ONC	4-5	0.2-0.4	3.5-5	70-150	6-20	15-20	40-300	-	-	-	-
Toxic level	-	-	-	-	-	700	425				

OCF Optimal chemical fertilization, ONC optimal nutrient concentrations

suggesting a general reducing condition during the entire growth period (Ören et al., 2018). Incorporation of large amounts of fresh carbon sources along with excessive Fe (5523 mg kg^{-1}) in the SS such as SS up to 120 t ha^{-1} can even support the redox reaction increasing Fe availability (Lindsay, 2001) higher than the redox sensitive Mn.

The deficiency of N in all treatments was likely to be related to the sampling time difference between the study and reported limits (Reuter et al., 1986). In general, the N concentration of leaves reaches a maximum around the end of the growth stage III where flowering and tuber formation starts (Thornton, 2020). From initiation of growth stage IV to maturation the leaf N concentration is to decrease upon translocation of the element towards tubers. Therefore, late leaf sampling resulted in N concentration below the sufficiency level. Otherwise, no plant showing extreme starvation, i.e. about 50% of the optimal level (Table 4), for any element cannot complete the life cycle with economically feasible yield. However, there are reports in the literature that the N content of potatoes can range between 0.5-6.4% depending on the sampling time and plant organ (Hopkins et al., 2020).

There was no extreme accumulation of essential nutrient elements in potato tubers. Most of the treatment-induced nutrient contents were highly similar to those of OCF treatment (Table 5). There was also no clear fact suggesting the heavy metal accumulation in the tuber. Therefore, the potatoes produced using SS were chemically safe for human nutrition. The significant point here is the plant regulates ion translocation to generative organs to ensure the formation of the next generations. Therefore, even if the growth media and subsequently the leaves have a high concentration of any element, the accumulation of potentially hazardous elements in generative organs are limited by the plants through differing mechanisms.

Conclusion

The effect of sewage sludge application on the yield and mineral composition of soil, plant, and tuber was evaluated in the third year after the treatments. Up to 80 ton ha^{-1} application rates SS may be incorporated into clay loam soil without any visual or analytic side-effects since comparable yield was obtained to OCF. In terms of potato yield, 20 ton ha^{-1} SS may be suggested for sustainable crop yield. Due to the potential risks of using SS, it should be taken special care for subsequent use in the same field to avoid the potential accumulation of either nutrient elements or heavy metals. Excessive usage of SS does not lead to a toxic concentration of any elements in any parts of the potato including the edible parts but excessive and repeated usage can cause environmental problems with potential risks in the plant as well. To solve this problem, the residual effects of the SS treatments should be followed for a longer period with repeated treatments.

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Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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RESEARCH PAPER

Effect of N fertilizing on gas exchange, leaf photosynthetic performance and nutrient concentrations of Sweet Cherry cv. 0900 ziraat

Kadir Uçgun[1]*, Bahar Türkeli[2], Murat Cansu[2], Mesut Altindal[2]

[1]Department of Plant and Animal Production, Technical Sciences Vocational School, Karamanoğlu Mehmetbey University, 70200 Karaman, Türkiye
[2]Fruit Research Institute, 32500 Isparta, Türkiye

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Corresponding Author
Tel.: +90 338 226 2000
E-mail: kadirucgun@gmail.com

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Abstract

In this study, the effect of nitrogen (N) fertilization on gas exchange and the photosynthetic performance of cherry leaves were investigated. In the study, 4 different doses of N were applied from the soil, and N, P, K, Ca, Mg, Fe, Mn, Zn, and B concentrations were determined in leaf samples taken from the middle part of the shoots 65-70 days after full flowering. Assimilation rate (A), the concentration of intercellular CO_2 (Ci), transpiration rate (Tr), stomatal conductance to water vapor (Gsw), total conductance to CO_2 (Gtc), and total conductance to water vapor (Gtw) were measured simultaneously with leaf collection for mineral analysis. Leaf water use efficiency (WUE) and instantaneous carboxylation efficiency (ICE) were calculated. N fertilizing affected the leaf accumulation of some macro (N, P, K, Ca, and Mg) and micro (B) nutrients. As N doses increased, N content of leaf increased, while decreasing leaf P, K, and B contents. N fertilizing negatively affected Tr, A, Gsw, Gtw, Gtc, and ICE. While there were negative correlations between leaf N concentration and gas exchange and leaf photosynthetic performance, they were positive for P and K. It means that changes in gas exchange and leaf photosynthetic performance were not related to increasing leaf N concentration, but decreasing leaf K and/or P concentrations depending on N fertilizing.

Introduction

Photosynthesis is a very important processes to acquisition carbon supply for plant growth and development (Cheng & Fuchigami, 2000). The epidermis of leaf is covered with a waxy cuticle to prevent water loss. But it lets diffusion of atmospheric CO_2 toward the inner photosynthetic tissues. Gas exchanges are primarily realized through stomata (Lebaudy et al., 2008). Stomata regulates leaf gas exchange (the uptake of CO_2) and the loss of water vapor in response to changing environmental conditions (Hetherington, 2001). The stomata occupy a central position in the pathways for both the loss of water from plants and the exchange of CO_2. It is commonly assumed that they therefore provide the main short-term control of both transpiration and photosynthesis (Jones, 1998).

Sweet cherry trees, like others fruit trees, need various nutrients to grow and produce high yields. Nitrogen (N), phosphorus (P) and potassium (K) are the most important nutrients and mineral nutrition can markedly affect photosynthesis (Longstreth & Nobel, 1980). Bottrill et al. (1970) stated that all nutrient disorders, excluding iron (Fe) and molybdenum (Mo), inhibit photosynthesis when chlorophyll was the basis of their calculation; manganese (Mn)-, copper (Cu)-, P-, and K-deficient plants had the greatest depression. Use of mineral fertilizer is the quickest way of increasing crop production. It is clear that the level of fertilizer applied influenced many processes like fruit quality in orchards (Bybordi, 2013). Hasanuzzaman et al. (2018) pointed that among all nutrients, K is one of the most vital elements used for plant growth and physiology. Physiological processes such as stomatal regulation and photosynthesis depend on K. Wang et al. (2012) reported that K deficiency increased the root abscisic acid (ABA) concentration of cotton. Hetherington (2001) showed that ABA regulates the aperture of stomatal pores. Basile et al. (2003) proved that K deficiency affected the leaf photosynthetic capacity through biochemical limitations.

Cultivars and rootstocks show different responses to nutrient deficiencies. Hu et al. (2016) realized that effect of K fertilization was more important on photosynthesis, chlorophyll fluorescence, and carbohydrates contents in sensitive-K cultivar of cotton. Fallahi et al. (2001) investigated effect of rootstocks on net photosynthesis, leaf nutrition of apple trees and determined rootstock was important on leaf photosynthesis and leaf mineral concentrations.

