KASTAMONU UNIVERSITY JOURNAL OF ENGINEERING AND SCIENCES

e-ISSN 2667-8209

VOL: 09 ISSUE:02

KASTAMONU UNIVERSITY JOURNAL OF ENGINEERING AND SCIENCES

e-ISSN 2667-8209

Kastamonu University Journal of Engineering and Science

Kastamonu University Journal of Engineering and Science publish as blind peer review and two times in a year.

Kastamonu University
Journal of Engineering and Science

Vol: 9 Issue: 2 December 2023 E-ISSN:2667-8209

Kastamonu University
Journal of Engineering and Science

Vol: 9 Issue: 2 December 2023 E-ISSN:2667-8209

Kastamonu University Faculty of Engineering and Architecture 37150 Kastamonu / TÜRKİYE
Tel: +(90)366 2802901 Fax: +(90)366 2802900

Web: https://dergipark.org.tr/tr/pub/kastamonujes
e-mail: kujes@kastamonu.edu.tr

This journal is published two times in a year.
June and December
Kastamonu University Journal of Engineering and Science
Indexed and Abstracted in: Dergipark

Kastamonu University
Journal of Engineering and Science

Vol: 9 Issue: 2 December 2023 E-ISSN:2667-8209

CONTENTS

doi:10.55385/kastamonujes.1333878 KUJES 9(2):46-52 2023

Kastamonu University
Journal of Engineering and Sciences
e-ISSN 2667-8209
http://dergipark.gov.tr/kastamonujes

Halobacillus trueperi CT7: A Spore-Forming, Gelatinase Producing, Salt-Tolerant Bacteria Isolated from Çankırı Salt Mine

Fevziye Işıl Kesbiç[a,*], Nejdet Gültepe[b], Naci Tüzemen[c]

[a] Central Research Laboratory, Kastamonu University, Kastamonu, Türkiye
[b] Department of Fisheries Fundamental Sciences, Fisheries Faculty, Ataturk University, Erzurum, Türkiye
[c] Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Kastamonu University, Kastamonu, Türkiye
***Corresponding Author:** ikesbic@kastamonu.edu.tr

Received: **July 28, 2023** ◆ Accepted: **November 15, 2023** ◆ Published Online: **December 26, 2023**

Abstract: Halophilic microorganisms are an extreme group of organisms that can spread at high salt concentrations, and a significant part of them consists of halophilic bacteria. Salt mines are important sources where halophilic bacteria are detected. In this study, *Halobacillus trueperi* CT7, a halophilic bacterium, was isolated from Çankırı Salt Mine. It was determined that the obtained strain showed 98.1% similarity with *Halobacillus trueperi* by DNA isolation and sequence analysis as well as biochemical analysis. In addition, two-dimensional (scanning electron microscopy) and three-dimensional (atomic force microscopy) images of *Halobacillus trueperi* were performed to reveal the cell morphology. In order to determine the industrial use potential of the microorganism, the minimum and maximum salt concentrations, temperature and pH ranges in which the species can grow, as well as the enzyme activities, where the species can grow, were determined qualitatively. The interest in halophilic organisms for their use in extreme industrial processes is increasing day by day. It is thought that this study will contribute to future studies on halophilic bacteria.

Keywords: Halophilic bacteria, *Halobacillus trueperi*, Salt Mine

Öz: Halofilik mikroorganizmalar yüksek tuz konsantrasyonunda yayılım gösterebilen ekstrem bir canlı grubudur ve bunların önemli bir bölümü halofilik bakterilerden oluşmaktadır. Tuz madenleri halofilik bakterilerin tespit edildiği önemli kaynaklardandır. Bu çalışmada bir halofilik bakteri olan *Halobacillus trueperi* CT7, Çankırı Tuz Madeni'nden izole edilmiştir. Elde edilen bu strainin, biyokimyasal analizlerin yanısıra DNA izolasyonu ve sekans analiziyle *Halobacillus trueperi*'ye %98,1 benzerlik gösterdiği tespit edilmiştir. Ayrıca *Halobacillus trueperi*'nin iki boyutlu (taramalı elektron mikroskobu) ve üç boyutlu (atomik kuvvet mikroskobu) görüntülemeleri gerçekleştirilmiştir. Mikroorganizmanın endüstriyel kullanım potansiyellerinin belirlenmesi amacıyla türün gelişim gösterebildiği minimum ve maksimum tuz konsantrasyonları, sıcaklık ve pH aralıklarının yanısıra enzim aktiviteleri de kalitatif olarak tespit edilmiştir. Ekstrem endüstriyel proseslerde kullanımları açısından halofilik organizmalara olan ilgi her geçen gün artmaktadır. Bu çalışmanın da halofilik bakterilerle ilgili gelecek çalışmalara katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Halofilik bakteri, *Halobacillus trueperi*, Tuz Madeni

1. Introduction

Extreme microorganisms are a very interesting group among all microorganisms. They are famous for being able to adapt to harsh conditions in which the vast majority of organisms can not survive. Among the extreme microorganisms, halophilic microorganisms are a different group of organisms as they can live in environments with high salt concentrations. This group consists of organisms with various genetic characteristics, with members in both Bacteria, Archaea and Eukarya domains. Halophilic bacteria are grown in salt-containing media to simulate their natural environment. Since environments with high salt concentration are not suitable for the growth of microorganisms other than halophilic group, the risk of contamination is also very low. Considering the reasons such as the easy and fast production of microorganisms and the ease of genetic modification, the importance of these microorganisms for industrial processes emerges.

Parameters such as oxidase, catalase, Gram stain, growth in a single carbon source, indole, nitrate production are quite important parameters in terms of biochemical characteristics of microorganisms [1]. However, today's technological developments enable us to detect species similarities with the data based on the base sequences of the species. After DNA isolation of pure cultures, 16 S rRNA sequences amplified by polymerase chain reaction are sequenced with next generation sequencing technologies. Following this, the obtained base sequences obtained are checked in databases and species similarity rates are revealed.

One of the areas where halophilic bacteria have industrial potential is their wide range of pigmentation properties [2,3,4]. These pigments, which can be yellow, orange, pink or red, play an important role in many fields such as

cosmetics, food, textiles, pharmaceuticals. Pigment-rich extracts obtained from halophilic microorganisms are also investigated for their antimicrobial, anticancer, antioxidant and cytotoxic properties [5,6,7]. In this context, it is thought that the biochemical and phenotypic properties of *Halobacillus trueperi*, which is a halophilic bacterium, isolated from the salt mine are very important in creating a study area for many medical and industrial processes.

2. Material and Method

Isolation of halophilic strain

Salt sample was obtained from salt mines in Çankırı (Türkiye). For enrichment, 1 gram of salt sample was dissolved in 90 ml of sterile 15% NaCl and incubated for several hours [8]. 1 mL of brine were mixed with 50 ml of MAM JCM 168 which contained (g/L): casamino acids (5), yeast extract (5), sodium glutamate (1), trisodium citrate (3), $MgSO_4x7H_2O$ (29.5), KCl (2), NaCl (175.5), $FeCl_2x4H_2O$ (0.036), $MnCl_2x4H_2O$ (0.36 mg). The medium pH was 7.0-7.2 before autoclaving. After that the samples were incubated at 37 ºC for several weeks until the appearance of colorful colonies was occurred. In order to obtain pure cultures, the culture medium was inoculated into petri dishes with the same medium composition and added 20g/L agar. After 5 days of 37°C incubation, colonies with different pigmentation and morphology were planted in different petri dishes and pure cultures were obtained.

Identification of halophilic strain

Biochemical tests

The isolated strain which has pale yellow colony pigmentation was coded as CT7 and analyzes were carried out to reveal its biochemical characteristics. By adding 10% NaCl to the staining solutions, gram staining was carried out according to the method by Dussault [9]. The ideal growing conditions were examined using a growth medium containing varied NaCl concentrations (0–25%) and yeast concentrations (0-0.1%), as well as various incubation temperatures (10–50 ºC) and pH values (5–10). By incorporating 1% KNO_3 into the growing medium, the conversion of nitrate to nitrite and the production of gas from nitrate were examined.

The amount of gas produced by nitrate was measured using Durham tubes submerged in the broths. In the growing medium that was supplemented with a 1% (w/v) substrate, glucose, lactose, maltose, fructose, mannitol, trehalose, galactose, and sucrose were used as the sole carbon sources. By introducing Kovac's reagent to the growth medium containing 1% tryptone, the synthesis of indole from tryptone was identified. Hydrolyses of starch, gelatin, casein, and Tween 80 were evaluated for amylase, gelatinase, caseinase, and esterase activities in addition to the strains' oxidase and catalase capabilities.

DNA isolation procedure and polimerase chain reaction conditions

The strains of halophilic bacteria were identified using phylogenetic analysis. The strains' DNA was isolated using the technique described by Neumann et al. [10]. Strains grown on MAM JCM 168 agar medium were taken into 1 mL STE buffer with loop, vortexed and homogenized. The cell suspension was centrifuged at 8 000 rpm for 2 minutes and the supernatant was removed.

The cell pellet was washed twice with 400 µL of STE, and after the last supernatant was removed, the cell pellet was resuspended with 200 µL of TE buffer and vortexed by adding 100 µL of tris-saturated phenol. The suspension, which was centrifuged at 13 000 rpm for 5 min at 4 ºC, was separated into two phases and transferred to a new 160 µL tube from the upper phase. By adding 40 µL of TE buffer and 100 µL of chloroform, it was centrifuged at 13 000 rpm for 5 minutes at 4 ºC, and the extraction process with chloroform continued until the color of the lysate became clear. 160 µL of the upper phase was taken into a new tube, 40 µL of TE buffer and 5 µL of RNase enzyme (10 mg/mL) were added and incubated at 37 ºC for 10 minutes. At the end of the incubation, 100 µL of chloroform was added to the tubes and centrifuged at 13 000 rpm for 5 minutes at 4 ºC, the upper phase containing pure DNA was transferred to a clean tube and stored at -20 ºC for use in future studies. DNA purity was checked by running 1% agarose gel electrophoresis at 80 V, 100 mA for 60 minutes.

The PCR primers used to amplify the 16S rRNA gene were bacterial forward primers 5′-AGAGTTTGATCCTGGCTCAG-3′, and reverse primers Bac 1493r′ 5′-ACGGCTACCTTGTTACGACTT-3′.

Table 1. The protocol of polimerase chain reaction

Content of reaction	First concentration	Volume in Reaction	Last concentration
TaqDNA polymerase 2x master mix	2X	25 µL	1X
Forward primer	1 µM	2.5 µL	0.05 µM
Reverse primer	1 µM	2.5 µL	0.05 µM
DNA	-	2.5 µL	-
Sterile double distilled water	-	17.5 µL	-
Total reaction volume	-	50 µL	-

Table 2. The protocol of thermalcycler

Temperature	Time	Cycle
98 ºC	3 min	1
98 ºC	10 sec	30
61 ºC	30 sec	30
72 ºC	30 sec	30
72 ºC	5 min	1
4 ºC	-	∞

The PCR products of the species were sequenced using the Sanger sequencing method and the base sequences were submitted to the NCBI database.

Two and three dimensional imaging of strain

A previously described technique by Kesbiç and Gültepe [11] was used to prepare the strains for scanning electron microscope (SEM) and atomic force microscope (AFM) imaging. Fresh cultures of the halophilic bacteria strains were transferred from the agar surface to the coverslip surface after being cultured at 37 °C for three days. The coverslips were treated overnight in a 2% glutaraldehyde solution after being allowed to dry by air. After allowing the coverslips to air dry, they were treated overnight in a 2% glutaraldehyde solution. To completely remove the water, the preparations were taken from the solution and immersed in an acetone series of 30, 50, 70, and 90% for 10 minutes and 30 minutes, respectively. The preparations were coated with Au-Pd using the Cressington Sputter Coater at 40 mA for 60 seconds before being immediately placed on the FEI Quanta FEG 250 SEM (running at 10 kV). For three-dimensional imaging, the same preparation procedure was employed in Bruker's Edge3 AFM.

Determination of pigment characteristic of strain

The cultures were cultivated at 37 ºC for three days before being centrifuged at 9000 rpm for 10 minutes to yield cell pellets. The pellets were treated with 2 mL of methanol before being sonicated in an ultrasonic bath for 60 minutes and centrifuged. The extract was obtained using a two-stage extraction technique and scanned between 300 and 600 nm in a UV–Vis spectrophotometer (BioTek-Epoch 2) [12].

3. Results

The biochemical characteristics of strain CT7 were listed in Table 1. The strain has rod shape and pale yellow pigmentation. It has been determined that strain can grow in salt-free environment and also at high salt concentration up to 20% salinity. In addition, it has been determined that the temperature tolerance is on a very wide scale. According to the enzyme hydrolysis test results, it was observed that it has gelatinase enzyme but not caseinase, esterase and amylase enzymes.

Table 3. Biochemical test results of *H. trueperi* CT7

	Halobacillus trueperi CT7
Colony color	Pale yellow
Gram stain	+
NaCl tolerance (%)	0-20
pH tolerance	6-9
Temperature tolerance (˚C)	10-40
Oxidase	+
Catalase	+
Indole	-
Gas from Nitrate	-
Nitrate to Nitrite	-
Nitrate	-
Glucose	+
Lactose	+
Maltose	+
Fructose	+
Mannitol	+
Trehalose	+
Galactose	+
Sucrose	+
Gelatin Hydrolysis	+
Starch Hydrolysis	-
Casein Hydrolysis	-
Tween 80 Hydrolysis	-

The sequences of *H. trueperi* CT7 were analyzed by using NCBI Blast, aligned with other reported gene sequences and was submitted to the NCBI database (GenBank) under accession number OR352511. A phylogenetic tree was reconstructed by the neighbor-joining method with MEGA11 software (Figure 1).

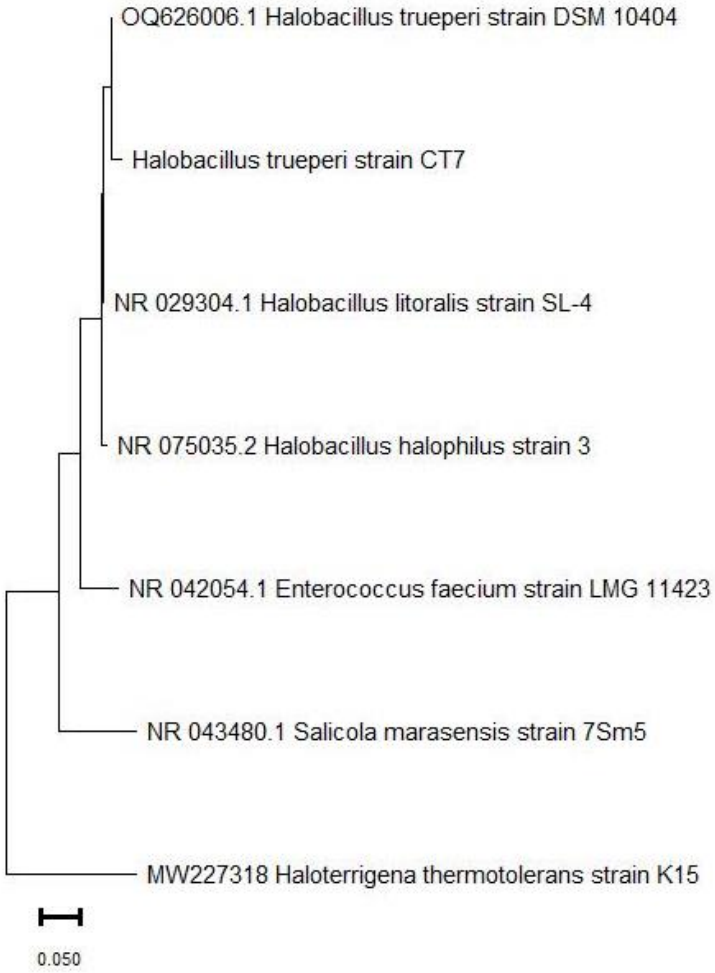

Figure 1. Phylogenetic tree of *Halobacillus trueperi* strain CT7 and its close relatives. *Haloterrigena thermotolerans* was used as an outgroup.

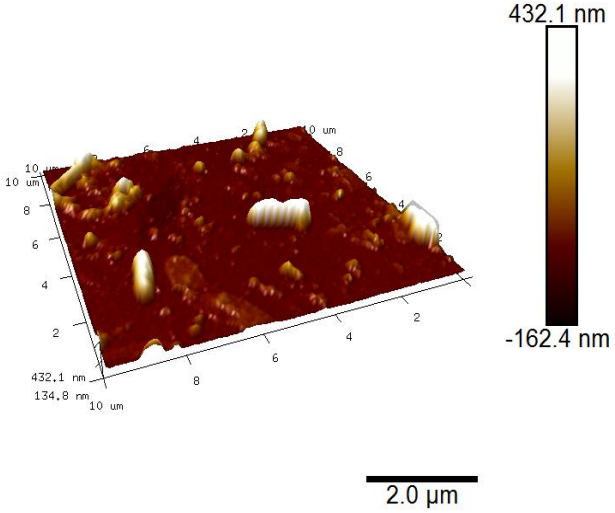

Figure 2. AFM image of *H. trueperi* CT7

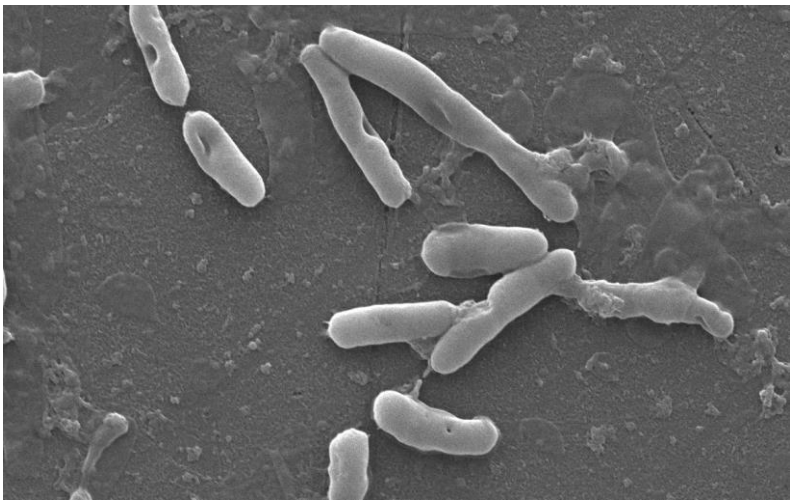

Figure 3. SEM image of spore-forming cells of *H. trueperi* CT7

The spectral scanning of *H. trueperi* extract are presented in Figure 4. According to the spectrum, maximum absorbances have been seen at 400, 425, 450, and 480 nm.