Boussadia et al. (2015) stated that most limiting factor for tree growth is N deficiency, which induced stunted growth and reduced yield and poor product quality. According to Cheng & Fuchigami (2002), N and carbohydrate metabolism are interrelated, and carbon assimilation depends on N metabolism to meet the needs of the photosynthetic machinery. There is a negative linear relationship between tree N concentration and total nonstructural carbohydrates concentration. Cheng & Fuchigami (2000) express that calculated intercellular CO_2 concentration, tended to decrease with increases in leaf N, indicating that stomatal conductance did not limit photosynthesis in leaves with low N concentration. Like K and N, P also affects some physiological process. Lauer et al. (1989) determined that low phosphate nutrition results in increased chlorophyll fluorescence, reduced photosynthetic rate, accumulation of starch and sucrose in leaves, and low crop yields. Bernardi et al. (2015) studied the effect of different doses of N, P, and K on photosynthesis. The results showed that the high levels of N photosynthesis negatively. When K was applied at intermediate fertilization levels, it had positive effects, but P had little effect.

The aim of this study was to investigate the effect of increasing N doses applied to soil on gas exchange and leaf photosynthetic performance of sweet cherry trees.

Material and Method

We carried out this study with '0900 Ziraat' sweet cherry cultivar grafted on Gisela 5 rootstocks. The experiment was carried out according to "Randomized Complete Block" design as 6 replicates and one tree in each replicate. We used 24 trees with 4 different N doses and 6 replications in each N dose. We planted the orchard used for the experiment in 2008 at 5x2 m planting distances. The orchard was full yield in 2014 and treatments were applied in 2015, 2016, 2017 and 2018, but took measurements only in 2018.

Ammonium nitrate (33.0.0), monopotassium phosphate (0.52.34) and potassium sulphate (0.0.50) used as fertilizer sources. Fertilizers were applied between April and June at approximately 15-day intervals (19th of April, 3rd of May, 24th of May and 7th of June). The required amounts were weighed for each tree at the recommended dose (0, 50, 125 and 250 g N) dissolved in water and applied beneath the tree canopy in 4 different periods. K (125 g K_2O/tree) and P (50 g P_2O_5/tree) were stable in all treatments.

Plant analysis: Leaf samples were collected 65 to 70 days after full bloom from the middle part of the shoots. Firstly, leaf samples were washed through tap water, then washed through HCl (0.1 normality) and finally washed with deionized water. We placed them in paper bags and dried at 65-70 °C in a drying chamber until a constant weight (for about 48 hours). Dried leaves were ground and weighed to determine N, P, K, Ca, Mg, Fe, Mn, Zn and B concentrations. Kjeldahl wet digestion (for N) and dry ashing methods (for P, K, Ca, Mg, Fe, Mn, Zn and B) were carried out the extraction of nutrients (Ryan et al., 2001) and determined by ICP-OES.

In the middle of the vegetative period, we carried out gas exchange and leaf photosynthetic measurements simultaneously and collected leaves for mineral analysis. For gas exchange and leaf photosynthetic, assimilation rate (A), concentration of intercellular CO_2 (Ci), transpiration rate (Tr), stomatal conductance to water vapor (Gsw), total conductance to CO_2 (Gtc), total conductance to water vapor (Gtw) were taken from the third fully expanded upper leaves between 10:00-11:00 am using Li-Cor 6800 Photosynthesis System (Li-Cor, Lincoln, NE, USA). Measurement conditions: photosynthetic photon flux density, 1000 μmol_{photon} m^{-2} s^{-1}, operational or chamber ambient CO_2 concentration, 400 $\mu molCO_2$ mol_{air}^{-1}. Leaf temperature and leaf to air vapor pressure deficit ranged from 25.6 to 28.8 °C and from 1.22 to 3.06 kPa, 27 °C, respectively. Leaf water use efficiency (WUE) and instantaneous carboxylation efficiency (ICE) were calculated respectively using the formula of WUE=A/Tr and ICE= (A/Ci).

Table 1. Effects of nitrogen treatments on leaf nutrient concentration of sweet cherry cv. 0900 Ziraat grafted on Gisela 5 rootstock and reaching full yield. All results were based on dry weight.

N doses (g/tree)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
0	1.84 ± 0.054 c	0.35 ± 0.035 a	2.10 ± 0.12 a	2.37 ± 0.085 a	0.48 ± 0.031 a
50	1.91 ± 0.048 c	0.26 ±0.012 b	2.16 ± 0.11 a	2.02 ± 0.075 b	0.44 ± 0.021 b
125	2.15 ± 0.081 b	0.18 ± 0.004 c	1.67 ± 0.078 b	2.13 ± 0.077 ab	0.47 ± 0.016 ab
250	2.43 ± 0.047 a	0.18 ± 0.004 c	1.48 ± 0.060 b	2.33 ± 0.097 a	0.50 ± 0.018 a
P value	P<0.01	P<0.01	P<0.01	P<0.05	P<0.05
N doses (g/tree)	**Fe (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Zn (mg kg^{-1})**	**B (mg kg^{-1})**
0	106 ± 6.27	10.2 ± 0.48	20.5 ± 1.87	11.0 ± 0.49	75 ± 4.22 a
50	107 ± 8.19	11.5 ± 0.79	23.8 ± 2.97	11.3 ± 0.73	73 ± 5.05 a
125	103 ± 8.45	11.0 ± 0.78	27.9 ± 4.34	11.5 ± 1.34	67 ± 5.56 b
250	101 ± 4.99	10.3 ± 0.26	29.3 ± 4.20	10.9 ± 0.49	66 ± 4.36 b
P value	NS	NS	NS	NS	P<0.01

NS: non-significant, ±: standard error mean

Table 2. Effects of nitrogen treatments on leaf photosynthetic performance of sweet cherry cv. 0900 Ziraat grafted on Gisela 5 rootstock and reaching full yield