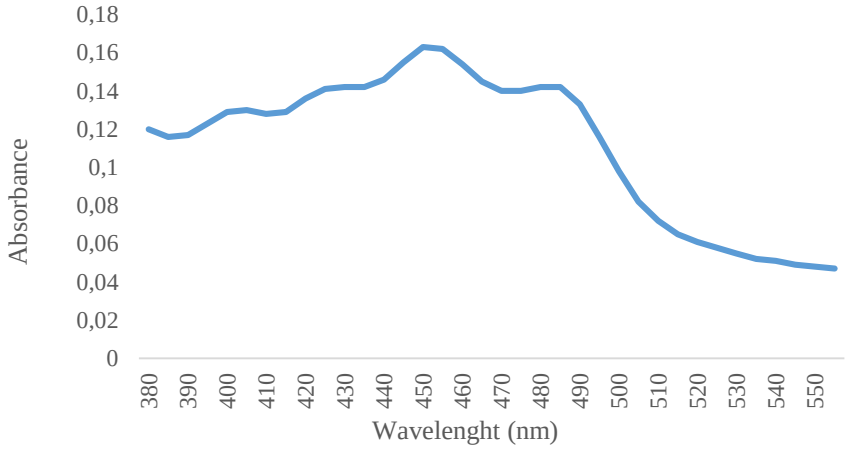

Figure 4. The spectral scanning of *H. trueperi* extract.

4. Discussion and Conclusion

It is known that *Halobacillus trueperi*, a halophilic bacterium, was first isolated from the Great Salt Lake in Utah, USA, and recorded as a new species. In the study, cells were observed with a phase contrast microscope and it was stated that the species was an endospore-forming bacterium [13]. However, in our study, *Halobacillus trueperi* species was examined for the first time with advanced technology microscopes such as SEM and AFM, and the endospore structure was detected as a hole in the SEM image in Figure 2 and the AFM image in Figure 3. Although the endospore structure is not seen in most bacteria, it is formed in some bacteria to provide resistance against adverse external conditions. Considering that the salt tolerance of *Halobacillus trueperi* is between 0-20%, it can be said that endospore structures were formed to protect itself from high salt concentrations. The nitrate production, starch hydrolysis, tween 80 hydrolysis and casein hydrolysis were determined as negative while the catalase, oxidase and gelatin hydrolysis test results were determined as positive in our research and these findings were shown similarity with the results of the research of Spring et. al. [13].

It was observed that the obtained bacterial extract obtained had a yellow-like color as well as the color of the colony. As a result of UV-VIS spectrophotometric scanning, peaks at 400, 425, 450 and 480 nm wavelengths were remarkable. The wavelengths of pigments of *Staphylococcus aureus* colonies, which were determined as yellow-orange or golden in a previous study, are similar to these wavelengths: Neo-4,4'-diaponeurosporene C, 430, 405, 428, 456 nm wavelengths, cis-4,4'-Diaponeurosporenoic acid at wavelengths of 453, 481 nm and Zeta carotene-like pigments at wavelengths of 400, 421, 452 nm [14]. It is thought that these wavelengths obtained in our study are similar to those of *Staphylococcus aureus*. It is possible to make pigment characterization with advanced chromatographic methods in future studies.

Halophilic microorganisms are a newly discovered group compared to non-extreme ones. For this reason, they continue to attract attention day by day thanks to their interesting features. There are already many patents on the use of halophilic microorganisms in medical and industrial processes [15-20]. Their enzymes, pigments and many secondary

metabolites that have not yet been identified are very promising. The wide temperature and salt tolerance range of *Halobacillus trueperi*, which was isolated and identified in our study, shows its potential to be an important brandy for industrial processes. In addition, the fact that the species has gelatinase enzyme indicates that it can be a source for gelatinase enzyme used in medicine and photography sectors [21]. With further studies, quantitative analyzes and enzyme purification of this enzyme can be performed. In addition, it is thought that it will be beneficial to shed light on the secondary metabolites of the species with advanced chromatographic methods.

Conflict of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethics Committee Approval

Ethics committee approval is not required.

Author Contribution

Conceptization: FIK, NG; methodology and laboratory analyzes: FIK; writing draft: FIK, NG; proof reading and editing: FIK, NG, NT. Other: All authors have read and agreed to the published version of manuscript.

Funding

This study was supported by Kastamonu University Scientific Research Project Fund (Project No: KUBAP01-2018-55).

5. References

[1] Oren, A., Ventosa, A., & Grant, W. D. (1997). Proposed minimal standards for description of new taxa in the order Halobacteriales. International journal of systematic and evolutionary microbiology, 47(1), 233-238.

[2] Thakur, M., & Modi, V. K. (2022). Biocolorants in food: Sources, extraction, applications and future prospects. Critical Reviews in Food Science and Nutrition, 1-40.

[3] Patkar, S., Shinde, Y., Chindarkar, P., & Chakraborty, P. (2021). Evaluation of antioxidant potential of pigments extracted from Bacillus spp. and Halomonas spp. isolated from mangrove rhizosphere. BioTechnologia, 102(2), 157.

[4] Litchfield, C. D. (2011). Potential for industrial products from the halophilic Archaea. Journal of Industrial Microbiology and Biotechnology, 38(10), 1635.

[5] Henciya, S., Vengateshwaran, T. D., Gokul, M. S., Dahms, H. U., & James, R. A. (2020). Antibacterial activity of halophilic bacteria against drug-resistant microbes associated with diabetic foot infections. Current Microbiology, 77, 3711-3723.

[6] Fariq, A., Yasmin, A., & Jamil, M. (2019). Production, characterization and antimicrobial activities of bio-pigments by Aquisalibacillus elongatus MB592, Salinicoccus sesuvii MB597, and Halomonas aquamarina MB598 isolated from Khewra Salt Range, Pakistan. Extremophiles, 23, 435-449.

[7] Chen, L., Wang, G., Bu, T., Zhang, Y., Wang, Y., Liu, M., & Lin, X. (2010). Phylogenetic analysis and screening of antimicrobial and cytotoxic activities of moderately halophilic bacteria isolated from the Weihai Solar Saltern (China). World Journal of Microbiology and Biotechnology, 26, 879-888.

[8] Enache, M., & Kamekura, M. (2013). Halophilic archaea in the Neogene salt massif from Slănic Prahova, Romania. Oltenia. Studii şi Comunicări. Stiinţele Naturii, 29, 237-243.

[9] Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. Journal of bacteriology, 70(4), 484.

[10] Neumann, B., Pospiech, A., & Schairer, H. U. (1992). Rapid isolation of genomic DNA from gram-negative bacteria. Trends in Genetics, 8(10), 332-333.

[11] Kesbiç, F. I., & Gültepe, N. (2022). C50 carotenoids extracted from Haloterrigena thermotolerans strain K15: Antioxidant potential and identification. Folia Microbiologica, 67(1), 71-79.

[12] Kesbiç, F. I., & Gültepe, N. (2023). Bioactive components, sun protective properties, and total phenolic contents of halobacterial extracts. Biochemical Systematics and Ecology, 108, 104647.

[13] Spring, S., Ludwig, W., Marquez, M. C., Ventosa, A., & Schleifer, K. H. (1996). Halobacillus gen. nov., with Descriptions of Halobacillus litoralis sp. nov. and Halobacillus trueperi sp. nov., and Transfer of Sporosarcina halophila to Halobacillus halophilus comb. nov. International Journal of Systematic and Evolutionary Microbiology, 46(2), 492-496.

[14] Marshall, J. H., & Wilmoth, G. J. (1981). Pigments of Staphylococcus aureus, a series of triterpenoid carotenoids. Journal of bacteriology, 147(3), 900-913.

[15] Giani, M., Montoyo-Pujol, Y. G., Peiró, G., & Martínez-Espinosa, R. M. (2023). Haloarchaeal carotenoids exert an in vitro antiproliferative effect on human breast cancer cell lines. Scientific Reports, 13(1), 7148.

[16] Caglayan, P., Birbir, M. E. R. A. L., Sánchez-Porro, C., & Ventosa, A. (2017). Screening of industrially important enzymes produced by moderately halophilic bacteria isolated from salted sheep skins of diverse origin. Journal of the American Leather Chemists Association, 112(06), 207-216.
[17] Kuchina, N. (2015). U.S. Patent Application No. 14/666,823.
[18] Kuchina, N. (2018). U.S. Patent Application No. 15/796,897.
[19] Herwig, C., Loranfty, B. & Porqueras, E. M. (2017). European Patent Aplication No. EP2712919B1.
[20] Umeno, T., Kobayashi, M., Misawa, N. & Takaichi, S. (2012). Worldwide Application No. WO2012169623A1
[21] Oren, A. (2010). Industrial and environmental applications of halophilic microorganisms. Environmental technology, 31(8-9), 825-834.

Kastamonu University
Journal of Engineering and Sciences
e-ISSN 2667-8209
http://dergipark.gov.tr/kastamonujes

Assessment of Afforestation Activities in Thrace Region using Some Oak (*Quercus* sp.) Varieties

Halil Barış Özel[a,*], Mesut Tandoğan[b], Nilüfer Şahin[b], Vedat Aslan[b], Mehmet Özdemir[b], Hakan Şevik[c]

[a] Department of Forest Engineering, Faculty of Forestry, Bartın University, Bartın, Türkiye
[b] Marmara Forestry Research Institute, İstanbul, Türkiye
[c] Department of Environmental Engineering, Faculty of Engineering and Architecture, Kastamonu University, Kastamonu, Türkiye
***Corresponding Author:** halilbarisozel@gmail.com

Received: **August 14, 2023** ◆ Accepted: **November 24, 2023** ◆ Published Online: **December 26, 2023**

Abstract: In this study, which is a preliminary assessment, afforestation studies established at different times with different oak species (*Quercus* sp.) in the Thrace Region were examined. Accordingly, as a result of the variance analysis applied to the data of the percentage of survival variable, which is important in terms of adaptation ability, it was determined that there was a statistically significant difference at the *P<0.05* confidence level in terms of afforestation areas. In this respect, as a result of Duncan test performed at *P<0.05* confidence level to create homogeneous groups, Ulukonak oak plantation area established with *Q. infectoria* with a survival rate of 89% is in the first group, and this group is classified with a survival percentage of 78%. Celaliye plantation area established with *Q. cerris* species followed. In the study, mean values were determined in terms of afforestation areas regarding the quantitative and morphological characters determined in the trial areas taken from the oak afforestation areas. When these data are examined, the mean diameter value varies between 2.3-5.1 cm in *Q. petraea* afforestation areas of different ages, 2.0-3.6 cm in *Q. infectoria* afforestation areas, 3.5 cm in *Q. frainetto* and *Q. cerris*. It was determined to be 4.9 cm in. Mean height value varies between 2.36-5.58 m in *Q. petraea* afforestation areas, 2.30-2.98 m in *Q. infectoria* afforestation areas, 3.62 m in *Q. frainetto* and 3.93m in *Q. cerris*. has been found to be. The crown symmetry is generally asymmetrical in all afforestation areas, regardless of the oak species. Stem straightness, on the other hand, was in the curve category for all afforestation areas. As a result of the variance analysis applied to the average volume values determined in the sampling areas, a statistically significant difference was determined between the forestation areas at the *P<0.05* confidence level. In this context, afforestation area established with *Q. petraea* oak species formed the first group with an average volume value of 0.00930 m^3, as a result of Duncan Range Test performed at *P<0.05* confidence level to create homogeneous groups in terms of afforestation areas.

Keywords: Oak, Afforestation, Survival Percentage, Adaptation, Growth

Öz: Ön değerlendirme niteliğindeki bu çalışmada Trakya Yöresinde farklı meşe türleri (*Quercus* sp.) ile değişik zamanlarda tesis edilmiş ağaçlandırma çalışmaları incelenmiştir. Buna göre, adaptasyon yeteneği açısından önemli olan yaşama yüzdesi değişkenine ait verilere uygulanan varyans analizi sonucunda ağaçlandırma alanları itibarıyla *P<0,05* güven düzeyinde istatistiki açıdan anlamlı farklılığın olduğu belirlenmiştir. Bu itibarla homojen grupları oluşturmak için *P<0,05* güven düzeyinde gerçekleştirilen Duncan testi sonucunda, %89'luk yaşama yüzdesi ile *Q. infectoria* ile tesis edilen Ulukonak meşe ağaçlandırma alanı ilk grupta yer alırken bu grubu, %78'lik yaşama yüzdesi değeri ile *Q. cerris* türü ile tesis edilen Celaliye ağaçlandırma alanı izlemiştir. Araştırmada meşe ağaçlandırma alanlarından alınan örnek alanlarda tespit edilen kantitatif ve morfolojik karakterlere ilişkin ağaçlandırma alanları itibarıyla ortalama değerler saptanmıştır. Bu veriler incelendiğinde ortalama çap değerinin farklı yaşlardaki *Q. petraea* ağaçlandırma alanlarında 2,3-5,1cm arasında değiştiği, *Q. infectoria* ağaçlandırma alanlarında 2,0-3,6cm arasında değiştiği, *Q. frainetto*'da 3,5cm ve *Q. cerris*'te 4,9cm olduğu belirlenmiştir. Ortalama boy değerinin *Q. petraea* ağaçlandırma alanlarında 2,36-5,58m arasında değiştiği, *Q. infectoria* ağaçlandırma alanlarında 2,30-2,98 m arasında değiştiği, *Q. frainetto*'da 3,62m ve *Q. cerris*'te 3,93m olduğu tespit edilmiştir. Tepe simetrisi ise tüm ağaçlandırma alanlarında meşe türleri fark etmeksizin genel olarak asimetrik niteliktedir. Gövde düzgünlüğü ise yine tüm ağaçlandırma alanları için genel olarak eğri kategorisinde yer almıştır. Örnekleme alanlarında belirlenen ortalama hacim değerlerine uygulanan varyans analizi sonucunda ağaçlandırma alanları arasında *P<0,05* güven düzeyinde istatistiki açıdan anlamlı farklılık belirlenmiştir. Bu kapsamda ağaçlandırma alanları itibarıyla homojen grupları oluşturmak için *P<0,05* güven düzeyinde gerçekleştirilen Duncan testi sonucunda 0,00930 m^3'lük ortalama hacim değeri ile *Q. petraea* meşe türü ile tesis edilen ağaçlandırma alanı ilk grubu oluşturmuştur.

Anahtar Kelimeler: Meşe, Ağaçlandırma, Yaşama Yüzdesi, Adaptasyon, Büyüme

1. Introduction

Successful regeneration activities should be conducted in our oak (*Quercus* sp.) forests because the continuity of natural forest resources consisting of this important species can be ensured and their productivity can be increased only through successful natural and artificial regeneration efforts [1]. Regeneration is an urgent silvicultural problem in the majority of

Türkiye's oak stands and, in a significant portion of these problems, it is necessary to make use of artificial means for stand establishment or implement artificial regeneration as a complement or auxiliary to natural regeneration [2].

Oak forests, which were managed as coppices, have suffered from pressure and indiscriminate use resulting in ecological imbalances, been deforested, and turned into erosion-prone areas and it is possible to restore their productivity by converting the existing coppices into managed stands, where appropriate oak species, along with compatible coniferous species, can be afforested using seedlings or seeds, with adaptation to the ecological conditions of oak forest openings [3,4]. Saatçioğlu [5], stated that converting degraded or coppice oak areas in the Belgrade forests into forests is a silvicultural necessity for quickly making them productive and the oak species with the highest silvicultural and economic value and capability should be prioritized during the establishment of forest stands.

Afforestation efforts were carried out with oak (*Quercus* sp.) species for various purposes in different forest habitats (Northern Thrace, Inner Thrace, and Çatalca) and management directorates (Kırklareli, Vize, Demirköy, Istanbul) in the Thrace Region. In the present study, the success performances of afforestation activities conducted with oak at 9 sample sites in different periods in the Thrace Region were evaluated. Measurements, observations, and records were made on 400 m^2 sample plots regarding diameter-height, spacing-distance, volume, and some site characteristics to assess the success of oak afforestation activities. Thus, initial evaluations were made regarding the oak afforestation practices established at different times in the Thrace Region and some preliminary information for practitioners is provided here.

2. Material and Method

Material

Presentation of study area

As stated by [6, 7], the afforestation areas in Kırklareli, Vize Kömürköy, Pınarhisar Evciler, Osmancık, and Demirköy Lafya Deresi are located within the "North Thrace Mountainous Habitat," whereas the afforestation areas in Yeniköy and Ulukonak in Kırklareli and Celaliye in Lüleburgaz are located within the "Inner Thrace Habitat" region. Furthermore, the Istanbul Hasdal afforestation area, which was examined as part of the research, is located within the "Çatalca Peninsula Habitat" region (Figure 1).