N doses (g/tree)	Tr mmol m^{-2} s^{-1}	A (μmol m^{-2} s^{-1})	Ci μmol mol^{-1}	Gsw mol m^{-2} s^{-1}
0	2.03 ± 0.31 a	8.67 ± 0.80 a	230 ±14.94	0.099 ± 0.016 a
50	1.62 ± 0.12 b	6.85 ± 0.38 b	236 ± 8.70	0.074 ± 0.005 b
125	1.19 ± 0.19 c	5.96 ± 0.93 b	223 ± 9.17	0.055 ± 0.008 bc
250	0.91 ± 0.12 c	4.34 ± 0.55 c	216 ± 6.41	0.041 ± 0.006 c
P value	P<0.01	P<0.01	NS	P<0.01
N doses (g/tree)	**Gtw mol m^{-2} s^{-1}**	**Gtc mol m^{-2} s^{-1}**	**WUE**	**ICE**
0	0.096 ± 0.015 a	0.060 ± 0.009 a	4.61 ±0.42	0.038 ±0.002 a
50	0.073 ± 0.005 b	0.046 ±0.003 b	4.35 ± 0.21	0.029 ± 0.002 b
125	0.054 ± 0.008 bc	0.034 ±0.005 bc	5.38 ± 0.85	0.028 ± 0.005 b
250	0.041 ± 0.006 c	0.026 ±0.003 c	4.76 ± 0.11	0.020 ±0.002 c
P value	P<0.01	P<0.01	NS	P<0.01

NS: non-significant, ±: standard error mean, A: assimilation rate, Ci: concentration of intercellular CO_2, Tr: transpiration rate, Gsw: stomatal conductance to water vapor, Gtc: total conductance to CO_2, Gtw: total conductance to water vapor, WUE: water use efficiency, ICE: instantaneous carboxylation efficiency

Statistical analysis: Data means were separated using one-way ANOVA with "JMP© 8.0" (SAS Institute, Inc.) according to LSD (Least Square Difference). Statistical differences based on *P*<0.05 and *P*<0.01. In addition, with pairwise correlations between the nutrients and physiological parameters were examined.

Results and Discussion

Nitrogen fertilization applied to the soil at different doses affected N, P, K, Ca, Mg and B leaf accumulations. As N doses increased, N concentration of leaves increased, but decreased nutrients such as P, K and B. While the highest N values (2.43%) were obtained from the highest N dose (250 g N/tree), the lowest values (1.84%) resulted from the lowest N dose (0 g N/tree). The situation contrasted with P, K and B concentrations of leaves and the highest values of P, K and B were obtained at the lowest N dose. In other words, while regression between leaf N and increasing N doses was linear and positive, it was linear and negative for P, K, B. Ca, and Mg had the same trend and we obtained the highest values of Ca and Mg at 0 g N/tree and 250 g N/tree treatments (Table 1). Assimilation rate (A), transpiration rate (Tr), stomatal conductance to water vapor (Gsw), total conductance to CO_2 (Gtc), and instantaneous carboxylation efficiency (ICE) were also affected from increasing N doses and while the highest values were determined at the lowest N dose, and the lowest ones were in the highest N dose (Table 2). According to correlation analysis, of all nutrients, N, P, K and Mg had effect on gas exchange and leaf photosynthetic performance. While correlations were negative for leaf N and Mg concentrations, they were positive for P and K (Table 3).

Fallahi et al. (2001a), Klein (2002), Prsa et al. (2007) and Souza et al. (2013) reported that as N supply increase, it results in high N concentration in the leaves. Fallahi et al. (1984) reported that they fertilized apple trees with N applied to the soil and determined increasing Mg concentration and reducing K and P concentration in apple leaves. Neilsen et al. (1984) realized N, which is applied from soil at different doses, increased the N and Mn concentration of the apple leaves. Klein et al. (1989) informed that fertilizer N applied to the soil reduced significantly the K amount of soil solution in top soil layer (0-30 cm) and the according to increasing N dose, N concentration of leaf increased. Neilsen et al. (1999) found that the N concentration of the leaves and fruits increased with increasing of applied N in the apple orchard, but the P concentration of the fruits and the K concentration of the leaves and fruits decreased. Yang et al. (2015) determined a significant negative correlation between the N and B concentrations in the leaves of litchi trees. Uçgun & Altindal (2021) determined that as N fertilization applied from soil increased, the nutrient levels of sweet cherry leaves changed with increasing N and Mn and decreasing P, K and B concentrations.

Cheng & Fuchigami (2000) determined that the

Table 3. Correlations between nutrient concentrations of leaf and photosynthetic performance of leaf of sweet cherry cv. 0900 Ziraat grafted on Gisela 5 rootstock and reaching full yield

	Tr	A	Ci	Gsw	Gtw	Gtc	WUE	ICE
N	-0.62**	-0.67**	-0.15	-0.61**	-0.61**	-0.61**	0.16	-0.66**
P	0.48*	0.60**	-0.16	0.50*	0.50*	0.50*	0.05	0.70**
K	0.48*	0.55**	0.11	0.50*	0.50*	0.50*	0.10	0.49*
Ca	-0.20	-0.08	-0.30	-0.18	-0.18	-0.18	0.14	0.09
Mg	-0.53**	-0.40*	-0.49*	-0.53**	-0.53**	-0.53**	0.18	-0.13
Fe	-0.24	-0.15	-0.01	-0.18	-0.19	-0.19	0.38	-0.15
Cu	-0.16	-0.19	-0.05	-0.19	-0.19	-0.19	0.05	-0.18
Mn	-0.14	-0.32	0.02	-0.18	-0.18	-0.18	-0.31	-0.36
Zn	-0.17	-0.32	0.07	-0.18	-0.18	-0.18	-0.22	-0.30
B	0.23	0.23	0.39	0.31	0.31	0.31	0.09	0.09

A: assimilation rate, Ci: concentration of intercellular CO_2, Tr: transpiration rate, Gsw: stomatal conductance to water vapor, Gtc: total conductance to CO_2, Gtw: total conductance to water vapor, WUE: water use efficiency, ICE: instantaneous carboxylation efficiency calculated intercellular CO_2 decreased with increasing leaf N and found curvilinear relationship between leaf N concentration and photosynthetic capacity in apple leaves. Tóth et al. (2002) performed a study to determined effect of the different N doses (30, 60, 90, 120 and 150 N kg/ha) on photosynthesis of in maize plants and found no significant differences. Cechin & De Fátima Fumis (2004) obtained that the CO_2 assimilation of the sunflower leaves for photosynthesis was remarkably increased by high nitrogen supply. N did not affect statically stomatal conductance, but high-N grown plants had lower intercellular CO_2 concentration. Reddy et al. (1996) characterized net photosynthetic rate, stomatal conductance and transpiration of cotton were positively correlated with leaf N concentration. Prsa et al. (2007) stated that the treatment with 80 kg N/ha (recommended dose in integrated apple production) had no or little effect on physiological parameters according to control (no fertilizer).

Hu et al. (2016) proved non-stomatal factors such as chlorophyll and decreased carboxylation efficiency supervised photosynthesis level when K was deficiency. Basile et al. (2003) determined that leaf potassium concentration didn't affect stomatal conductance significantly and leaves having low potassium had the highest calculated internal CO_2 concentrations. Zhao et al. (2001) stated that photosynthetic rate of cotton (*Gossypium hirsutum* L.) grown in K deficiency-environment was only 23% of the control plants receiving a full K supply. It was mainly associated with mainly low chlorophyll concentration, poor chloroplast ultrastructure, and restricted saccharide translocation. There was no relationship between stomata conductance and photosynthetic rate. Fallahi et al. (2001b) revealed the scion leaf net photosynthesis and leaf mineral concentrations were affected by rootstock. Bud.9 rootstock had lower net photosynthesis, higher Ca and Mn but lower K concentrations than those on the other rootstocks. Bednarz & Oosterhuis (1999) indicated that reductions in leaf physiological processes and growth of cotton plants occur after the petiole K concentration fell below 0.88% on a dry weight basis. According to Terry & Ulrich (1974)'s results, low K apparently decreased photosynthesis through an increase in mesophyll resistance to CO_2 (rm). Kanai et al. (2007) stated that K had a positive effect on biomass of tomato plants and K deficiency decreased severely biomass of all organs and depressed leaf photosynthesis and transport of ^{13}C assimilates. Behboudian & Anderson (1990) also showed that K deficiency caused lower rate of photosynthesis in tomato plants. This decreasing effect in -K leaves were due to impairment of photosynthetic capacity and not to stomatal closure. Peaslee & Moss (1966) stated that photosynthetic capacity in maize leaves were primarily associated with leaf K concentration and the critical level was about 2 mg/g for K in fresh weight. Normal-appearing leaves living K deficiency showed a sharply decreasing in photosynthesis rates. Reddy & Zhao (2005) determined photosynthesis rate decreased in cotton with decreasing K levels.

In the study of Fujita et al. (2003), P-deficiency treatment affected negatively leaf photosynthesis, stomatal conductance of tomato plants. Kondracka & Rychter (1997) stated that phosphate deficiency affects plant growth and the rate of photosynthesis. Bernardi et al. (2015) evaluated the effect of N, P and K fertilizing on gas exchange and leaf photosynthetic performance in sweet orange. The results indicated that photosynthesis rate was depressed by the high levels of N, improved by K at intermediate fertilization levels and affected a little by P. Li et al. (2021) studied the nutrient uptake and distribution in mycorrhizal cuttings of Populus × canadensis 'Neva' under drought stress and determined that gas exchange parameters positively correlated with the concentrations of leaf P, K, Ca, Fe, Mn, Cu, and Zn while negatively with N.

In many studies as mentioned above, it was revealed nutrients have positive or negative effects on gas exchange and gas exchange and leaf photosynthetic performance. These effects may occur directly or indirectly. The excessive or deficiency of a mineral element affects some enzyme activities and hormone syntheses and these enzymes and hormones regulate some physiological process affecting gas exchange and leaf photosynthetic performance.

Conclusion

While N fertilization affected positively the leaf accumulation of N, its effect was negative on the leaf accumulation of P and K. As for Tr, A, Gsw, Gtw, Gtc, and ICE, they decreased with increasing N fertilizing. There were negative correlations between decreasing gas exchange and leaf photosynthetic performance and increasing leaf N level, but contrarily for P and K. It is known that there is antagonistic and synergic interaction between mineral nutrients. We determined an antagonistic effect of N fertilization applied to the soil on the accumulation of P and K. It shouldn't be forgotten that overfertilizing with any nutrient causes environmental pollution, soil salinization (which also precludes the absorption of mineral nutrients), decreased yield, and decreases gas exchange and leaf photosynthetic performance. Increased fertilization also has a high cost decreasing profit margins for fruit producers.

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Author Contributions

KU: Conceptualization, data curation, investigation, methodology, visualization, initial drafts, writing, review and editing; **BT:** data curation, investigation; **MC:** investigation; **MA:** investigation, writing, initial drafts, review and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper

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RESEARCH PAPER

The effects of humic acid and chelate applications on some morphophysiological properties and antioxidant enzyme activities of ornamental cabbage (*Brassica oleracea* var. *acephala*) under boron stress

Şenel Birceyudum Eman Gökseven[1]*, Sevinç Kıran[2], Ş. Şebnem Ellialtioğlu[3]

[1]Ministry of Agriculture and Forestry, Directorate General for European Union and Foreign Relations, Ankara, Türkiye
[2]Soil, Fertilizer and Water Resources Central Research Institute, 06172 Ankara, Türkiye
[3]Ankara University Teknopolis, Doqutech Academy LLC, 06830 Ankara, Türkiye

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Corresponding Author
Tel.: +90 312 287 3360
E-mail: beman2023@gmail.com

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Abstract

The increase of industrialization, the overuse chemical fertilisers and mining activities are brought about heavy metal-led environment pollution, especially agricultural land. This leads to more boron (B) contamination and accumulation in the soil. This study was carried out to evaluate B uptake from the soil of ornamental cabbage grown as a hyperaccumulator plant under B stress conditions in a controlled greenhouse on plant morphology, physiology, antioxidant enzyme activity the effects of humic acid (50mg kg^{-1} B + 2% humic acid -HA) and chelate [0.5 g kg^{-1} chelate (EDTA)] applications. According to the results, especially chelate application significantly increased the B uptake of the plant, and B accumulation was higher in the plant shoot than in the root. However, HA and chelate applications brought out the negative effects of B stress on growth and physiological characteristics and reinforced the increases in malondialdehyde (MDA) content and superoxide dismutase (SOD) and catalase (CAT) enzyme activities. In conclusion, this study shows that HA and chelate additions increase the efficiency of the use of ornamental cabbage to remove excess boron from the soil. According to these results, it is possible to increase the use of ornamental cabbage for phytoremediation purposes, especially with chelate application.