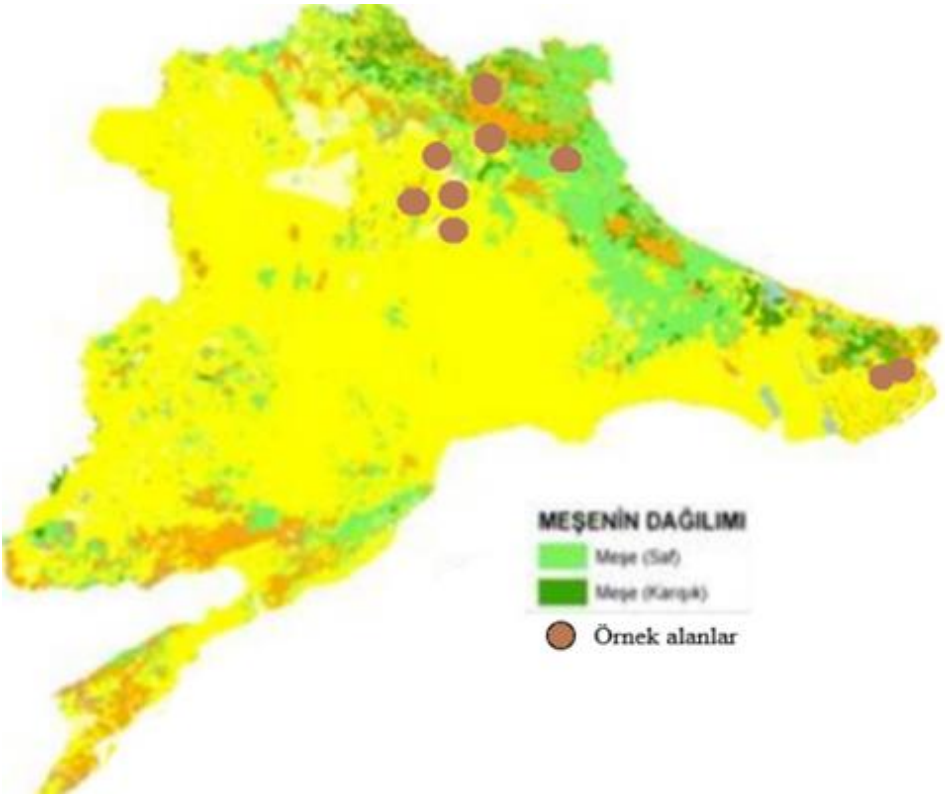

Figure 1. Study area [8]

Physiographical characteristics of the sample areas of 20 m x 20 m, which were selected by using the random block experimental design from the oak afforestation areas to best represent the afforestation areas established in different years and where the study was carried out are presented in Table 1.

Table 1. Presentation of afforestation areas

Business Administration	Location	Species	Planting area (ha)	Slope exposure	Height (m)	Slope (%)	Sample area (m^2)
Kırklareli - Pınarhisar	Evciler	*Q. petraea*	10.5	GB	512	40-45	400
Kırklareli – Lüleburgaz	Yeniköy	*Q. petraea*	21.5	GD-D	145	10-20	400
Kırklareli – Pınarhisar	Osmancık	*Q. petraea*	58	B-KB	187	10-30	400
Kırklareli – Lüleburgaz	Celaliye	*Q. cerris*	6	KB	164	0-20	400
Kırklareli – Merkez	Ulukonak	*Q. infectoria*	17	K-G	150	0-20	400
Demirköy – İncesırt	İncesırt	*Q. frainetto*	21.5	GB	388	0-20	400
Vize – Kömürköy	Kızılağaç	*Q. petraea*	10.1	K	180	30-40	400
İstanbul - Merkez	Hasdal 1-2	*Q. infectoria*	13.3	GB	180	0-20	400

Moreover, the current status of the afforestation areas, which were established with different oak species in different years and constitute the research area, and which also serve as a preliminary assessment, is presented in Figure 2.

Figure 2. Kırklareli-Yeniköy, b-Vize-Kömürköy-Kızılağaç, c-Demirköy-Lafyaderesi oak afforestation areas

Information regarding the edaphic characteristics specified in the implementation projects of the afforestation areas established with different oak species in different years was utilized in order to determine the edaphic characteristics of the oak afforestation areas where the study was carried out. Accordingly, except for the area in Demirköy characterized as "clay mud," the sample areas are generally characterized as "sandy mud" or "sandy-clay mud." Some soil properties of the oak afforestation areas, as well as the land preparation operations performed, are presented in Table 2. Furthermore, the climate types of the afforestation areas determined according to [9] are shown in Table 3.

Table 2. Some soil characteristics of oak afforestation areas by afforestation implementation projects

Locations	Years	Species	Soil processing	Bedrock	Depth (cm)	Soil species
Evciler	1997	Q. petraea	DTM+Pal. TR+2'li rip.	Gnays-Şist	M 31-60/F 60 üz	sandy loam
Yeniköy	2001	Q. petraea	DTM+Pal. TR+2'li rip.	Sediment	M, F 60 üz	Sandy-clay loam
Osmancık	1991	Q. petraea	DTM+Pal. TR+2'li rip.	Sediment	M, F 60 üz	sandy loam
Celaliye	1994	Q. cerris	DTI+Pal. TR+2'li rip.	Sediment	M, F 60 üz	sandy loam
Ulukonak	2004	Q. infectoria	DTM+Pal. TR+2'li rip.	Sediment	M, F 60 üz	Sandy-clay loam
Demirköy	1993	Q. frainetto	DTI + Pal TR+3'lü rip.	Effusive	M, F 60 üz	clay loam
Kömürköy	1983-1984	Q. petraea	DTM+Pal. TR+2'li rip.	Metamorphic	M 31-60/F 60 üz	sandy loam
Hasdal 1-2	2010-2011	Q. infectoria	DTM+ Pal. TR+3'lü rip.	Sediment	M, F 60 üz	sandy loam

Table 3. Climate types of oak afforestation areas determined according to [9]

Locations	Annual avg. precipitation (mm)	Annual avg. load. temp (C)	Climate type
Evciler	630.1	18.7	Semi-Moist
Yeniköy	575.8	18.4	Semi-Moist
Osmancık	575.8	18.4	Semi-Moist
Celaliye	575.8	18.4	Semi-Moist
Ulukonak	575.8	18.4	Semi-Moist
Demirköy	818	17.3	Mosit
Kömürköy	639.2	18.2	Semi-Moist
Hasdal 1-2	1088.8	17.4	Too Moist

Digital transportation network to be used in the implementation of the analysis and the evaluation of the analysis result; Data of the locations of Health Service Centers; border and population data of the neighborhoods within the study area; The vector data of the houses in the study area were used. Digital road network data of the study area has been obtained, and corrections have been made.

Method

Measurements and determinations made in afforestation areas

In this preliminary study carried out in order to determine the growth and adaptation capabilities of afforestation activities conducted with different oak species in different years, quantitative characteristics such as diameter at breast height (DBH) and total height were measured in sample plots taken randomly from afforestation areas by making use of the systematic sampling method. In addition, quality parameters such as stem straightness and crown condition were evaluated by using binary and ternary indices. The average values of DBH, height, and calculated volume per hectare were calculated for afforestation areas and oak species. The average volumes of oak species were calculated by using the yield table prepared by [10]. Furthermore, the survival percentage was calculated by utilizing the individual counts determined and reported per hectare based on the planting spacing distances applied to determine the adaptation performance of afforestation areas because the survival percentage (success rate) is an important variable used in order to evaluate afforestation investments from the early years, especially from a technical and biological perspective [11].

Statistical Analyses

The data related to average volume and survival percentage variables were included in statistical analyses for growth performance and adaptation ability due to age differences in afforestation areas, as well as the differences in planting spacings. For this purpose, the Kolmogorov-Smirnov (K-S) normality test was first applied to the data related to average volume and survival percentage variables. After determining that the data showed a normal distribution by using this test, the One-Way Analysis of Variance (ANOVA) was conducted in order to determine if there was a statistically significant difference between afforestation areas in terms of average volume and survival percentage variables. In case of a statistically significant difference between afforestation areas in terms of average volume and survival percentage variables found ANOVA, the Duncan test was used at a confidence level of $P < 0.05$ in order to determine the homogeneous groups. The SPSS 22.0 statistical package program was used in all these statistical analyses. The data of the survival percentage variable were subjected to Arc.Sin transformation before being subjected to statistical analyses.

3. Result

The number of trees per hectare and the survival percentage values determined in afforestation areas established with different oak species in different years are presented in Table 4. As a result of the variance analysis applied to the survival percentage data, a statistically significant difference at a confidence level of $P < 0.05$ was found between afforestation areas. Therefore, based on the results of the Duncan test conducted at a confidence level of $P < 0.05$ to form homogeneous groups, the Ulukonak oak afforestation area established with *Q. infectoria* with an 89% survival percentage was found to be in the first group, followed by the Celaliye afforestation area established with *Q. cerris* with a 78% survival percentage (Table 4).

Table 4. Duncan test results and individual count and survival percentage values by afforestation areas

Locaiton	Species	Range × Distance (m)	Individual number (ha/piece)	Survival percentage (%)
Pınarhisar	*Q. petraea*	2×1.5	2225	66bc
Yeniköy	*Q. petraea*	2×1.5	1575	47d
Osmancık	*Q. petraea*	2×1.5	1650	49d
Celaliye	*Q. cerris*	3×1.25	2075	78b
Ulukonak	*Q. infectoria*	3×1.25	2375	89a
Demirköy	*Q. frainetto*	2×1.0	3750	75b
Kömürköy	*Q. petraea*	1.0×1.0	5400	54c
Hasdal 1	*Q. infectoria*	1×1.5	3050	46d
Hasdal 2	*Q. infectoria*	2×1.5	1500	45d

The average values of quantitative and morphological characteristics determined in sample plots taken from oak afforestation areas are given for each oak afforestation area in Table 5. Given these data, it was determined that the average diameter ranged between 2.3 and 5.1 cm in different-aged *Q. petraea* afforestation areas, between 2.0 and 3.6 cm in *Q. infectoria* afforestation areas, 3.5 cm in *Q. frainetto*, and 4.9 cm in *Q. cerris* (Table 5). The average height was found to vary between 2.36 and 5.58 m in *Q. petraea* afforestation areas, between 2.30 and 2.98 m in *Q. infectoria* afforestation areas, 3.62 m in *Q. frainetto*, and 3.93 m in *Q. cerris* (Table 5). As a result of the variance analysis applied to the average volume values found in the sample plots, a statistically significant difference was found between afforestation areas at a confidence level of $P < 0.05$. In this context, the Duncan test conducted at a confidence level of $P < 0.05$ resulted in the first group being formed by the oak afforestation area established with *Q. petraea* with an average

volume of 0.00930 m^3. The crown symmetry is generally "asymmetric" in all afforestation areas, regardless of oak species. Stem straightness is categorized as "curved" for all afforestation areas (Table 5).

Table 5. Average values of quantitative and morphological characteristics of oak species in afforestation areas

Locaiton	Species	Years	Range × Distance (m)	Average diameter (cm)	Average size (m)	Average volume (m^3)	Vertex symmetry	Body smoothness
Pınarhisar	Q. petraea	21	2×1.5	2.3	2.36	0.00246e	Asymmetric	Curve-Too Curve
Yeniköy	Q. petraea	17	2×1.5	4.3	3.33	0.00546c	Asymmetric	Curve - Smooth
Osmancık	Q. petraea	27	2×1.5-3×1.25	3.2	2.92	0.00338d	Asymmetric	Curve
Celaliye	Q. cerris	24	3×1.25	4.9	3.93	0.00741b	Asymmetric	Curve
Ulukonak	Q. infectoria	14	3×1.25	3.6	2.98	0.00416cd	Asymmetric	Too Curve
Demirköy	Q. frainetto	25	2×1.0	3.5	3.62	0.00508c	Asymmetric	Too Curve
Kömürköy	Q. petraea	35	1.0×1.0	5.1	5.58	0.00930a	Symmetric	Curve
Hasdal 1	Q. infectoria	8	1×1.5	1.6	2.47	0.00247e	Asymmetric	Curve
Hasdal 2	Q. infectoria	9	2×1.5	2	2.3	0.00209e	Asymmetric	Curve

In addition, the diameter and height performances of sessile oak and Hungarian oak were analyzed using the yield tables prepared by Şahin [12] and Şahin et al. [13], respectively. It was determined that the average diameter and height values were below the normal growth performances according to all yield tables (Table 6).

Table 6. Average diameter and height values of sessile oak and Hungarian oak in afforestation areas

					Average diameter (cm)			Average size (m)		
Order	Locations	Species	Years	Bonitet	Measurement	Eraslan [9]	Şahin et al. [13]	Measurement	Eraslan [9]	Şahin et al. [13]
1	Pınarhisar	Q. petraea	21	IV	2.3	4.36	2.8	2.36	18.5	5.60
2	Yeniköy	Q. petraea	17	III	4.3	3.24	3.6	3.33	22.5	6.00
3	Osmancık	Q. petraea	27	III	3.2	6.95	5.9	2.92	22.5	8.27
6	Demirköy	Q. frainetto	25	II	3.5	6.95	8.0	3.62	26.5	8.50
7	Kömürköy	Q. petraea	35	III-IV	5.1	6.45	9.5	5.58	18.5	11.75

4. Discussion

Within the scope of the research, it was determined in the preliminary assessment process that the afforestation areas established with different oak species in different years generally showed a development trend in the right direction in terms of fulfilling the projected or planned forest functions. It can be said that the afforestation functions anticipated or advocated by various researchers [14-16] Saatçioğlu, [5] were observed in the oak afforestation areas, which constitute the research material, in the Trakya Region. Considering the actual growing conditions, it is also of significant importance in terms of maintaining the ecological balance in the region and ensuring the continuity of the forest ecosystem.

Phenotypic traits of all living organisms are shaped by genetic structure [17-19] and environmental conditions [20]. Environmental conditions consist primarily of climatic [21, 22] and edaphic [23] conditions. Indeed, when evaluated for the current actual structure, it can be stated that the edaphic conditions, which are one of the important ecological factors for the development of oak species from the early years, as in all plants, do not pose significant problems or create a significant development and adaptation issue for oak afforestation areas. As [24] and [12] stated, the region where afforestation was carried out exhibits a suitable structure in terms of all ecological factors and especially edaphic characteristics regarding oak afforestation studies.

In light of the initial and preliminary assessments, although the initial values, especially in terms of the survival percentage (success rate) that is the most important indicator of adaptability, had relatively low values for oak species used in afforestation activities and afforestation areas (Table 4), it should be noted that it is still difficult to reach a definitive judgment based on these evaluations. In terms of this variable, oak species, which have different varieties with a wide geographic variation in different growing conditions, can occasionally have low survival percentage values due to their

sensitive characteristics in terms of plant nutrition [25-28]. However, especially considering the negative effects of global warming on all organism populations, this finding further emphasizes the importance of oaks and oak forests both for the continuity of other organism groups in the forest ecosystem and for soil preservation characteristics [29, 30]. Actually, previous studies emphasized that the most significant effects of global warming will manifest itself in the form of a general increase in temperature and drought [31, 32]. In this process, oaks, which stand out with their drought tolerance and high adaptability, have a special importance.

The oak afforestation carried out using different oak species, which constitute the research material, has not yet exhibited very highperformance values in terms of quantitative and morphological characteristics that are quite important indicators of growth performance, as given in Table 5. Especially considering the site differences in terms of breast height diameter, height, and volume variables, it tends to show a performance below the expected level. However, it should be noted that different conditions may arise in afforestation areas due to the changing effects of both biotic and abiotic factors each year, and especially the influence of these factors on growth performance may vary [33]. Saatçioğlu [2] stated that site productivity can have a significant effect on the development of oak afforestation and the Site II areas in Belgrad Forest are suitable conditions for oaks. Accordingly, it would be suitable to test the growth performance of oak varieties under conditions suitable for the Site II in the Thrace Region.

5. Conclusion

Considering the results obtained from the present preliminary research, it can be stated that the afforestation activities conducted with different oak varieties in the Thrace region are highly beneficial and significant in terms of preserving the ecological balance, nature, and multidimensional forest ecosystems of the region. Therefore, it is crucial to ensure the preservation and continuity of these afforestation projects, which will provide high benefits for the health of the local community and soil preservation. Consequently, all necessary measures, especially maintenance works, should be conducted in afforestation areas in accordance with the appropriate technical procedures. Oak afforestation areas, which are not yet able to fully perform their ecological and technical functions due to social pressures and grazing damages, must be particularly protected. In addition, aiming to enhance the contributions of this species to the national economy, oak afforestation should be continued in suitable areas determined using multidimensional decision-making techniques in order to meet the raw material needs of the local population and the country's forest industry. Furthermore, it should always be kept in mind that the success of afforestation- efforts involving sensitive broad-leaved species like oak is remarkably affected by the quality of seedlings. In this context, successful local origins to be used in such oak afforestation should be identified, improved seed sources should be established from them, and high-quality and durable seedlings raised from seeds collected from these sources should be used for afforestation and artificial regeneration practices in open areas, both in our Thrace region and in different parts of our country.

Conflict of Interest

The authors declare that they have no competing interests.

Ethics Committee Approval

N/A

Author Contribution

Conceptualization: H.B.Ö., M.T., N.Ş. V.A., M.Ö., H.Ş.; Investigation: H.B.Ö., M.T., N.Ş. V.A., M.Ö., H.Ş.; Material and Methodology: H.B.Ö., M.T., N.Ş.; Visualization: V.A., M.Ö., H.Ş.; Writing-Original Draft: V.A., M.Ö., H.Ş.; Writing-review & Editing: V.A., M.Ö., H.Ş.; Other: All authors have read and agreed to the published version of manuscript.

Funding

The author declared that this study has received no financial support.

Acknowledgements

This article was presented as an oral presentation at the 2nd International Oak Workshop held by the General Directorate of Forestry of the Republic of Türkiye and the Directorate of Marmara Forestry Research Institute on 10-12 May 2022. We would like to express our sincere gratitude to Mr. Nüfer YAVUZ, Mr. Hikmet İHTİYAR, and Mrs. Nihan İNAN, officials and technical personnel from Kırklareli Forest Management Directorate, Lüleburgaz Forest Nursery Directorate, and Marmara Forestry Research Institute Directorate, for their assistance and great support during the research process related to this article. We'd also like to thank Mr. A. Ayhan KUL, Mr. Hayali GEÇİT (retired Forest Management Director), and Mrs. Vicdan ALADAĞ (retired Nursery Director).

6. References

[1] Özel, H. B., & Ertekin, M. (2010). İstanbul-Bentler yöresindeki doğal sapsız meşe (Quercus petraea (mattuschka) lieb.) gençliklerinde ilk büyümelerin değerlendirilmesi. Bartın Orman Fakültesi Dergisi, 12(18), 67-76.

[2] Saatçioğlu, F. (1979). Silvikültür Tekniği (Silvikültür II), İ.Ü Orman Fakültesi, (Silvikültür II), 2490(268), 556.

[3] Taşdemir, C., & Karatay H. (2007). Elazığ Yöresinde İran Palamut Meşesi (Quercus brantii Lindl.) ve Saçlı Meşe (Quercus cerris L.)'de Bazı Fidanlık ve Ağaçlandırma Tekniklerinin Araştırılması. Güneydoğu Anadolu Ormancılık Araştırma Enstitüsü Müdürlüğü, Araştırma Bülteni, 23049.
[4] Saatçioğlu, F. (1952). Türkiye'de Ağaçlandırmanın Önemine ve Problemlerine Toplu Bakış, İstanbul Üniversitesi Orman Fakültesi Dergisi, Seri A,2(1), 60-70.
[5] Saatçioğlu, F., (1960). Belgrad Ormanı'nda Çeşitli Aletlerle Yapılan Meşe Dikimlerine Ait Denemeler ve Sonuçları. İ.Ü. Orman Fakültesi Dergisi.
[6] Kantarcı, M. D. (1976). Trakya Orman Mıntıkalarının Bölgesel Orman Yetişme Muhiti Özelliklerine Göre Doğal Ağaç Ve Çalı Türleri Đle Sınıflandırılması, Đstanbul Üniversitesi Orman Fakültesi Dergisi, Seri. İstanbul Üniversitesi Orman Fakültesi Dergisi, Seri: A, 26(2).
[7] Kantarcı, M. D. (2018). Kuzey Trakya Yetişme Ortamı Bölgesi Ormanları ve İklim Değişikliği İle Hava Kirliliğinin Orman Ekosistemlerine Etkileri Üzerine Ekolojik Bir Değerlendirme, İstanbul Orman Bölge Müdürlüğü Yayını.
[8] Şahin, A. (2016). Marmara Bölgesindeki meşe ormanlarının yayılışı, işletme amaçları ve planlanması. Orman Genel Müdürlüğü Marmara Ormancılık Araştırma Enstitüsü, Uluslararası Katılımlı Meşe Çalıştayı Bildiriler Kitabı, s. 27-51. 18-20 Ekim, İğneada–Kırklareli.
[9] Erinç, S. (1965). Yağış Müessiriyeti Üzerine Bir Deneme ve Yeni Bir İndis, İstanbul Üniversitesi, Coğrafya Enstitüsü, 41.
[10] Eraslan, İ. (1954). Trakya ve bilhassa Demirköy mıntıkası meşe ormanlarının amenajman esasları hakkında araştırmalar, Meşe Hasılat Tablosu. OGM Yayınları, 132(13).
[11] Ürgenç, S. (1986). Ağaçlandırma Tekniği, İstanbul Üniversitesi Orman Fakültesi Dergisi 3314(375), 5250.
[12] Şahin, A., 2020. Marmara Bölgesindeki Sapsız Meşe (Quercus petraea (Mattuschka) Liebl.) Meşcerelerinin Hasılatı ve Amenajman Esasları. İstanbul Üniversitesi-Cerrahpaşa Lisansüstü Eğitim Enstitüsü. Doktora Tezi. İstanbul.
[13] Şahin, A., Özdemir, E., Aylak Özdemir, G., Biricik, Y., Korkmaz, Ü., Saraçoğlu, Ö. 2021. Macar Meşesi (Quercus frainetto Ten.) Meşcerelerinin Hasılatı (Proje No: 10.3201/2014-2018-2020). Marmara Ormancılık Araştırma Enstitüsü Müdürlüğü, Araştırma Projesi Sonuç Raporu, 237 sayfa, İstanbul.
[14] Uğurlu, S., & Çevik, I. (1991). Güneydoğu Anadolu Bölgesinde bazi meşe türlerinin ekim yoluyla ağaçlandirma tekniği. Ormancilik Araştirma Enstitüsü Müdürlüğü.
[15] Boydak, M., & Çalışkan, S. (2014). Ağaçlandırma, Ormancılığı Geliştirme ve Orman Yangınları ile Mücadele Hizmetlerini Destekleme Vakfı.
[16] Kalkan, B., Karakurt, H., Özkan, K., Tiryaki, O., & Güneş, Ö. (2021). Elazığ yöresinde meşe ekimlerinin gelişimi ile bazı yetişme ortamı özellikleri arasındaki ilişkiler. Ormancılık Araştırma Dergisi, 8(1), 27-41.
[17] Imren, E., Kurt, R., Yucedag, C., Bilir, N., Ozel, H. B., Cetin, M., & Sevik, H. (2021). Selection of superior clones by the multi-dimensional decision making techniques in Scots pine seed orchard. Journal of Forests, 8(1), 13-22.
[18] Özel, H. B., Şevik, H., Onat, S. M., & Yigit, N. (2022). The effect of geographic location and seed storage time on the content of fatty acids in stone pine (Pinus pinea L.) seeds. BioResources, 17(3), 5038.
[19] Macnair, M. R. (1987). Heavy metal tolerance in plants: a model evolutionary system. Trends in Ecology & Evolution, 2(12), 354-359.
[20] Liang, J., Chen, C., Song, X., Han, Y., & Liang, Z. (2011). Assessment of heavy metal pollution in soil and plants from Dunhua sewage irrigation area. International journal of electrochemical science, 6(11), 5314-5324.
[21] Isinkaralar K. (2022). Some atmospheric trace metals deposition in selected trees as a possible biomonitor. Romanian Biotechnological Letters, 27(1), 3227-3236.
[22] Yayla, E. E., Sevik, H., & Isinkaralar, K. (2022). Detection of landscape species as a low-cost biomonitoring study: Cr, Mn, and Zn pollution in an urban air quality. Environmental monitoring and assessment, 194(10), 687.
[23] Işınkaralar, Ö., & Şevik, H. (2023). A Statistical Analysis of Traffic-Related Pollution and City Center Dynamics in Ankara: The Cases of Ulus and Kızılay. Journal of Ankara Studies, 11(1), 25-35.
[24] Pirinc Bayraktar, E., Isinkaralar, O., & Isinkaralar, K. (2022). Usability of several species for monitoring and reducing the heavy metal pollution threatening the public health in urban environment of Ankara. World Journal of Advanced Research and Reviews, 14(3), 276-283.
[25] Işınkaralar, K. (2022). Evaluation of environmental barium concentration biomonitoring in tree rings. Turkish Journal of Agriculture-Food Science and Technology, 10(4), 754-759.
[26] Rajkumar, M., Sandhya, S., Prasad, M. N. V., & Freitas, H. (2012). Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnology advances, 30(6), 1562-1574.
[27] Gamalero, E., Lingua, G., Berta, G., & Glick, B. R. (2009). Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Canadian journal of microbiology, 55(5), 501-514.
[28] Işınkaralar, K., & Erdem, R. (2022). The effect of atmospheric deposition on potassium accumulation in several tree species as a biomonitor. Environmental Research and Technology, 5(1), 94-100.

[29] Badr, N., Fawzy, M., & Al-Qahtani, K. M. (2012). Phytoremediation: An ecological solution to heavy-metal-polluted soil and evaluation of plant removal ability. World Applied Sciences Journal, 16(9), 1292-1301.
[30] Malik, R. N., Husain, S. Z., & Nazir, I. (2010). Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. Pak J Bot, 42(1), 291-301.
[31] Jung, M. C. (2008). Heavy metal concentrations in soils and factors affecting metal uptake by plants in the vicinity of a Korean Cu-W mine. Sensors, 8(4), 2413-2423.
[32] Nabi, R. B. S., Tayade, R., Hussain, A., Kulkarni, K. P., Imran, Q. M., Mun, B. G., & Yun, B. W. (2019). Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. Environmental and Experimental Botany, 161, 120-133.
[33] Shaw, B. P., Sahu, S. K., & Mishra, R. K. (2004). Heavy metal induced oxidative damage in terrestrial plants. Heavy metal stress in plants: from biomolecules to ecosystems, 84-126.

doi:10.55385/kastamonujes.1355695 KUJES 9(2):61-66, 2023

Kastamonu University
Journal of Engineering and Sciences
e-ISSN 2667-8209
http://dergipark.gov.tr/kastamonujes

Comparative Performance Analysis of Ensemble Learning Algorithms for Rock Classification

Ebru Efeoğlu

Department of Software Engineering, Faculty of Engineering, Kütahya Dumlupınar University, Kütahya, Türkiye
***Corresponding Author:** ebru.efeoglu@dpu.edu.tr

Received: **September 05, 2023** ◆ Accepted: **November 30, 2023** ◆ Published Online: **December 26, 2023**

Abstract: Knowing the physical and mechanical properties of rocks is important for engineering studies. Because determining the properties and type of rocks affects the safety of engineering structures. Therefore, this study is important in terms of minimizing possible errors in engineering studies. Moreover, Automatic detection of rock types reduces the workload of engineers. In this study, the types of rocks were determined by using some physical and mechanical properties of rocks measured in the laboratory. Rep tree algorithm and ensemble learning algorithms were used in the study. The success of ensemble learning algorithms in classification was compared. As a result, it was understood that ensemble learning algorithms increase success. When the logitboost algorithm was used together with the rep tree algorithm, the Tp rate increased to 0.82. Precision Recall values were 0.80, MCC and AUC were 0.95, kappa was 0.80. In addition, the FP rate decreased to 0.04. The most successful algorithm in rock classification was the Logistboost algorithm. The highest performance metrics were obtained in the classification made with the Logistboost algorithm. In addition, 4 different metric types were calculated to determine the error rates of the algorithms. Logistboost algorithm classified with the lowest error rate.

Keywords: Clasification, Ensemble learning algorithms, Machine learning, Rep tree, Rock

Öz: Kayaların fiziksel ve mekanik özelliklerinin bilinmesi mühendislik çalışmaları açısından önemlidir. Çünkü kayaların özelliklerinin ve türünün belirlenmesi mühendislik yapılarının güvenliğini etkilemektedir. Kaya türlerinin otomatik tespiti mühendislerin iş yükünü azaltır. Bu çalışmada kayaçların laboratuvarda ölçülen bazı fiziksel ve mekanik özellikleri kullanılarak kaya türleri belirlenmiştir. Çalışmada rep ağacı algoritması ve topluluk öğrenme algoritmaları kullanılmıştır. Topluluk öğrenme algoritmalarının sınıflandırmadaki başarısı karşılaştırıldı. Sonuç olarak topluluk öğrenme algoritmalarının başarıyı arttırdığı anlaşıldı. Kaya sınıflandırmasında en başarılı algoritma Logistboost algoritması oldu. Logistboost algoritması ile yapılan sınıflandırmada en yüksek performanslı metrikler elde edildi. Ayrıca algoritmaların hata oranlarını belirlemek için 4 farklı metrik türü hesaplandı. Logistboost algoritması en düşük hata oranına ile sınıflandırmayı gerçekleştirdi.

Anahtar Kelimeler: Sınıflandırma, Topluluk öğrenme algoritmaları, Makine öğrenimi, Rep ağacı, Kaya

1. Introduction

The determination of the properties of the rocks is important in terms of determining the usage area of the rock. The usage area is determined according to the type of rock. For example, basalt is often used as an insulation and durable building material because it is resistant to heat and frost. Basalt as a storage material for high-temperature concentrated solar power plants was investigated by experiments [1]. Fiber reinforced polymeric composite materials were examined [2]. To determine the mechanical properties of basalt, uniaxial compressive strength (UCS) and Young's modulus were evaluated [3]. The geological and mineralogical properties of basalt in Karakalpakstan were investigated [4]. Research has been done on the use of various rocks in tunnel work. Rock tunnel performance under blast loading was investigated by finite element analysis [5]. A three-dimensional finite element analysis of the tunnel was performed under static loading condition and the effect of rock weathering was examined [6]. A simulation model of a rock tunnel with mudstone and sandstone layers was made [7]. The dynamic stability of the subway tunnel in layered weathered sandstone was analyzed [8]. Another name for shale is clay stone and it is generally used in the construction industry. It is used to ensure that the paint is more permanent on the surfaces on which it will be applied. It is used in the manufacture of glass products and materials. In addition, it is of great importance in the extraction and processing of oil, which is very important in the energy sector. The complex conductivity of graphitic schists and sandstones was studied [9]. Granite is used in kitchen counters, sinks in the bathroom, exterior cladding, table and coffee table production, garden stones, decoration decorations. A study was conducted on the use of waste marble and granite dust in structural applications [10]. Experimental studies were carried out on the physical and mechanical changes of hot granite under different cooling processes [11]. Geochemical controls of uranium release from neutral-pH rock drainage produced by the weathering of

granite, gneiss and schist [12]. Sandstone is used in construction, paving of roads and pavements, limestone is used in soda production, glass industry and sugar production.

Machine learning methods, which have a wide usage area, were also used in studies related to rocks. Artificial neural network models were used to estimate the unconfined compressive strength of the rock [13]. Supervised machine learning techniques were used to estimate the tunnel boring machine penetration rate [14]. Petrographic classification of sand and sandstone was done [15]. Machine learning methods were used for lithology classification using geophysical data [16]. In this study, it was aimed to automatically detect different rock types using their mechanical and physical properties. and 7 different rock types were identified in the study. Ensemble learning algorithms were used to achieve the best performance.

2. Material and Method

In this study, 7 different rock types collected in Kocaeli were collected [17]. Cylindrical samples with a length of 110–115 mm and a diameter of 54 mm [18,19,20] were prepared from the rock types collected. Ultrasonic pulse velocity, ultrasonic pulse velocity (UPV), uniaxial compressive strength (UCS), resistivity (Ro), chargeability (M) and porosity (n) values of the samples were measured [17]. To measure the UPV value, Proseq acoustic pulse velocity test device was used in accordance with ISRM 1981 and ASTM 1978 standards [17]. The measurements were used to classify rocks. The rocks used and their properties are given in Table 1.

Table 1. Rocks and their properties

Rocks	The properties of the rocks
Lime stone	It is a type of sedimentary rock with at least 90% calcium carbonate ($CaCO_3$) in its chemical composition and at least 90% calcite mineral in its mineralogical composition.
Arkose	It is a type of sandstone. They derive from granitoid igneous rocks. It contains feldspar and mica fractures.
Sandstone	It is a type of sedimentary rock. Contains feldspar and quartz.
Basalt	It is a type of igneous rock. It contains high amounts of MgO and CaO and low amounts of $Na_2O + K_2O$.
Granite	Granite is a hard, crystalline mineralized igneous rock. It contains silicate, quartz and feldspar minerals.
Amphibolite	It is a metamorphic rock containing amphibole hornblende and actinolite.
Schist	It is a metamorphic rock

Rep Tree

REP Tree algorithm is a fast algorithm and works with regression tree logic. The information gain criterion is used to construct the regression tree. It creates multiple trees in iterations and then chooses the best one from the trees it creates. The pruning method is used to minimize the error.

Bagging

Bagging algorithm is an ensemble learning method for creating a classifier ensemble by combining basic learning algorithms trained on different samples of the training set [21]. The Bagging algorithm is based on the principle that each basic learning algorithm that makes up the community is trained on different training sets. Thus, diversity increases. It is aimed to create different training sets from the data set. Simple random substitution sampling method is generally used to create the training set. Then, the outputs of the classification methods trained with the training sets are combined through majority voting.

Adaboost

AdaBoost algorithm is one of the most basic Boosting methods. This algorithm focuses more on samples that are difficult to classify. The purpose of the algorithm is to increase the classification success [22]. Weight values are changed with iterations. In each iteration, the weight values of the correctly classified samples are decreased and the weight values of the incorrectly classified samples are increased. This allows more iterations to be allocated to data samples that are difficult to classify.

Logitboost

AdaBoost algorithm can be affected by noises and overfitting problem may occur. It therefore recommends using LogitBoost for noisy data [23]. Training errors can be reduced by using the Loogitboost algorithm.