Introduction

Boron (B) is an essential element for the vital activities of plants (Hussain et al., 2017). Boron element is included a great number of processes in plants such as: photosynthesis, the cell walls and the lignification process, the transport of sugars, the ascorbate/glutathione cycle, metabolism of phenolic compounds, pollen tube formation, the plasma membrane integrity and its function, the nitrogen metabolism. In addition, it can create a stress affect at higher concentrations than a certain dose range. In case of excess boron in the soil, many physiological and biochemical processes in the life cycle of plants are negatively affected and occurred significant losses in yield and quality (Princi et al., 2016; Garcia-Sanchez et al., 2020). B toxicity is an important problem on plants, especially in regions where boron mines are located and B contamination can cause severe damage to local ecosystems (Stiles et al., 2011). It is highly important for sustainability to clean and improve boron-contaminated areas through the use of B-tolerant plant species in the revitalization of ecosystems that are in danger of extinction (Rámila et al., 2016). At this point, phytoremediation technique emerges as a cost efficient and environmentally friendly application for on-site improvement in areas excess of B (Eman Gökseven & Kıran, 2021). The use of hyperaccumulator plants with high B tolerance is important for the success of this technique, especially in areas where pollution is intense. The use of agents such as chelate, and humic acid is also seen as an effective approach in order to support the accumulative properties of plants and increase their capacity. As a matter of fact, it is known that ethylene diamine tetra acetic acid (EDTA) as a synthetic chelator has an effect on the metal bioavailability potential of plants (Arshad et al., 2020; Konkolewska et al., 2020; Saffari & Saffari, 2020). Moreover, it is stated that humic acids, which are considered the most active components of soil and compost organic matter, demonstrate physiological, morphological, biochemical and genetic effects on plants by forming strong bonds with toxic heavy metal ions (Ferrara & Brunetti, 2008; Shehata et al., 2019), and are also effective in mitigating the negative effects of heavy metals on plants (Özkay et al., 2016). In addition, humic acid improves the morphological characteristics of plants grown under B stress conditions and increases the amount of nutrients (Karaman et al., 2017). In addition, it is observed that plants grown under heavy metal stress (Cu, Cd, Pb, and Zn) conditions help reduce the effect of oxidative stress by regulating antioxidant enzyme activities such as SOD, CAT, GR, and APX (Kıran et al., 2014). Furthermore, humic acid improves growth by reducing oxidative stress in plants due to its organic structure. Therefore, it can increase the effect of phytoremediation in soils contaminated with heavy metals (Canal et al., 2022).

Oxidative stress caused by heavy metals in plants may vary depending on the plant species, the type and concentration of the heavy metal (Olaniya et al., 1998). Many researchers have reported that some vegetables species have the capacity to accumulate heavy metals in the soil. Especially, certain *Brassica* spp. are mostly evaluated as hyperaccumulator plants because of their high accumulation of heavy metals in their tissues (Kusznierewicz et al., 2012; Ning et al., 2015; Haghighi et al., 2016; Eman Gökseven & Kıran, 2021). While it is not preferred to accumulate heavy metals, which can have negative effects on human health, in edible plant species, the use of species that are not consumed as food or feed is preferred for phytoremediation. Therefore, in our study, *B. oleracea* var. *acephala*, a subspecies of *Brassica oleracea* and considered as an ornamental plant, was used and evaluated as a potential hyperaccumulator plant in the improvement of B-contaminated areas. In this study, it was studied to increase the efficiency of use for phytoremediation in soils containing B by supporting the hyperaccumulator property of *B. oleracea* var. *acephala*. The specific aim of this study was to determine changes in some morphological and biochemical properties of ornamental cabbage by applying humic acid and chelate (EDTA) to plants grown in soil contaminated with boron.

Material and Methods

Plant materials and treatments

This study was carried out in the greenhouse of Soil, Fertilizer and Water Resources Central Research Institute with automatic temperature and relative humidity control (at 13-17°C 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, Türkiye. Ornamental cabbage (*Brassica oleracea* var. *acephala*) was used as plant material. Table 1 shows physical and chemical properties of studies soil. Studied subjects were used 1. Control (only 50 mg kg^{-1} B dose), 2. 50 mg kg-^{1} B + Chelate (EDTA), 3. 50 mg kg^{-1} B + Humic Acid (HA) in this experiment. Ornamental cabbage (*Brassica oleracea* var. *acephala)* seedlings with 3-4 true leaves were planted in pots 15×17×22 cm (a plant per pot) in January 25, 2018. One week after planting, 50 mg l^{-1} B was applied to the pots except control group. Boron was applied as boric acid (H_3BO_3, 17.5% B) with irrigation water. In order to support B uptake in plants, chelate (0.5 g kg^{-1} EDTA by spraying) and humic acid (HA) (2% powdered humic acid (48.34% organic matter + 60.47% humic+fulvic acid)) were added to the potting soil one week after B application. The plants were irrigated at the level of field capacity with tap water. Plants were grown for eight weeks. In order to make observations and measurements, at the end of the 8th week, three plants from each subject were harvested and measurements were made and samples were taken for analysis.

Table 1. Physical and chemical properties of studies soil

Properties	
Texture class	Clay Loam (CL)
EC* (dS m^{-1})	0.92
pH*	7.49
$CaCO_3$(%)	37.6
Available P (P_2O_5) (kg ha^{-1})	11.0
Available K (K_2O) (kg ha^{-1})	78
Organic Matter(%)	1.86
Available Fe (mg kg^{-1})	15.44
Available Cu (mg kg^{-1})	2.59
Available Zn (mg kg^{-1})	2.42
Available Mn (mg kg^{-1})	16.68
Available B (mg kg^{-1})	0

Determination of plant growth characteristics

Each plant is divided into two as roots and shoots and weighed weekly in grams on a precision scale. After their fresh weights measured, the samples were dried in the oven set at 65°C until they reach constant weight then their dry weights were measured.

Measurements of physiological properties

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.

Stomatal conductance (gs)

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.

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

$$RWC (\%) = [(FW-DW)/(TW-DW)] \times 100$$

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 65°C. Plant shoots and roots were ground finely to pass through a 200 µm sieve for analysis. 0.25 g of shoots and root samples were first digested with nitric acid (HNO_3) in a microwave device, then these samples were transferred to 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 & 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 & İnal, 2008).

Lipid Peroxidation

Lipid peroxidation is called damage to cell membranes. The method developed by Lutts et al., (1996) was followed in order to determine the amount of malondialdehyde (MDA), which is a product of lipid peroxidation. Fresh tissues (0.5 g) were homogenized in 10 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 15.000 g for 5 min. Assay mixture containing 1 mL of the supernatant and 4 mL of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA was heated at 95°C for 30 min and rapidly cooled in an ice bath. After centrifugation (10.000 g for 10 min), the absorbance of the resulting supernatant was measured at 532 and 600 nm wavelengths. The concentration of MDA in the solution was calculated as MDA (nmol/ml) = [A532-A600)/155000] x 106 by Sairam & Saxena, 2000.

Assessment of the Antioxidant Enzymes Activity: Superoxide dismutase (SOD), Catalase (CAT)

To assess enzyme activity, approximately 1 g fresh leaf tissue was crushed in liquid nitrogen in a porcelain mortar and homogenized in extraction medium containing 5 mL of 0.1 M Na-phosphate, pH 7.5; 0.5 mM Na-EDTA and 1 mM ascorbic acid. The homogenate was centrifuged at 18.000 g for 30 min at 4 °C. The supernatant was used to assess catalase (CAT) and superoxide dismutase (SOD). Measurements were performed in an Analytical Jena 40 model spectrophotometer. The CAT activity was calculated as the rate of decomposition of H_2O_2 during at 240 nm (E=39.4 mM cm^{-1}). The reaction mixture (2.5 mL) contained 0.05 M phosphate buffer (pH 7.0), 1.5 mM H_2O_2 and 0.2 mL enzyme extract (Jebara et al., 2005). Superoxide dismutase (SOD) activity was adjusted by the nitroblue tetrazolium (NBT) method (Rahnama & Ebrahimzadeh, 2005).