3. Result

In this study, laboratory test results were used to determine the physical and mechanical properties of 7 different rock types. Rep tree algorithm and ensemble learning algorithms from machine learning techniques were used to detect the types of rocks. The tree structure created by the software for the rep tree algorithm is given in Figure 1. Accordingly, the root node was chosen as M and the first branching was done. The first branching was done according to whether the M value was less than 14.25 or greater than 14.25. If the M value is greater than or equal to 14.25, the Ro value was checked and the rock type was determined. In the other part of the tree, if the n value is greater than or equal to 0.08, the upv value was checked. If the UPV value was less than 4035 and Schist was larger, it was classified as Amphibolite. In other branches, n and UCS values were checked and other samples were classified.

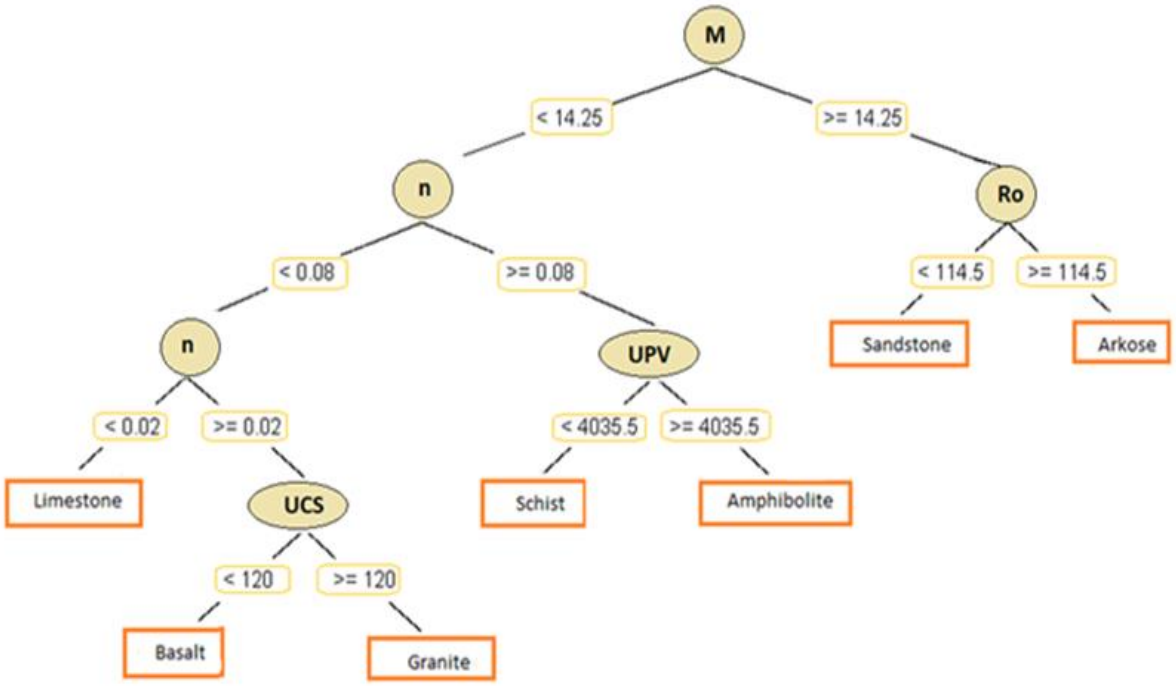

Figure 1. Rep Tree

To improve the performance of the rep treee algorithm, 3 different ensemble learning algorithms were used and their results were compared. For comparison, firstly, the individual confusion matrices of the algorithms are given in Table 2,3,4,5, respectively. It was seen in Confusion matrices that the number of correctly predicted instances increased when ensemble learning algorithms were used with the Rep tree algorithm. The highest increase in the number of samples was seen when the Logitboost algorithm was used.

Table 2. Confusion matrix of Rep tree

		Predicted						
		Limestone	Arkose	Sandstone	Basalt	Granite	Amphibolite	Schist
Actual	Limestone	**10**	0	0	2	0	0	0
	Arkose	0	**10**	8	0	0	0	0
	Sandstone	0	10	**10**	0	0	0	0
	Basalt	0	0	0	**9**	1	0	0
	Granite	0	0	0	1	**9**	0	0
	Amphibolite	0	0	0	0	0	**13**	1
	Schist	0	0	0	0	0	0	**12**

Table 3. Confusion matrix of Bagging+Rep Tree

		Predicted						
		Limestone	Arkose	Sandstone	Basalt	Granite	Amphibolite	Schist
Actual	Limestone	**10**	0	0	2	0	0	0
	Arkose	0	**6**	12	0	0	0	0
	Sandstone	0	6	**14**	0	0	0	0
	Basalt	0	0	0	**10**	0	0	0
	Granite	0	0	0	0	**10**	0	0
	Amphibolite	0	0	0	0	0	**13**	1
	Schist	0	0	0	0	0	0	**12**

Table 4. Confusion matrix of Adaboost+rep tree

		Predicted						
		Limestone	Arkose	Sandstone	Basalt	Granite	Amphibolite	Schist
Actual	Limestone	**10**	0	0	2	0	0	0
	Arkose	0	**11**	7	0	0	0	0
	Sandstone	0	8	**12**	0	0	0	0
	Basalt	0	0	0	**9**	1	0	0
	Granite	0	0	0	1	**9**	0	0
	Amphibolite	0	0	0	0	0	**13**	1
	Schist							**12**

Table 5. Confusion matrix of Logitboost+rep tree

		Predicted						
		Limestone	Arkose	Sandstone	Basalt	Granite	Amphibolite	Schist
Actual	Limestone	**12**	0	0	0	0	0	0
	Arkose	0	**8**	10	0	0	0	0
	Sandstone	0	9	**11**	0	0	0	0
	Basalt	0	0	0	**10**	0	0	0
	Granite	0	0	0	0	**10**	0	0
	Amphibolite	0	0	0	0	0	**14**	0
	Schist	0	0	0	0	0	0	**12**

Other performance metrics of the algorithms were calculated and given in Table 6. The farther the metric values are from 0, the more successful the algorithm is. These values can be at most 1. The lowest metric values were obtained when Ensemble learning algorithms were not used. Bagging, Adaboost and Logit boost algorithms increased all performance metrics. The highest TP Rate was 0.82, the highest Precision, recall, F-Score was 0.80, and the highest AUC was 0.95 when Logitboost+Reptree algorithms were used. The accuracy rates obtained by the algorithms in classification are given in Figure 2. According to the figure, the highest accuracy value is 80%. This value was obtained when the loogit boost algorithm and the Reeptree algorithm were used together.

Table 6. Performance metrics of algorithms

	TP Rate	FP Rate	Precision	Recall	F-Score	MCC	AUC	Kappa
Rep Tree	0.76	0.05	0.76	0.76	0.76	0.71	0.91	0.71
Bagging+Rep Tree	0.78	0.05	0.78	0.78	0.77	0.73	0.94	0.74
Adaboost+Reptree	0.79	0.04	0.79	0.79	0.79	0.74	0.95	0.75
Logitboost+Reptree	0.82	0.04	0.80	0.80	0.75	0.95	0.95	0.76

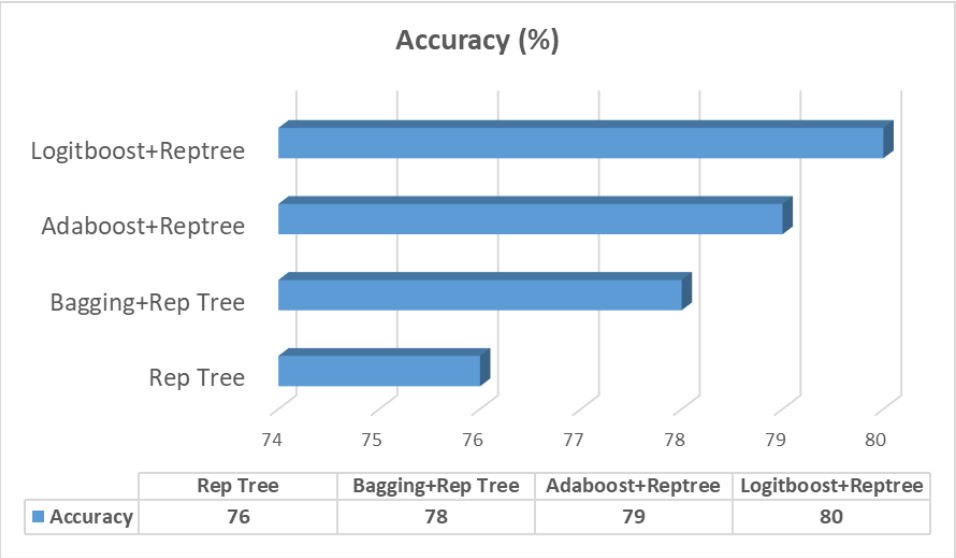

Figure 2. The accuracy of the algorithms

Another metric used in performance evaluation is the error metric. There are many error metrics developed for this. In the study, the error rates of the algorithms were calculated using 4 different metrics and are given in Table 7. It was seen in the table that the lowest error value was obtained with the Loogit boost algorithm.

Table 7. Error metrics of algorithms

	Mean absolute error	Root mean squared error	Relative absolute error	Root relative squared error
Rep Tree	0.0682	0.2186	28.1285	62.7637
Bagging+Rep Tree	0.0766	0.2053	31.5813	58.9681
Adaboost+Reptree	0.0625	0.2253	25.7863	64.707
Logitboost+Reptree	0.0527	0.183	21.7339	52.5597

4. Discussion and Conclusion

In this study, 7 different rock types were classified using machine learning techniques. Rep tree algorithm, a decision tree algorithm, was used in classification. For classification, a data set containing ultrasonic pulse velocity, ultrasonic pulse velocity, uniaxial compressive strength, resistivity, chargeability and porosity values, which are used to determine the physical and mechanical properties of rocks, was used. As a result of the classification, various performance metrics were calculated to determine the performance of the algorithm. TP rate, Precision Recall and F-Score values were found to be 0.76, MCC and Kappa values were 0.71, AUC value was 0.91 and FP Rate value was 0.05. Three different Ensemble Learning Algorithms were then used to improve the performance of the algorithm. As a result, it was seen that the three algorithms used increased the classification performance. logitboost algorithm was the best performing algorithm among Ensemble Learning Algorithms. When the logit boost algorithm was used together with the rep tree algorithm, the Tp rate increased to 0.82. Precision Recall values were 0.80, MCC and AUC were 0.95, kappa was 0.80. In addition, the FP rate decreased to 0.04. For this reason, it is recommended to use the rep teree algorithm and logitboost algorithm together in rock classification.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contribution

We declare that all Authors equally contribute.

Funding

The author declared that this study has received no financial support.

5. References

[1] Nahhas, T., Py, X., & Sadiki, N. (2019). Experimental investigation of basalt rocks as storage material for high-temperature concentrated solar power plants. Renewable and Sustainable Energy Reviews, 110, 226-235.

[2] Fegade, V., Ramachandran, M., Madhu, S., Vimala, C., Malar, R. K., & Rajeshwari, R. (2022, May). A review on basalt fibre reinforced polymeric composite materials. In AIP Conference Proceedings 2393(1). AIP Publishing.

[3] Aldeeky, H., Al Hattamleh, O., & Rababah, S. (2020). Assessing the uniaxial compressive strength and tangent Young's modulus of basalt rock using the Leeb rebound hardness test. Materiales de Construcción, 70(340), e230-e230.

[4] Mustapaevich, D. K. (2021). Geological-Geochemical and Mineralogical Properties of Basalt Rocks of Karakalpakstan. International Journal on Integrated Education, 4(10), 205-208.

[5] Sadique, M. R., Zaid, M., & Alam, M. M. (2022). Rock tunnel performance under blast loading through finite element analysis. Geotechnical and Geological Engineering, 40(1), 35-56.

[6] Zaid, M. (2021). Three-dimensional finite element analysis of urban rock tunnel under static loading condition: effect of the rock weathering. Geomechanics and Engineering, 25(2), 99-109.

[7] Zhao, D., He, Q., Ji, Q., Wang, F., Tu, H., & Shen, Z. (2023). Similar model test of a mudstone-interbedded–sandstone-bedding rock tunnel. Tunnelling and Underground Space Technology, 140, 105299.

[8] Shoeb, M., Khan, S. A., Alam, T., Ali, M. A., Gupta, N. K., Ansari, M. M., ... & Dobrota, D. (2023). Dynamic stability analysis of metro tunnel in layered weathered sandstone. Ain Shams Engineering Journal, 102258.

[9] Abdulsamad, F., Revil, A., Ghorbani, A., Toy, V., Kirilova, M., Coperey, A., ... & Ravanel, L. (2019). Complex conductivity of graphitic schists and sandstones. Journal of Geophysical Research: Solid Earth, 124(8), 8223-8249.

[10] Nayak, S. K., Satapathy, A., & Mantry, S. (2022). Use of waste marble and granite dust in structural applications: A review. Journal of Building Engineering, 46, 103742.

[11] Kang, F., Jia, T., Li, Y., Deng, J., & Huang, X. (2021). Experimental study on the physical and mechanical variations of hot granite under different cooling treatments. Renewable Energy, 179, 1316-1328.

[12] Skierszkan, E. K., Dockrey, J. W., Mayer, K. U., Bondici, V. F., McBeth, J. M., & Beckie, R. D. (2020). Geochemical Controls on Uranium Release from Neutral-pH Rock Drainage Produced by Weathering of Granite, Gneiss, and Schist. Minerals, 10(12), 1104.

[13] Barham, W. S., Rabab'ah, S. R., Aldeeky, H. H., & Al Hattamleh, O. H. (2020). Mechanical and physical based artificial neural network models for the prediction of the unconfined compressive strength of rock. Geotechnical and Geological Engineering, 38, 4779-4792.

[14] Xu, H., Zhou, J., G. Asteris, P., Jahed Armaghani, D., & Tahir, M. M. (2019). Supervised machine learning techniques to the prediction of tunnel boring machine penetration rate. Applied sciences, 9(18), 3715.

[15] Garzanti, E. (2019). Petrographic classification of sand and sandstone. Earth-science reviews, 192, 545-563.

[16] Bressan, T. S., de Souza, M. K., Girelli, T. J., & Junior, F. C. (2020). Evaluation of machine learning methods for lithology classification using geophysical data. Computers & Geosciences, 139, 104475.

[17] Sertçelik, İ., Kurtuluş, C., Sertçelik, F., Pekşen, E., & Aşçı, M. (2018). Investigation into relations between physical and electrical properties of rocks and concretes. Journal of Geophysics and Engineering, 15(1), 142-152.

[18] ASTM Standard 1984Standard test method for unconfined compressive strength of intact corespecimens soil and rock building stones Annual Book of ASTM Standards 4.08

[19] ASTM Standard 2001Standard Practice for Preparing Rock Core Specimens and Determining Dimensional and Shape Tolerances (Philadelphia, PA: American Society for Testing and Materials) D4543

[20] ISRM 2007 The Complete ISRM Suggested Methods for Rock Characterization Testing and Monitoring:1974–2006 ed R Ulusay and J A Hudson (Ankara: Kozan Ofset Press)

[21] Breiman, L. (1996). Bagging predictors. Machine learning, 24(2), 123-140.

[22] Freund, Y., & Schapire, R. E. (1996, July). Experiments with a new boosting algorithm. In Icml 96, 148-156.

[23] Friedman, J., Hastie T., & Tibshirani, R. (2000) Additive Logistic Regression: a Statistical View of Boosting, Annals of Statistics, 28, 337-407.

doi:10.55385/kastamonujes.1357436 KUJES 9(2):67-72, 2023

Kastamonu University
Journal of Engineering and Sciences
e-ISSN 2667-8209
http://dergipark.gov.tr/kastamonujes

Microstructure and Hardness Properties of Ni-Si_3N_4 Composite Materials Produced by Powder Metallurgy Method

Hasaneen Houssain[a,b], Serkan Islak[c], Uğur Çalıgülü[d,*]

[a] Department of Materials Science and Engineering, Institute of Science, Kastamonu University, Kastamonu, Türkiye
[b] Department of Chemical Engineering, Faculty of Engineering, Al Muthanna University, Samawah, Iraq
[c] Department of Mechanical Engineering, Faculty of Engineering and Architecture, Kastamonu University, Kastamonu, Türkiye
[d] Department of Metallurgy and Materials Engineering, Faculty of Technology, Fırat University, Elazığ, Türkiye
***Corresponding Author:** ugurcaligulu@gmail.com

Received: **September 08, 2023** ◆ Accepted: **December 4, 2023** ◆ Published Online: **December 26, 2023**

Abstract: In this study, the effect of the amount of Si_3N_4 on the microstructure and hardness properties of nickel (Ni) matrix silicon nitride (Si_3N_4) reinforced composite materials were investigated. Si_3N_4 was added to Ni at 5%, 10% and 15% by volume. The samples were produced using cold pressing and pressureless sintering technique. The sintering temperature was 1200 °C and the sintering time was 1 hour. SEM-EDS and XRD analyzes were used for the microstructure and phase formation analysis of the composites. The densities of the composites were measured according to the Archimedean principle. To determine the effect of Si_3N_4 on hardness properties, the microhardness of the samples was measured as Vickers. It was determined from XRD analysis that Ni, β_1 (Ni_3Si) and ε-Ni_3Si_2 phases were formed in the microstructure. As the amount of Si_3N_4 increased, both the experimental densities and relative densities decreased, and the amount of pores partially increased. Due to the distribution strengthening effect of Si_3N_4 in the Ni matrix, there was an increase in the hardness values and the highest hardness was determined as 135 HV2 with the addition of 15% Si_3N_4.

Keywords: Ni, Si_3N_4, composite, microstructure, hardness

Öz: Bu çalışmada, nikel (Ni) matrisli silisyum nitrür (Si_3N_4) takviyeli kompozit malzemelerin mikroyapı ve sertlik özellikleri üzerine Si_3N_4 miktarının etkisi araştırılmıştır. Ni içerisine Si_3N_4 hacimce % 5, % 10 ve % 15 oranında ilave edilmiştir. Numuneler soğuk presleme ve basınçsız sinterleme tekniği kullanılarak üretilmiştir. Sinterleme sıcaklığı olarak 1200 °C ve sinterleme süresi olarak 1 saat seçilmiştir. Kompozitlerin mikroyapı ve faz oluşumu analizi için SEM-EDS ve XRD analizleri kullanılmıştır. Kompozitlerin yoğunlukları Arşiment prensibine göre ölçülmüştür. Si_3N_4'ün sertlik özelliklerine etkisini belirlemek için numunelerin mikrosertlikleri Vickers olarak ölçülmüştür. Mikroyapıda Ni, β1 (Ni_3Si) ve ε-Ni_3Si_2 fazlarının oluştuğu XRD analizinden tespit edilmiştir. Si_3N_4 miktarının artmasıyla hem deneysel yoğunluklarda hem de bağıl yoğunluklarda azalma meydana gelmiş olup, kısmen gözenek miktarı artmıştır. Si_3N_4'ün Ni matris içerisinde dağılım mukavemetlendirme etkisi göstermesinden dolayı sertlik değerlerinde artış olmuş ve en yüksek sertlik %15 Si_3N_4 ilavesinde 135 HV2 olarak tespit edilmiştir.

Anahtar Kelimeler: Ni, Si_3N_4, kompozit, mikroyapı, sertlik

1. Introduction

Metal matrix composites (MMCs) are an engineering combination of a continuous metallic matrix and a reinforcement, usually ceramic. While aluminum, titanium, copper, nickel, magnesium and their alloys are used as matrix, oxide, carbide, nitride and borides are used as ceramic reinforcement [1-4]. Ni-based alloys are widely used in chemical plant, nuclear power plant, oil field and some corrosive environments due to their superior corrosion resistance [5]. However, Ni has poor mechanical properties for high temperature applications. This drawback can be overcome by producing Ni matrix composites [6]. There are studies conducted by producing MMCs to improve mechanical properties. Jiang et al. [7] produced Ni matrix graphene reinforced composites by in-situ method and stated that graphene in Ni increased the strength. Islak et al. [8] by adding TiC to the Ni-based alloy powder, they produced the NiCrBSi matrix TiC reinforced composite by hot pressing technique, one of the powder metallurgy methods, and stated that the friction coefficients decreased and the hardnesses increased as the TiC ratio increased. Islak et al. in a different study [9], they produced hybrid composites by powder metallurgy method by adding carbon nanofiber (CNF) and boron carbide (B_4C) to the nickel matrix. They reported that the hardness increased by 44% and the wear rate decreased by approximately 10 times in the Ni-10B_4C-2CNF composite compared to neat Ni.

Covalently bonded silicon nitride (Si_3N_4) ceramics have been studied in recent years for shaping, sintering and microstructure control in high temperature and wear resistant applications due to their attractive combination of

mechanical and thermal properties [10]. Because of its superior properties and because it has never been added to the Ni matrix in previous studies, we planned to produce Ni-Si_3N_4 composite and examine some of its properties in this study.

2. Material and Method

In this study, Ni powder with 2 µm grain size and 99.5% purity and Si_3N_4 powder with 10 µm grain size and 99.9% purity were used to produce Ni-Si_3N_4 composites. Ni powder was obtained from Nanokar Nanotechnology company, and Si_3N_4 powder was obtained from Nanografi Nanotechnology company. The powders were mixed in the turbula for 1 hour in the proportions given in Table 1, and were cold pressed in the hydraulic press at 600 MPa pressure in the form of pellets with a diameter of 20 mm and a thickness of 5 mm. Then, the pellets were sintered in a tube furnace at 1200 ºC for 1 hour in an argon atmosphere.

Table 1. Powder composition rates in composite production

Sample group	Composition (vol.%)	
	Ni	Si_3N_4
N0S	100	0
N5S	95	5
N10S	90	10
N15S	85	15

The samples were sanded using 320-2000 mesh grit sandpaper and polished with diamond solution. Then, it was etched for microstructure imaging in 100 ml distilled water + 25 ml hydrochloric acid + 8 g iron (III) chloride solution. FEI QUANTA 250 FEG brand scanning electron microscope, and X-ray diffraction (Bruker D8 Advance) analyzes were used to examine the microstructure of samples and determine phase compound. The experimental densities of the samples were measured according to the Archimedes principle, as specified in ASTM B 962 [11]. The hardness of the samples was determined using the Shimadzu HMV-G21 model microhardness device according to the ASTM E92-17 standard [12] at a load of 2 kg and a dwell time of 15 seconds. Measurements were made from 5 different points for each test and average values presented in the study.

3. Result and Discussions

SEM-EDS analysis of the pure Ni matrix is given in Figure 1. It is clearly seen from the SEM photo in Figure 1a that very little porosity occurs in the sample. Again, according to the SEM photo, it is understood that a compact structure has been formed. However, scratches formed during metallographic sample preparation are also noticeable. In the EDS analysis of the N0S sample, it is seen that the structure consists of 100% Ni and there is no oxygen in the structure. The absence of oxygen caused the Ni powder particles to bond better with each other during the sintering process.

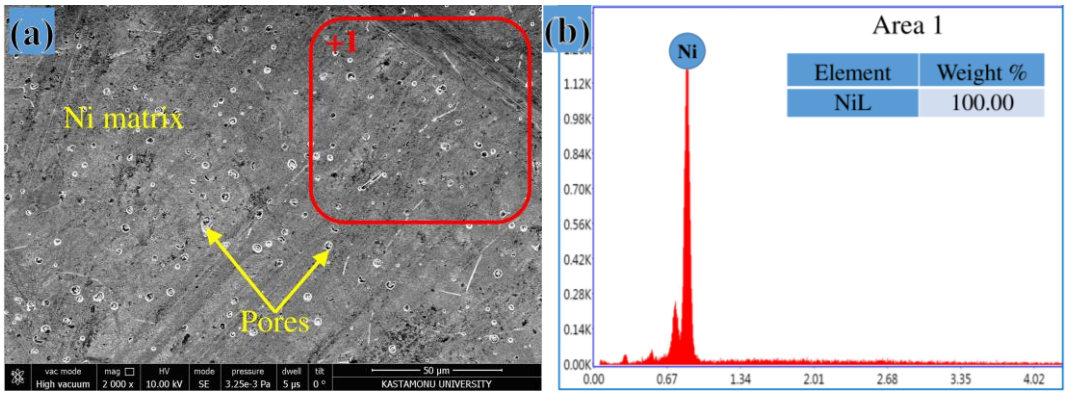

Figure 1. For the N0S (Ni) sample: (a) SEM photograph, and (b) EDS analysis

Figure 2 shows general SEM, detailed SEM and EDS analysis of Ni-15%Si_3N_4 composite. It is understood from SEM photographs that a phase transformation microstructure is formed. The reason for this may be that there is partial melting during the sintering process. It can be seen from Figure 2a that the microstructure has a dendritic morphology. ε-Ni_3Si_2 precipitates were formed between the Ni+β1-Ni_3Si phase dendritic arms (Figure 2b). It is clear from the EDS analysis of point 1 and point 2 in Figure 2c that the mentioned structures are formed. The formation of these phases is given in the XRD graph in Figure 3. When Si_3N_4 was added to Ni, the amount of Ni phase decreased and β_1-Ni_3Si and ε-Ni_3Si_2 phases became evident. Xie et al. [13] in their study, it was determined that dendritic structures were formed and the above-mentioned phases were formed. Oxidation has occurred in the dendritic arms. The presence of oxygen in the EDS analysis at point 3 also supports this. The oxide formation here may have occurred in the form of SiO_2. Shen et al. [14] reported that black mottled SiO_2 structure was formed by oxidation of silicon in their study.

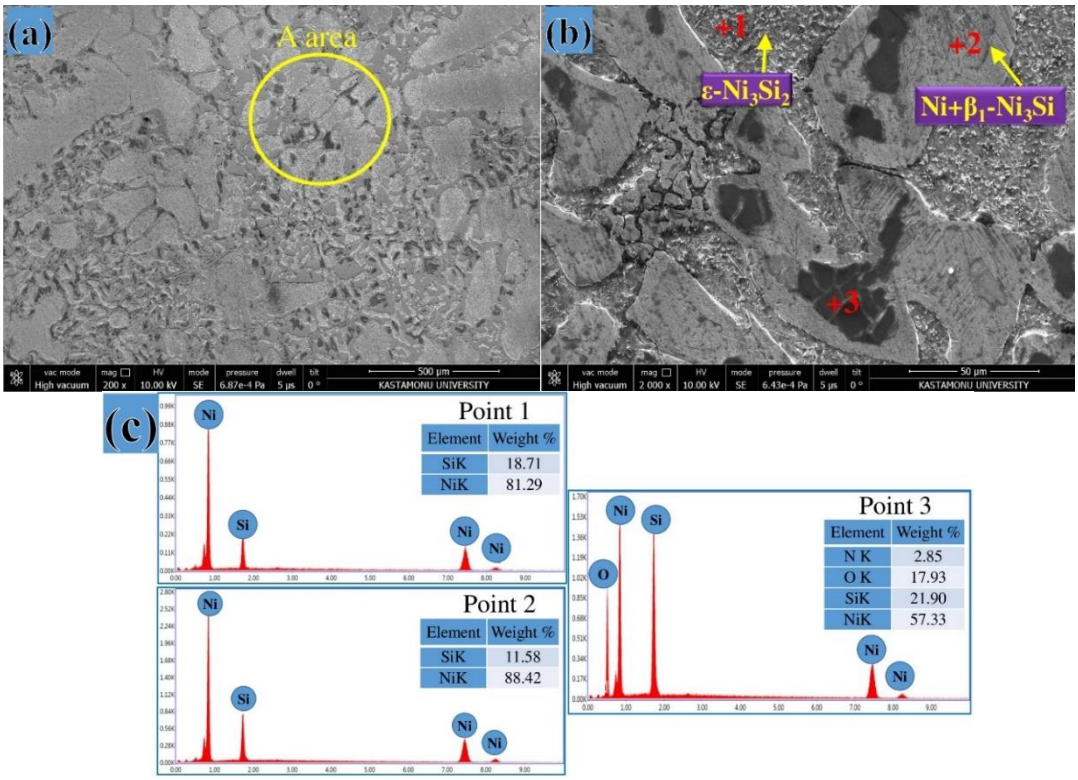

Figure 2. For the N15S (Ni-15%Si_3N_4) sample: (a) general SEM photograph, (b) detailed SEM photograph of area A, and (c) EDS analysis

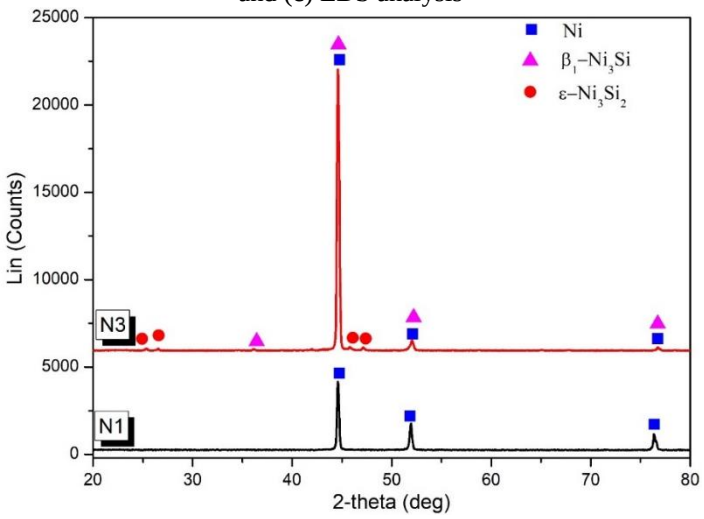

Figure 3. XRD analysis graph of N0S and N15S samples

Figure 4 gives a graph showing the effect of Si_3N_4 addition on experimental density, relative density and porosity of Ni-Si_3N_4 composites. According to the graph, experimental density and relative density decreased and porosity increased with increasing the addition of Si_3N_4. While the experimental densities for N0S, N5S, N10S and N15S were 8.64 g/cm^3, 7.95 g/cm^3, 7.55 g/cm^3 and 7.21 g/cm^3, respectively, the relative densities were calculated as 97.06%, 92.30%, 90.67% and 89.68%, respectively. The reason for the decrease in density is that the density of the reinforcing element Si_3N_4 (3.17 g/cm^3) is quite low compared to the density of the Ni matrix (8.9 g/cm^3). Islak and Çelik [15] added B_4C to bronze and produced bronze-B_4C diamond sockets and reported that the densities decreased as the amount of B_4C increased. Kriewah and Islak [16] reported that reinforcement elements with low density compared to the matrix reduce the density of the composite. The reason for the decrease in relative densities is based on two fundamentals. The first of these is that the ceramic particles added to the metallic matrix negatively affect the sinterability and prevent the matrix particles from necking. The other is the melting temperature difference between the matrix and the reinforcement elements [17]. The porosity rates were calculated as 2.94%, 7.70%, 9.33% and 10.32% for N0S, N5S, N10S and N15S, respectively. The increase in porosity can be associated with the reasons for the decrease in relative density.

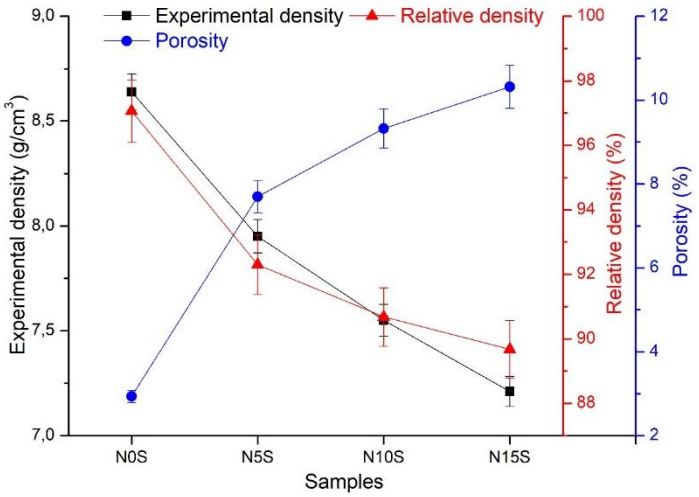

Figure 4. Experimental density, relative density, and amount of porosity of the samples.

Figure 5 shows the effect of Si_3N_4 reinforcement on hardness values, which is the most basic mechanical property determination test for Ni-Si_3N_4 composites. While the hardness value measured for the pure N0S sample is 105 HV2, the hardness values for N5S, N10S and N15S with the addition of Si_3N_4 are 110 HV2, 123 HV2 and 134 HV2, respectively. The increase in hardness is clearly seen with the addition of Si_3N_4. With the addition of 15% Si_3N_4, the hardness increase was 28% compared to the sample without additives. It can be said that this increase in hardness is caused by the distribution of the Si_3N_4 reinforcement element in the matrix [18]. In other words, the increase in hardness can be explained by the mixing rule. Mixing rule for materials with high relative density (Equation 1):

$$H_C = H_m f_m + H_r f_r \quad (1)$$

Here, H_c is the hardness of the composite, H_m is the hardness of the matrix, H_r is the hardness of the reinforcement element, and f_m and f_r are the volumetric ratio of the matrix and reinforcement element, respectively [19-21]. Buytoz et al. [22] stated that TiC particles in Cu-TiC composites produced by hot pressing technique caused higher dislocation density in the composite. This causes an increase in hardness. Additionally, ceramic particles added to composites cause an increase in strength by preventing the movement of dislocations [23, 24].

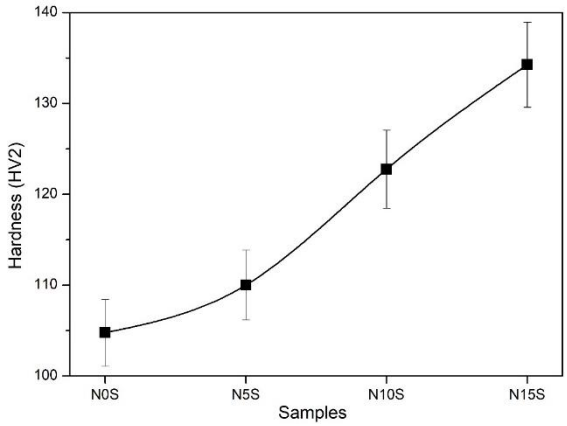

Figure 5. Hardness graph of Ni-Si_3N_4 composites

4. Conclusion

The following results were obtained in the study on the properties of Ni matrix Si_3N_4 reinforced composites produced by powder metallurgy.

A compact and less porous microstructure was obtained in the pure Ni sample. With Si_3N_4 addition, the microstructure had a dendritic morphology.

According to XRD analysis, Ni, β_1-Ni_3Si and ε-Ni_3Si_2 phases were formed in the microstructure. Ni+β_1-Ni_3Si phase was formed in the dendrite arms, and ε-Ni_3Si_2 phase was formed between the dendrite arms. The formation of these phases is also supported by EDS analysis.

With the increase of Si_3N_4 contribution, there was a decrease in experimental and relative densities, an increase in porosity, and a significant increase in hardness values. With the addition of 15% Si_3N_4, there was a 28% increase in hardness compared to the sample without additive.