Statistical analysis

Experiments were carried out randomized plots with a factorial design with 3 replications. All data was statistically analysed using the MSTAT-C (Freed et al., 1989). The significant differences were compared with LSD test at $P \leq 0.05$.

Results

Shoot -root fresh and dry weights

The effect of humic acid and chelate applications with B on plant shoot-root fresh and dry weights parameters are shown in Table 2. Differences between B applications was found statistically significant ($P \leq 0.05$) in terms of shoot-root fresh weights and root dry weight, while shoot dry weight was not significant ($P > 0.05$). All application forms significantly reduced shoot fresh weight of the plants to the control plants. The highest shoot fresh weights in plant were obtained

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

Source of variation	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	gc	Chlorophyll (SPAD value)	RWC
Application forms	**	NS	**	**	**	**	NS
CV (%)	2.85	17.88	6.94	16.29	5.12	6.00	3.75

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 humic acid (HA) and chelate associated with B application on plant shoot-root fresh and dry weights, stomatal conductance (gc), chlorophyll and relative water content (RWC)

Application forms	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})	gc (mmol $(m^2s)^{-1}$)	Chlorophyll (SPAD value)	RWC (%)
Control	109.79±1.22 a	11.27±0.95	43.11±2.42 a	7.69±0.85 a	102.10±3.24a	36.72±1.62a	77.38±2.21
50 mg kg^{-1} B	80.56±2.00 b	12.06±1.85	38.03±2.28 b	5.44±0.47 b	86.92±7.39b	27.50±2.18b	74.43±2.14
50 mg kg^{-1}B+Chelate	76.83±2.57 b	9.00±2.00	20.88±1.64 d	4.34±0.62 b	63.43±5.36c	25.53±1.16b	76.37±1.71
50 mg k^{-1}g B+HA	70.33±2.52 c	9.45±1.65	27.87±1.80 c	3.74±0.98 b	59.93±4.58c	25.60±4.13b	74.00±3.46
LSD (5%)	4.80	-	4.50	1.73	7.99	3.46	-

*: Means with the different letter within same column are significantly different (P≤0.05)

from 'B' (80.56±2.00 g plant^{-1}) and 'B + Chelate' (76.83±2.57 g plant^{-1}), which were statistically same group (Table 3). The lowest shoot fresh weight value was determined in the 'B + HA' (70.33 ±2.52 g plant^{-1}) (Table 3). This application method draws attention as the application method in which plants most exploit the boron element from the soil, thus affecting the development most negatively compared to the control. It was determined that the root fresh weight of the plants decreased significantly compared to the control plants in all applications. The highest root fresh weight was obtained from 'B' (38.03±2.28 g plant^{-1}). After 'B' application, root fresh weights were obtained from 'B + HA' (27.87±1.80 g plant^{-1}) and 'B + Chelate' (20.88±1.64 g plant^{-1}), respectively (Table 3). The highest root dry weights were not statistically different between 'B' (5.44±0.47 g plant^{-1}), 'B + Chelate' (4.34±0.62 g plant^{-1}) and 'B + HA' (3.74±0.98 g plant^{-1}) applications, respectively (Table 3).

Stomatal conductance (gc), chlorophyll and relative water content (RWC)

The differences between the B applications were not statistically significant in terms of relative moisture content (P>0.05), while the differences between the applications were found to be important in terms of gc (P≤0.05) (Table 2). In ornamental cabbage, gc was significantly reduced in all B application forms compared to the control plant. While the highest gc was determined in the 'B' (86.92±7.39 mmol m^2s^{-1}) application, the lowest gc was found in the combinations of 'B + Chelate' (63.43±5.36 mmol m^2s^{-1}) and 'B + HA' (59.93±4.58 mmol m^2s^{-1}), which were statistically in the same group (Table 3). Chlorophyll content of the plants in all B application forms was decreased compared to control plants. The highest chlorophyll content was obtained from the 'B' (27.500±2.18 SPAD).

Boron contents

The differences between the application forms ('Control','B','B+ Chelate', 'B + HA') was found to be statistically significant the amounts of B accumulated in the shoot, root and whole plant (P≤0.05) (Table 4). The highest B accumulation occurred 'B + Chelate' in terms of the shoot, root and whole plant. B applications led to increase in the amount of B in shoot, root and the whole plant. The increases were found to be significant when compared to the control plants. It was determined that B accumulation in the shoot was higher that the accumulation in the root, in 'B', 'B + Chelate' and 'B+ HA applications. After 'B + Chelate' application, the highest B accumulations in shoot, root and whole plant were followed by 'B + HA' and 'B' applications, respectively (Figure 1, Figure 2 and Figure 3).

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
Application forms	**	**	**
CV(%)	2.57	5.51	23.84