Conflict of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethics Committee Approval

N/A

Author Contribution

Conceptization: HH, SI, UÇ; methodology and laboratory analyzes: HH; writing draft: HH, SI, UÇ; proof reading and editing: HH, SI, UÇ. Other: All authors have read and agreed to the published version of manuscript.

Funding

The author declared that this study has received no financial support.

5. References

[1] Kondoh, K. (2015). Titanium metal matrix composites by powder metallurgy (PM) routes. In Titanium powder metallurgy. Butterworth-Heinemann, 277-297.

[2] Samer, N., Andrieux, J., Gardiola, B., Karnatak, N., Martin, O., Kurita, H., Chaffron, L., Gourdet, S., Lay, S., & Dezellus, O. (2015). Microstructure and mechanical properties of an Al–TiC metal matrix composite obtained by reactive synthesis. Composites Part A: Applied Science and Manufacturing, 72, 50-57.

[3] Rohatgi, P. K. (1993). Metal matrix composites. Defence science journal, 43(4), 323-349.

[4] Evans, A., San Marchi, C., Mortensen, A., Evans, A., San Marchi, C., & Mortensen, A. (2003). Metal matrix composites. Springer US, 9-38.

[5] Kumar, B. A., Ananthaprasad, M. G., & Krishna, K. G. (2015). A review on corrosion behavior of nickel matrix composites. International Journal of Emerging Technology and Advanced Engineering, 5(10), 342-346.

[6] Kumar, B. A., Ananthaprasad, M. G., & GopalaKrishna, K. (2016). A review on mechanical and tribological behaviors of nickel matrix composites. Indian Journal of Science and Technology, 9(2), 1-7.

[7] Jiang, J., He, X., Du, J., Pang, X., Yang, H., & Wei, Z. (2018). In-situ fabrication of graphene-nickel matrix composites. Materials Letters, 220, 178-181.

[8] Islak, S., Ulutan, M., & Buytoz, S. (2020). Microstructure and Wear Properties of Hot-Pressed NiCrBSi/TiC Composite Materials. Russian Journal of Non-Ferrous Metals, 61(5), 571-582.

[9] Islak, S., Koç, V., & Gariba, A.M.M. (2022). Wear and microstructural properties of Ni-B_4C/CNF composites. Science of Sintering, 54(4), 439-448.

[10] Han, I. S., Seo, D. W., Kim, S. Y., Hong, K. S., Guahk, K. H., & Lee, K. S. (2008). Properties of silicon nitride for aluminum melts prepared by nitrided pressureless sintering. Journal of the European Ceramic Society, 28(5), 1057-1063.

[11] ASTM B962-08. (2008). Standard test methods for density of compacted or sintered powder metallurgy (PM) products using Archimedes' principle.

[12] ASTM E92-17. (2017). Standard test methods for Vickers hardness and Knoop hardness of metallic materials. West Conshohocken (PA): ASTM International.

[13] Xie, H., Jia, L., & Lu, Z. (2009). Microstructure and solidification behavior of Cu–Ni–Si alloys. Materials Characterization, 60(2), 114-118.

[14] Yu, L., Shen, F., Fu, T., Zhang, Y., Cui, K., Wang, J., & Zhang, X. (2021). Microstructure and oxidation behavior of metal-modified Mo-Si-B alloys: A review. Coatings, 11(10), 1256.

[15] Islak, S., & Çelik, H. (2015). Effect of sintering temperature and boron carbide content on the wear behavior of hot pressed diamond cutting segments. Science of Sintering, 47(2), 131-143.

[16] Kriewah, O. A. E., & Islak, S. (2022). Synthesis of Cu-Cr-B_4C-CNF hybrid composites. Kastamonu University Journal of Engineering and Sciences, 8(2), 90-97.

[17] Rahimian, M., Ehsani, N., Parvin, N., & reza Baharvandi, H. (2009). The effect of particle size, sintering temperature and sintering time on the properties of Al–Al_2O_3 composites, made by powder metallurgy. Journal of Materials Processing Technology, 209(14), 5387-5393.

[18] Min, K. H., Lee, B. H., Chang, S. Y., & Do Kim, Y. (2007). Mechanical properties of sintered 7xxx series AI/SiC_p composites. Materials Letters, 61(11-12), 2544-2546.

[19] Kim, H. S. (2000). On the rule of mixtures for the hardness of particle reinforced composites. Materials Science and Engineering: A, 289(1-2), 30-33.

[20] Kumar, G. V., Rao, C. P., & Selvaraj, N. (2011). Mechanical and tribological behavior of particulate reinforced aluminum metal matrix composites–a review. Journal of minerals and materials characterization and engineering, 10(01), 59-91.

[21] Okay, F., & Islak, S. (2022). Microstructure and mechanical properties of aluminium matrix boron carbide and carbon nanofiber reinforced hybrid composites. Science of Sintering, 54(2), 125-138.

[22] Buytoz, S., Dagdelen, F., Islak, S., Kok, M., Kir, D., & Ercan, E. (2014). Effect of the TiC content on microstructure and thermal properties of Cu–TiC composites prepared by powder metallurgy. Journal of Thermal Analysis and Calorimetry, 117, 1277-1283.

[23] Yang, S., Guo, Z., & Xia, M. (2018). Effect of TiB content on the properties of Al-TiB composites. Science of Sintering, 50(2), 237-244.

[24] Islak, S. (2019). Mechanical and corrosion properties of AlCu matrix hybrid composite materials. Science of Sintering, 51(1), 81-92.

doi:10.55385/kastamonujes.1371765 KUJES 9(2):73-82, 2023

Kastamonu University
Journal of Engineering and Sciences
e-ISSN 2667-8209
http://dergipark.gov.tr/kastamonujes

The Feasibility of using Annual Rings to Monitor Changes in Boron Concentrations in Air

Burak Arıcak[a, *], **Şemsettin Kulaç**[b]

[a] Faculty of Forestry, Department of Forest Engineering, Bursa Technical University Bursa, Türkiye
[b] Faculty of Forestry, Department of Forest Engineering, Düzce University Düzce, Türkiye
***Corresponding Author:** burak.aricak@btu.edu.tr

Received: **October 05, 2023** ◆ Accepted: **December 7, 2023** ◆ Published Online: **December 26, 2023**

Abstract: Boron is one of the most important elements on the agenda in recent years, both because it is a micronutrient for plants and because it is used in more than 250 fields in industry. Due to its intensive use and the continuous expansion of these areas of use, the concentrations of boron, which is produced more and more, are constantly increasing in receiving environments. However, boron, one of the important heavy metals, can cause significant health problems when inhaled from the air and taken into the human body. Therefore, monitoring the changes in boron concentration in the air is of great importance. In this study, the change of boron concentration in *Pinus pinaster, Cupressus arizonica, Picea orientalis, Cedrus atlantica* and *Pseudotsuga menziesii* species growing in Düzce, which is among the 5 most polluted cities in Europe, was examined on the basis of species, organ, direction and period in the last 40 years. The results of the study generally showed that boron concentrations did not change significantly on the basis of direction and year interval. Similarly, boron concentration in wood did not change significantly by species, whereas the change in boron concentration in bark was found to be statistically significant. According to the mean values, the lowest values were obtained in *P. menziesii* and the highest values were obtained in *C. arizonica.*

Keywords: Heavy metal, boron, biomonitor

Öz: Bor, hem bitkiler için mikro besin olması hem de sanayide 250'den fazla alanda kullanılması nedeniyle son yılların gündemdeki en önemli unsurlardan biridir. Yoğun kullanımı ve bu kullanım alanlarının sürekli genişlemesi nedeniyle her geçen gün daha fazla üretilen borun, alıcı ortamlardaki konsantrasyonları sürekli artmaktadır. Ancak önemli ağır metallerden biri olan bor, havadan solunarak insan vücuduna alındığında önemli sağlık sorunlarına yol açabilmektedir. Bu nedenle havadaki bor konsantrasyonundaki değişimlerin izlenmesi büyük önem taşımaktadır. Bu çalışmada Avrupa'nın en kirli 5 şehri arasında yer alan Düzce'de yetişen *Pinus pinaster*, *Cupressus arizonica*, *Picea orientalis*, *Cedrus atlantica* ve *Pseudotsuga menziesii* türlerinde bor konsantrasyonunun değişimi tür, organ, son 40 yılın yönü ve dönemi incelenmiştir. Çalışma sonuçları genel olarak bor konsantrasyonlarının yön ve yıl aralığına göre önemli bir değişim olmadığını göstermiştir. Benzer şekilde odundaki bor konsantrasyonu türlere göre önemli bir değişim göstermezken, ağaç kabuğundaki bor konsantrasyonunun değişimi istatistiksel olarak anlamlı bulunmuştur. Ortalama değerlere göre en düşük değerler *P. menziesii*'de, en yüksek değerler ise *C. arizonica*'da elde edilmiştir.

Anahtar Kelimeler: Ağır metal, Bor, Biyomonitör

1. Introduction

Plants are the most important living groups that form the basis of life on earth due to their ability to photosynthesize [1-3]. Plant growth is largely dependent on climatic and edaphic factors [4-6]. One of the most important factors shaping plant growth is nutrients. Nutrients are classified as macro and micronutrients and are absolutely essential for plant growth [7-9].

Boron (B) is the only nonmetal element among microelements. Boron enables the formation of cell walls and tissue regeneration in plants, activates some dehydrogenase enzymes, and plays a role in carbohydrate biosynthesis. It is effective on nucleic acid and protein metabolism. It plays a role in the replacement of sugars within the plant [10]. However, B is also a heavy metal [11]. Its high concentration in soil and water has a toxic effect on plants. In old leaves, leaf tips turn yellow and necrosis occurs. Symptoms then spread to the leaf margins and midrib. Leaves take a burnt appearance and fall off early [10]. Boron is used in more than 250 areas of industry, including construction, health, ceramics, ceramics, glass, cleaning, metallurgy, metallurgy, aerospace and aeronautics, as it increases the physical, chemical and technological properties of the materials it is a component of due to its ability to compound with organic and inorganic materials [12]. Therefore, the concentration of boron in receiving environments such as air, water and soil is constantly increasing.

Studies have shown that heavy metals, even those necessary as nutrients for living organisms, are harmful to health at high concentrations [13-15]. Especially heavy metals inhaled from the air can cause many health problems and even death in humans [16-20]. Studies reveal that approximately 7 million human deaths and more than 4 million premature births are associated with air pollution every year worldwide [17]. According to data from the European Environment Agency, air pollution is the single biggest environmental problem in Europe [21]. Worldwide, environmental pollution is shown as the most important global problem along with global warming [22-24] and urbanization [25-26]. Heavy metals are the most dangerous and harmful components of air pollution [27]. Therefore, it is of great importance to monitor the changes in heavy metal concentrations in the air and reduce the concentrations in polluted areas [28]. It is known that plants are the most effective tool for monitoring and reducing heavy metal pollution [29-30]. Previous studies have shown that tree annual rings can be used effectively in monitoring heavy metal pollution in air [31-32]. However, it is emphasized that the plant species that can be used in monitoring the change of each heavy metal pollution and reducing pollution should be determined separately [33]. In this study, it was aimed to determine the most suitable tree species for monitoring and reducing the change of Boron pollution in the air over the years.

2. Material and Method

The study was conducted on *Pinus pinaster* (Pp), *Cupressus arizonica* (Cpa), *Picea orientalis* (Po), *Cedrus atlantica* (Cda) and *Pseudotsuga menziesii* (Pm) species growing in Düzce city center. The species subject to the study are frequently used in landscape studies, especially in Europe. Düzce, where the study was conducted, is among the 5 most polluted cities in Europe according to the 2021 World Air Pollution report [34]. Previously, the changes of Cr [28], Bi [35] and Tl [36] elements in the city center of Düzce province were determined using these tree species. Within the scope of the study, the materials and methods used in these studies were used. At the end of the vegetation season in 2022, the main trunks of the trees subject to the study were cut at a height of about 50 cm from the ground by marking the north direction and trunk stump samples with a thickness of about ten cm were taken and Boron concentrations were determined using ICP-OES after pre-treatment. This method is one of the most frequently used methods in recent years to determine heavy metal concentrations in plant organs such as soil [37-40], leaves [41-42] and wood [43]. The values obtained as a result of the study were evaluated with the help of SPSS package program, analysis of variance and Duncan test were applied to the data. Thus, the variation of boron concentration (ppb) on the basis of species, organ, direction and year range was determined.

3. Result

The variation of B concentration by species and direction is given in Table 1.

Table 1. Variation of B concentration by species and direction

Species	North	East	South	West	Average
Pp	4204.9^{a}	7329.7^{b}	5988.8	2953.3^{a}	5119.1^{ab}
Cpa	12246.0^{c}	9395.7^{c}	5179.3	5708.4^{a}	8132.3^{c}
Po	4053.6^{a}	6142.2^{ab}	3877.7	14808.4^{b}	7277.2^{bc}
Cda	4035.9^{b}	4864.6^{a}	6014.1	5949.4^{a}	6716.0^{abc}
Pm	4794.0^{a}	4091.5^{a}	2893.3	6878.7^{a}	4694.5^{a}
F Value	25.1***	8.8***	2.0 ns	2.8*	3.3*

According to results of Duncan's test, values followed by the different letters (a and b) refer significant differences among species within each direction. ns = not significant; * = p≤0.05; *** = p≤0.001.

When the values in the table are examined, it was determined that the change in B concentration on species basis was statistically significant in all directions except south. In the north direction, the highest value was obtained in Cpa, while the lowest values were obtained in Pp, Po and Pm species. In the east direction, the highest value was observed in Cpa while the lowest values were observed in Cda and Pm. In the west direction, the highest value was found in Po species. According to the average values, the lowest value was obtained in Pm species. The variation of B concentration by species and organ is given in Table 2.

According to the results of analysis of variance, it was determined that the change in B concentration was statistically significant only in the barks on species basis. In the outer bark, the highest value was observed in Cpa, while the lowest value was observed in Pp and Po. In the inner bark, the highest value was obtained in Cpa while the lowest value was obtained in Pm. According to the average values, the lowest value was observed in the inner bark and wood, while the highest value was obtained in the outer bark. The variation of B concentration by period and direction is given in Table 3.

Table 2. Variation of B concentration by species and organ

Species	Outer bark	Inner bark	Wood	Average
Pp	7508.1^{a}	7191.3^{b}	4561.5	4561.5
Cpa	18523.3^{c}	10432.6^{c}	56545.9	6545.9
Po	7056.5^{a}	7668.9^{bc}	7254.4	7254.4
Cda	13612.4^{b}	7682.5^{bc}	5733.1	5733.1
Pm	11499.6b	2964.2a	4017.9	4017.9
F Value	24.1***	8.3***	1.9 ns	1.9 ns
Average	11640.0^{B}	7187.9^{A}	5622.2^{A}	

According to results of Duncan's test, values followed by the different letters (a and b) refer significant differences among species within each direction. ns = not significant; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

Table 3. Variation of B concentration by period and direction

AGE	North	East	South	West	F Value	Average
2018-2022	6775.8^{a}	4470.5^{ab}	3380.7^{ab}	4653.0^{a}	1.8ns	4858.8^{a}
2013-2017	4526.3^{a}	3825.1^{a}	2997.0^{ab}	3999.2^{a}	1.0ns	3837.6^{a}
2008-2012	5231.1^{a}	6392.2^{abc}	8601.8^{c}	4622.5^{a}	1.1ns	6211.9^{ab}
2003-2007	5053.9^{Ba}	4868.9^{Bab}	2466.0^{Aab}	2768.9^{Aa}	4.7**	3789.4^{a}
1998-2002	8663.1^{Ba}	7394.1^{Bbc}	1954.7^{Aa}	3590.9^{Aa}	8.6***	5400.7^{a}
1993-1997	4705.6^{Aa}	4891.6^{Aab}	3543.7^{Aab}	24183.4^{Ab}	3.7*	9331.1^{b}
1988-1992	7685.8^{a}	8010.8^{c}	6253.3^{bc}	3782.2^{a}	1.9ns	6433.0^{ab}
1983-1987	5819.5^{Ba}	6059.3^{Babc}	2672.3^{Aab}	5335.2^{Ba}	3.5*	4914.3^{a}
F Value	1.3 ns	2.6*	3.3**	3.2**		2.2*

According to results of Duncan's test, values followed by the different letters (a and b) refer significant differences among species within each direction. ns = not significant; * = p≤0.05; ** = p≤0.01; *** = p≤0.001.

Considering the above values, the change in B concentration is statistically significant in all periods except 1988-1992, 2008-2012, 2013-2017 and 2018-2022. In all directions except the north, the change in B concentration on a period basis is statistically significant. The highest value in the eastern direction was observed in the period 1988-1992, the highest value in the southern direction in the period 2008-2012 and the highest value in the western direction in the period 1993-1997. According to the mean values, the highest value was obtained in the period 1993-1997. Changes in B concentration by organ and direction are given in Table 4.

Table 4. Variation of B concentration by organ and direction

ORGAN	North	East	South	West	F Value
Outer bark	13094.3^{c}	11162.7^{b}	10415.9^{b}	11887.1^{a}	0.6 ns
Inner bark	9113.2^{b}	6243.5^{a}	5620.9^{a}	7773.9^{a}	2.5 ns
Wood	6057.6^{a}	5853.8^{a}	3983.7^{a}	6616.9^{a}	1.8 ns
F Value	15.2***	12.7***	11.5***	0.8 ns	
Average	7066.9	6473.2	4790.7	7259.6	2.5ns

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. ns = not significant; *** = p≤0.001.