**: P≤0.01 is significant at probability level

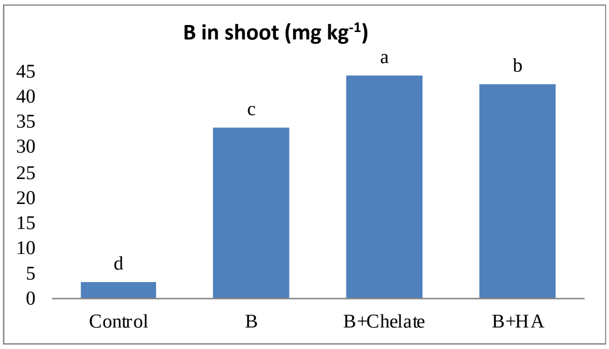

Figure 1. B content in shoot depending on application forms

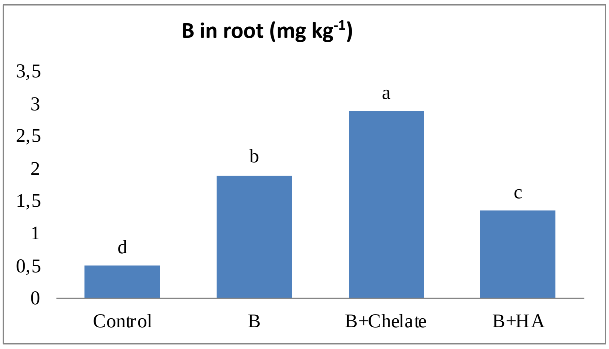

Figure 2. B content in root depending on application form

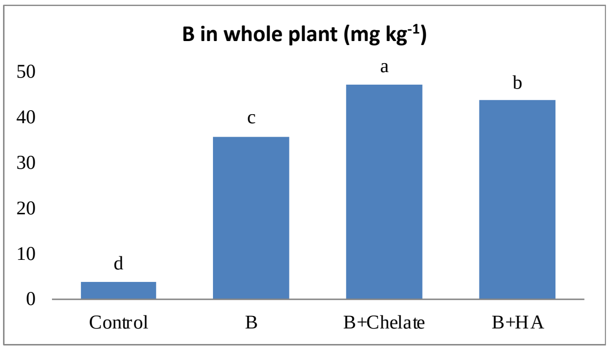

Figure 3. B content in the whole plant depending on application forms

Lipid peroxidation and activities of antioxidant enzymes

The amount of malondialdehyde (MDA), which is a product of lipid peroxidation, and antioxidative enzyme activities (SOD and CAT) were found to be statistically significant in terms of B application forms (P≤0.05) (Table 5). Compared to control, B application brought about an increase in MDA levels by creating oxidative stress in plants. The differences between all B applications were in the same statistical group. The highest MDA value was obtained from the 'B + Chelate'(12.96±0.88 µmol g^{-1}) and followed by 'B' (10.01±0.54 µmol g^{-1}) and 'B + HA' (9.48±0.58 µmol g^{-1}), respectively (Table 6).

Table 5. ANOVA for MDA contents and activities of antioxidant enzymes (SOD and CAT)

Source of variation	MDA	SOD	CAT
Application forms	**	**	**
CV (%)	7.18	6.71	1.33

**: P≤0.01 is significant at probability level. MDA: Malondialdehyde SOD: Superoxide dismutase, CAT: Catalase.

The highest SOD antioxidative enzyme activity was determined as 'B + Chelate' (77.90±6.06 U min^{-1} mg^{-1}) and this application was followed by 'B + HA' (71.07±7.79 U min^{-1} mg^{-1}), which was in the same statistical group. 'B' (60.15±2.03 U min^{-1} mg^{-1}) application was lower SOD enzyme activity compared to other B applications. In general, all B applications showed an increase compared to the control (Table 6). Compared to control, B applications led to increases in CAT activity. The highest CAT activity was determined as 'B + Chelate' (705.50±8.23 µmol min^{-1} mg^{-1}) and this application was followed by 'B + HA' (526.26±8.62 µmol min^{-1} mg^{-1}) and 'B' (520.00±5.00 µmol min^{-1} mg^{-1}), which were in the same statistical group (Table 6).

Table 6. The effect of chelate and humic acid (HA) associated with B application on MDA contents and activities of antioxidant enzymes SOD and CAT)

Application forms	MDA (μmol g^{-1})	SOD (U min^{-1} mg^{-1})	CAT (μmol min^{-1} mg^{-1})
Control	3.44±0.19b	11.61±0.62c	179.19±5.07c
50 mg kg^{-1} B	10.01±0.54a	60.15±2.03b	520.00±5.00b
50 mg kg^{-1} B+Chelate	12.96±0.88a	77.90±6.06a	705.50±8.23a
50 mg kg^{-1} B+HA	9.48±0.58a	71.07±7.79a	526.26±8.62b
LSD (5%)	5.14	7.77	13.51

*: Means with the different letter within same column are significantly different (P≤0.05)

Discussion

In this study, it was determined that the shoot fresh and dry weights of ornamental cabbage were higher in 50 mg kg^{-1} B application compared to '50 mg kg^{-1} B+HA''. However, chelate addition (B+chelate) had a similar effect to B application (50 mg kg^{-1} B) in terms of shoot fresh weight. On the other hand, HA supplementation caused significant reductions in trunk fresh weights compared to control and B application (50 mg kg^{-1} B) with the effect of toxic level of B accumulation. Similar results were obtained by Tursun (2014), on parsley, it was reported that '150 ppm B' and '150 ppm B + HA' application significantly reduced leaf fresh and dry weights. It has been reported that in boron pollution in rapeseed, HA and EDTA applications brought about decreases in root yield due to dose increase and repetitions (Esringü, 2012). In addition, it was stated that the effects of B toxicity and humic substances applications on biomass yield in cotton were positive, but this could not be observed clearly and there were differences between the application doses (Kaptan, 2013).

According to the findings obtained in this study, 'B+ Chelate' and 'B+ HA' applications significantly reduced root fresh and dry weights compared to the control. In terms of root fresh weight, these losses can be seen more clearly in HA applications. Root fresh weight losses, especially with chelate application, were revealed by B accumulated more in the root due to the addition of chelate. Moreover, EDTA, which is a chelate, has the potential to increase the phytoextraction of metal (Saffari & Saffari, 2020). Similar result was obtained by Göker (2019) who reported that EDTA application decreased root fresh and dry weight in corn plant compared to the control in chromium pollution. Moreover, our results were in line with the findings of Karabulut (2020), who notified that there was a decrease in chelate application root fresh weight and root dry weight of rosemary plant in pots contaminated with lead. Our results consistent with finding that 150 ppm B and 150 ppm B + HA applications reduced root fresh and dry weights compared to control on parsley plants. However, it has been also reported that HA has no effect on the prevention of boron toxicity, especially in terms of growth parameters (Tursun, 2014).

The stomatal conductance decreased during stress in all B applications compared to the control. In general, a decrease in stomatal conductance was determined in all B applications, while additional chelate and HA applications revealed losses in gc of plants more evident. There was a similar finding between chelate and HA applications on gc. It has been reported that HA increases stomatal activity and leaf K content in potato (Lopez, 1993), lettuce (Haghighi et al., 2012) and strawberry (Ameri & Tehranifar, 2012) plants grown in B-free conditions.

In our study, the findings obtained with stomatal conductivity differed, and B, which accumulated more on the leaves with HA application, prevented gas diffusion and led to a decrease in stomatal conductivity. Canal et al. (2022) reported that HA increases the metal accumulation in the plant. According to previous similar studies, reductions in stomatal conductivity were observed in plants exposed to excess B (Lovatt & Bates, 1984; Papadakis et al., 2004). Pereira et al. (2000) hypothesize that one of the possible causes of decreased photosynthesis by excess B is structural damage to thylakoids. Pereira et al. (2000) also explained this situation by changing the electron transport rate and affecting CO_2 photoassimilation, which can also be limited by stomatal reduction (Landi et al., 2012).

Chlorophyll content decreased in all B applications compared to control. There was no difference in the effects of additional chelate and HA applications in the decreases in the amount of chlorophyll content emerged by the effect of B toxicity. Due to the increase in the boron uptake capacity of the plant with HA and chelate applications, increased B concentrations in the shoot may have been effective in structural damage to chloroplasts. Güllüce et al. (2012) reported that different doses of HA led to a significant decrease in chlorophyll content in radish grown in areas contaminated with Pb and Cd. Additional EDTA application to As-contaminated soil significantly reduced the chlorophyll content of corn leaves (Abbas, 2013). A similar result was obtained by Wang et al. (2019), on the toxicity of Cd in lettuce plant, with a decrease in chlorophyll content with fulvic acid application.

Boron analysis in plants

Small amount of EDTA rates can be a helpful factor in exceeding the limits for metals uptake by plants. (Chen et al., 2004; Meers et al., 2005). Vanlı (2007), reported that increases in B uptake were observed in plants according to the chelate dose in canola, corn, and sunflower to which different amounts of chelate were added. Similar findings were obtained in our study, EDTA application led to more B accumulation in the shoot and root of ornamental cabbage, resulting in higher B concentrations. Although previous studies have shown that chelates applied to increase the uptake of B element by the plant have positive effects, it has also been noted that there are decreases according to the increase in the dose and number of repetitions of chelators (Esringü, 2012). Except EDTA, regulators such as citric, tartaric, and humic acids applied to the soil also caused an increase in heavy metal uptake in plants. (Eren, 2019a; Eren, 2019b). In our study, it was determined that HA application also had a positive effect on B uptake in the shoot and root, but this effect remained at lower levels compared to chelate application. Increasing the uptake of nutrients by plants may be associated with the chelating properties of humic substances on micronutrients and their hormone-like effects in the soil. It has been reported that the addition of different amounts of HA and different B doses in Vetiver (*Vetiveria zizanioides*) have a positive effect on B uptake in roots and shoots (Angin et al., 2008). Kaptan (2013), notified those humic substances increased the available B content in the soil, but its reflection on plant boron contents could not be determined clearly.

Lipid peroxidation and activities of antioxidant enzymes

Lipid peroxidation is an indicator of oxidative damage. Many researchers bring forward that there is a relationship between the occurence of reactive oxygen species as a result of oxidative stress and increased lipid peroxidation concentration. (Karabal et al., 2003; Han et al., 2009). Under stress conditions, MDA is generally used to evaluate lipid peroxidase or membrane damage. By detecting the MDA content, both the degree of lipid peroxidase and thus the degree of stress are determined (Onbaşı, 2017). In our study, B stress led to significant increases in the amount of MDA. Similar results have ben reported in vine (Güneş et al., 2006), apple rootstock (Molassiotis et al., 2006), tomato (Cervilla et al., 2007), patato (Ayvaz, 2009), barley plants (Onbaşı, 2017). Our results are consistent with also those of Barışık Kayın (2020), who notified that there are significant increases in MDA contents in plants under B toxicity. The use of chelate increased the B uptake of ornamental cabbage from the soil and brought about more B accumulation in the plant compared to '50 mg kg^{-1} B'. As a result of, MDA content increased because of oxidative damage in the plant.

Increases in SOD activity have been reported with an increase in reactive oxygen species (ROS) production due to stress. (Mittler, 2002; Ayvaz, 2009). The increased activity of SOD can be expressed as an increasing index of superoxide (O_2-) production in plants under B stress. It has been reported by many researchers that superoxides and toxic O_2 formation occur under B stress (Karabal et al., 2003; Kobayashi and Matoh 2004; Cervilla et al., 2007; Pandey and Archana, 2013). Increases in SOD enzyme activity in plants under boron toxicity were observed in chickpea (Ardıç, 2006), apple root shoots (Molassiotis et al., 2006; Sotiropoulos et al., 2006) and goldentop (Han et al., 2009), *Brassica juncea* (Giansoldati et al., 2012), pepper (Barışık Kayın, 2020). Varshney et al. (2015), it was determined that the highest increase in antioxidant enzyme activity was at the dose of 60 mg kg^{-1} B. In our study, the SOD activity increased with the administration of '50 mg kg^{-1}' B. In this regard, Varshney et al. (2015) and our findings are similar. The use of copper together with EDTA in rapeseed caused changes in antioxidant enzyme activity, and it was reported that EDTA application increased SOD activity (Habiba et al., 2015). In our study, chelate application increased B uptake from the soil to the plant. As a result, the SOD enzyme was activated more in order to eliminate the effect of oxidative damage due to excessive accumulation of B in the plant. It was reported that plants under boron toxicity increased CAT enzyme activity in chickpea (Ardıç, 2006), apple rootstocks (Sotiropoulos et al., 2006), *Citrus grandis* L. plants (Han ve ark., 2009) and rapeseed (*Brassica* sp.) (Pandey &Archana, 2013). Varshney et al. (2015), increases in CAT activity were reported at a dose of 60 mg kg^{-1} B. EDTA application activated CAT antioxidant enzyme more in Brassica napus L. plant under Cu stress (Habiba et al., 2015). Significant increases in CAT activity were also recorded by Barışık Kayın (2020) with the B application.

Conclusion

According to the results of this study, *B. oleracea* var. *acephala* as an ornamental plant, showed a significant potential against stress, toxicity, and accumulation of B. The application of HA and chelate as chelators negatively affected shoot fresh-dry weights and root fresh-dry weights. While the stomatal conductivity and chlorophyll content decreased in HA and chelate addition applications were added, there was an insignificant increase in the relative moisture content only in the 'B + Chelate' application. However, B applications caused an increase in MDA levels compared to the control by creating stress in plants and led to cell membrane damage. We found that chelate addition can be used to increase the efficiency of use of ornamental cabbage plant for removing excess boron from the soil, since it has an effect on increasing the B uptake of the plant. 'B + Chelate' application provided the highest MDA increase compared to other B applications during B stress. Antioxidative enzyme activities increased compared to control. On the other hand, the increase in the activities of antioxidative enzymes revealed that the tolerance mechanism in the cells was activated at a high level despite the damage. High B applications with HA added took place after the chelated applications, but the effect remained at lower levels. It can be said that testing different doses of both HA and chelate applications is likely to increase the effectiveness. It is considered that it would be beneficial to carry out this study, which was carried out in controlled conditions and in the form of pot trials, in field conditions in larger areas. It is thought that studies on the removal of element B by phytoremediation method and the application of different doses to understand the mechanisms of the toxic effects of element B in applications such as humic acid and chelate will eliminate the deficiencies in the literature on this subject.

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Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Author Contribution

Conceptualization: SK, SSE, Data Curation: SBEG, SK, SSE, Formal Analysis: SBEG, SK, SSE, Investigation: SBEG, SK, Methodology: SK, SSE, Project Administration: SK, SSE, Resources: SK, SSE, Supervision: SK, SSE, Visualization: SBEG, SK, SSE, Writing -original draft: SBEG, SK, SSE, Writing -review and editing: SBEG, SK, SSE.

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