When the results are examined, the change in B concentration in all organs on the basis of direction is not statistically significant. It is statistically significant on organ basis in all directions except west. For the north direction, it is possible to rank wood<inner bark<outer bark. In the east and south directions, the highest value was seen in the outer bark, while the lowest value was seen in the inner bark and wood. The variation of B concentration in *P. pinaster* by organ and direction is given in Table 5.

Table 5. Variation of B concentration by organ and direction in *P. pinaster*

ORGAN	North	East	South	West	F Value	Average
Outer bark	8315.2^{Cb}	9367.7^{D}	6084.6^{A}	6265.0^{Bc}	1334.4***	7508.1
Inner bark	10730.2^{Dc}	8616.0^{C}	4045.7^{A}	5373.4^{Bb}	4767.7***	7191.3
Wood	2875.4^{Aa}	6914.1^{B}	6219.7^{B}	2236.8^{Aa}	5.2**	4561.5
F Value	111.6***	0.8 ns	0.0 ns	489.7***		3.0 ns
Average	4204.9^{AB}	7329.7^{C}	5988.8^{BC}	2953.3^{A}	4.8**	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. ns = not significant; ** = p≤0.01; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

When the results in the table are examined, it is determined that the change in B concentration in *P. pinaster* is statistically significant in all organs on the basis of direction. In all directions except north and west, the change in B concentration is not statistically significant. For the outer bark, we can make the order south<west<north<east. For the inner bark, this order is south<west<east<north. In wood, the highest values were observed in the east and south directions, while the lowest values were obtained in the west and north directions. According to the mean values, the lowest value was observed in the west and the highest value in the east. According to the mean values, there is no statistically significant difference between the organs. The variation of B concentration in *P. pinaster* by period and direction is given in Table 6.

Table 6. Variation of B concentration in *P. pinaster* wood by period and direction

AGE	North	East	South	West	F Value	Average
2018-2022	2644.7^{Bd}	3191.5^{Db}	2891.2^{Ce}	2478.6^{Af}	557.8***	2801.5^{a}
2013-2017	2499.1^{Bc}	2824.4^{Da}	2736.1^{Cd}	1912.7^{Ab}	335.5***	2493.1^{a}
2008-2012	2778.2^{Ae}	15313.7^{Bh}	30022.2^{Cg}	2669.3^{Ag}	73391.6***	12695.8^{b}
2003-2007	1523.8^{Ab}	8561.2^{Dg}	1998.6^{Bc}	2255.2^{Cd}	16490.6***	3584.7^{a}
1998-2002	4007.6^{Cg}	6056.2^{Dd}	1853.6^{Ab}	2273.0^{Bd}	8690.3***	3547.6^{a}
1993-1997	4361.8^{Bh}	4934.9^{Cc}	5650.7^{Df}	1822.8^{Aa}	3721.4***	4192.5^{a}
1988-1992	3859.6^{Cf}	7687.5^{Df}	1748.1^{Aa}	2087.2^{Bc}	1281.2***	3845.6^{a}
1983-1987	1328.4^{Aa}	6743.5^{De}	2857.2^{Ce}	2395.7^{Be}	7976.9***	3331.2^{a}
F Value	2635.4***	4185.3***	78024.4***	340.6***		6.5***
Average	2875.4^{A}	6914.1^{B}	6219.7^{B}	2236.8^{A}	5.2**	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. ** = p≤0.01; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

According to the results of analysis of variance, it was determined that the change of B concentration in all woods was statistically significant in all periods on the basis of direction and in all directions on the basis of period. The highest value in the north direction was obtained in the period 1993-1997, while the highest value in the west, east and south directions was obtained in the period 2008-2012. According to the average values, the lowest value was observed in the north and west directions, while the highest value was observed in the east and south directions. The variation of B concentration in *C. arizonica* by organ and direction is given in Table 7.

Table 7. Variation of B concentration in *C. arizonica* by organ and direction

ORGAN	North	East	South	West	F Value	Average
Outer bark	16103.8^{B}	23256.9^{Db}	19407.3^{Cc}	15325.2^{Ab}	1038.8***	18523.3^{c}
Inner bark	10879.2^{B}	7609.3^{Aa}	7344.3^{Ab}	15897.4^{Cb}	1502.7***	10432.6^{b}
Wood	11934.6^{C}	7886.3^{Ba}	3130.2^{Aa}	3232.7Aa	31.8***	6545.9^{a}
F Value	0.8 ns	20.8***	635.1***	955.8***		33.6***
Average	12246.0^{C}	9395.7^{B}	5179.3^{A}	5708.4^{A}	11.3***	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. ns = not significant; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

Considering the results, it was determined that the change in B concentration in *C. arizonica* was statistically significant in all directions except north, organ-wise and in all organs, direction-wise. For the outer bark, it is possible to make the order of west<north<south<east. In the inner bark, the highest value was observed in the west direction, while the lowest values were observed in the east and south directions. In wood, the lowest value was obtained in the south and west directions, while the highest value was obtained in the north direction. According to the average values, we can sort wood<inner bark<outer bark. In addition, when we look at the average values, the lowest values were obtained in the south and west directions, while the highest value was obtained in the north direction. The variation of B concentration in *C. arizonica* by period and direction is given in Table 8.

Table 8. Variation of B concentration in *C. arizonica* wood by period and direction

AGE	North	East	South	West	F Value	Average
2018-2022	19174.4^{Dg}	6615.8^{Cc}	3922.6^{Be}	2392.4^{Aa}	78162.1***	8026.3^{ab}
2013-2017	11638.7^{Dd}	6754.0^{Cc}	4208.6^{Ag}	4366.7^{Bg}	7505.4***	6742.0^{ab}
2008-2012	5389.5^{Db}	3002.7^{Ba}	4032.4^{Cf}	2481.6^{Ab}	14076.4***	3726.6^{a}
2003-2007	7801.7^{Cc}	4270.2^{Bb}	2155.5^{Aa}	3469.4^{ABe}	22.2***	4424.2^{a}
1998-2002	21696.9^{Dh}	9613.5^{Cd}	2387.1^{Ab}	4010.1^{Bf}	35215.5***	9426.9^{b}
1993-1997	11994.0^{De}	9521.0^{Cd}	3420.4^{Bd}	2750.2^{Ac}	3634.2***	6921.4^{ab}
1988-1992	13281.8^{Cf}	17234.9^{De}	2487.1^{Ac}	3212.7^{Bd}	12200.8***	9054.1^{b}
1983-1987	4499.5^{Ca}	6078.8^{Dc}	2428.1^{Abc}	3178.2^{Bd}	7351.5***	4046.1^{a}
F Value	9074.5***	145.8***	838.9***	1172.4***		2.5*
Average	11934.6^{C}	7886.3^{B}	3130.2^{A}	3232.7^{A}	31.8***	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. * = p≤0.05; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

When the values were examined, it was determined that the change of B concentration in *C. arizonica* wood was statistically significant in all periods on the basis of direction and in all directions on the basis of period. The highest value in the north direction was obtained in the period 1998-2002, the highest value in the east direction was obtained in the period 1988-1992, and the highest value in the south and west directions was obtained in the period 2013-2017. According to the average values, the highest value was observed in the period 1988-1992. According to the average values, the lowest value was observed in the south and west directions, while the highest value was observed in the north direction. The variation of B concentration in *P. orientalis* by organ and direction is given in Table 9.

Table 9. Variation of B concentration in *P. orientalis* by organ and direction

ORGAN	North	East	South	West	F Value	Average
Outer bark	9435.4^{Dc}	4709.2^{A}	6599.0^{Bb}	7482.5^{C}	503.8***	7056.5
Inner bark	5303.4^{Ab}	6301.0^{B}	10245.5^{Dc}	8825.9^{C}	10065.3***	7668.9
Wood	3224.6^{Aa}	6354.5^{AB}	2741.5^{Aa}	16472.0^{B}	2.9*	7254.4
F Value	27.9***	0.9 ns	263.5***	0.1 ns		0.0 ns
Average	4053.6^{A}	6142.2^{A}	3877.7^{A}	14808.4^{B}	3.0*	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. ns = not significant; * = p≤0.05; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

According to the results in the table, the change in B concentration in *P. orientalis* is statistically significant in all organs on the basis of direction. In all directions except the north and south directions, the change in B concentration is not statistically significant. For the north direction, we can make the order of wood<inner bark<outer bark. In the south direction, this order is wood<outer bark<inner bark. The highest value in the outer bark was obtained in the north direction, the highest value in the inner bark in the south direction and the highest value in the wood in the west direction. According to the average values, the lowest values were observed in the north, south and east directions, while the highest value was observed in the west direction. The variation of B concentration in *P. orientalis* by period and direction is given in Table 10.

Table 10. Variation of B concentration in *P. orientalis* wood by period and direction

AGE	North	East	South	West	F Value	Average
2018-2022	5481.0^{Cf}	UL	3121.4^{Be}	2330.9^{Aa}	2757.1***	3644.4^{a}
2013-2017	2355.0^{Bb}	UL	2298.9^{Ac}	3900.5^{Ce}	3234.0***	2851.5^{a}
2008-2012	2671.2^{Bd}	5701.5^{Db}	1992.5^{Ac}	3137.0^{Cc}	11658.6***	3375.6^{a}
2003-2007	2369.5^{Ab}	6013.5^{Dc}	2715.0^{Cd}	2608.1^{Bb}	7782.1***	3426.5^{a}
1998-2002	2564.2^{Bc}	6967.5^{Dd}	1780.1^{Aa}	6204.9^{Cf}	3507.1***	4379.2^{a}
1993-1997	1475.6^{Aa}	5695.2^{Cb}	3491.0^{Bg}	106874.5^{Dg}	538800.5***	29384.1^{b}
1988-1992	3025.6^{Ae}	10392.5^{Ce}	3132.6^{Be}	3117.9^{Bc}	26494.4***	4917.2^{a}
1983-1987	5854.8^{Cg}	3356.7^{Aa}	3400.8^{Af}	3602.0^{Bd}	873.9***	4053.6^{a}
F Value	2802.1***	3820.3***	867.9***	466546.0***		3.2**
Average	3224.6^{A}	6354.5^{AB}	2741.5^{A}	16472.0^{B}	2.9*	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. UL = Undetectable limit; * = p≤0.05; ** = p≤0.01; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

When the variance analysis results were examined, it was determined that the change of B concentration in *P. orientalis* wood was statistically significant in all periods on the basis of direction and in all directions on the basis of period. The highest value in the northern direction was observed in the period 1983-1987, the highest value in the eastern direction in the period 1988-1992, and the highest value in the southern and western directions in the period 1993-1997. According to the average values, the highest value was obtained in the period 1993-1997.

Again, according to the average values, the lowest value was observed in the north and south directions, while the highest value was observed in the west direction. In addition, the change in B concentration in the eastern direction in the periods 2013-2017 and 2018-2022 was below the determinable limits. The variation of B concentration in *C. atlantica* by organ and direction is given in Table 11. When the results are examined, it is seen that the change in B concentration is statistically significant in all organs on the basis of direction. In all periods except north and west, the change in B concentration is not statistically significant. For the outer bark, we can rank east<south<north<west. For the inner bark, the order is south<east<west<north. In wood, the highest values were observed in the north direction, while the lowest values were observed in the east, south and west directions.

According to the average values, the lowest values were obtained in the east, south and west directions, while the highest value was obtained in the north direction. The variation of B concentration in *C. atlantica* by period and direction is given in Table 12. When the values were examined, it was determined that the change of B concentration in *C. atlantica* wood was statistically significant in all periods on the basis of direction and in all directions on the basis of period. The highest value in the north and east directions was observed in the period 1998-2002, while the highest value in the south direction was observed in the period 1988-1992. In the west direction, the highest value was obtained in the period 1988-1992, while the lowest value was obtained in the period 2013-2017. According to the average values, the highest value was observed in the 1988-1992 period and the lowest value was observed in the 2013-2017 period. Again, according to the average values, the lowest value was obtained in the east, south and west directions, while the highest value was found in the north direction. The variation of B concentration in *P. menziesii* by organ and direction is given in Table 13.

Table 11. Variation of B concentration in *C. atlantica* by organ and direction

ORGAN	North	East	South	West	F Value	Average
Outer bark	14789.9^{Cb}	7685.9^{A}	10627.6^{B}	21346.2^{Db}	10228.1***	13612.4^{b}
Inner bark	16248.4^{Db}	5121.6^{B}	4053.2^{A}	5306.8^{Ca}	39509.0***	7682.5^{a}
Wood	8665.1^{Ba}	4479.7^{A}	5682.6^{A}	4105.2^{Aa}	6.3**	5733.1^{a}
F Value	6.3**	2.7 ns	1.3 ns	127.3***		16.4***
Average	10035.9^{B}	4864.6^{A}	6014.1^{A}	5949.4^{A}	6.9***	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. ns = not significant; ** = p≤0.01; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

Table 12. Variation of B concentration in *C. atlantica* wood by period and direction

AGE	North	East	South	West	F Value	Average
2018-2022	2322.9^{Aa}	3604.3^{Bd}	3910.0^{Cd}	4794.6^{Df}	2813.5***	3658.0^{ab}
2013-2017	2531.9^{Bb}	3168.3^{Cc}	3974.6^{De}	2367.7^{Aa}	772.4***	3010.6^{a}
2008-2012	11044.2^{Dd}	3039.2^{Ab}	4861.5^{Cf}	4439.0^{Be}	16343.9***	5846.0^{ab}
2003-2007	11602.7^{De}	3181.7^{Bc}	3485.7^{Cc}	2587.3^{Ab}	26829.6***	5214.4^{ab}
1998-2002	13199.1^{Dh}	9009.8^{Cf}	2050.5^{Aa}	3211.0^{Bc}	5285.2***	6867.6^{b}
1993-1997	4003.0^{Dc}	3126.4^{Ac}	3467.5^{Bc}	3616.6^{Cd}	195.3***	3553.3^{ab}
1988-1992	12595.9^{Cg}	2650.0^{Aa}	20636.0^{Dg}	8607.9^{Bg}	13267.5***	11122.4^{c}
1983-1987	12021.2^{Df}	8058.3^{Ce}	3075.1^{Ab}	3217.2^{Bc}	21018.9***	6592.9^{b}
F Value	5223.1***	19455.8***	110403.5***	2397.0***		5.9***
Average	10035.9^{B}	4864.6^{A}	6014.1^{A}	5949.4^{A}	6.9***	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. *** = p≤0.001. Capital letters indicate the difference between average values within organs.

Table 13. Variation of B concentration in *P. menziesii* by organ and direction

ORGAN	North	East	South	West	F Value	Average
Outer bark	16827.0^{Db}	10793.8^{Cb}	9361.0^{Bb}	9016.8^{A}	2056.3***	11499.6^{b}
Inner bark	2405.0^{Aa}	3569.4^{Ba}	2416.1^{Aa}	3466.2^{B}	152.4***	2964.2^{a}
Wood	3588.5^{Aa}	3061.5^{Aa}	2144.5^{Aa}	7038.0^{B}	16.8***	4017.9^{a}
F Value	123.3***	38.9***	211.5***	1.4 ns		35.1***
Average	4794.0^{A}	4091.5^{A}	2893.3^{A}	6878.7^{B}	15.9***	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. ns = not significant; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

Table 14. Variation of B concentration in *P. menziesii* wood by period and direction

AGE	North	East	South	West	F Value	Average
2018-2022	4256.0^{Be}	UL	3058.2^{Af}	11268.7^{Cg}	4063.3***	6194.3^{cd}
2013-2017	3607.0^{Cd}	2553.9^{Bc}	1767.1^{Ac}	7448.6^{De}	2596.5***	3844.1^{abc}
2008-2012	4272.0^{Be}	4904.1^{Cd}	2100.7^{Ae}	10385.8^{Df}	5719.1***	5415.6^{bcd}
2003-2007	1971.8^{Ac}	2317.9^{Bb}	1975.5^{Ad}	2924.7^{Cc}	3570.1***	2297.5^{a}
1998-2002	1847.6^{Bb}	5323.4^{De}	1702.5^{Abc}	2255.7^{Cb}	11822.9***	2782.3^{a}
1993-1997	1693.5^{Ba}	1180.7^{Aa}	1689.2^{Bb}	5852.8^{Cd}	667.7***	2604.1^{a}
1988-1992	5666.1^{Dg}	2088.9^{Bb}	3263.0^{Cg}	1885.1^{Aa}	5112.8***	3225.8^{ab}
1983-1987	5393.8^{Bf}	UL	1600.3^{Aa}	14282.6^{Ch}	32543.0***	7092.2^{d}
F Value	3835.1***	477.9***	881.9***	6798.8***		4.3***
Average	3588.5^{A}	3061.5^{A}	2144.5^{A}	7038.0	16.8***	

According to results of Duncan's test, numbers followed by the different letters (a and b) indicate significant differences among species within each direction. UL = Undetectable limit; *** = p≤0.001. Capital letters indicate the difference between average values within organs.

According to the results of analysis of variance, the change in B concentration in *P. menziesii* was statistically significant in all organs by direction and by organ in all directions except west. The highest values in the north and south directions were observed in the outer bark and the lowest values in the inner bark and wood. In the east direction, the lowest value was observed in the wood, while the highest value was obtained in the outer bark. For the outer bark, it is possible to make the order of west<south<east<north. For the inner bark and wood, the lowest values were observed in the north and south directions. According to the average values, the highest value was obtained in the outer bark. The variation of B concentration in *P. menziesii* by period and direction is given in Table 14.

When the values in the table are examined, it is determined that the change in B concentration is statistically significant in all periods on the basis of direction and in all directions on the basis of period. The highest value in the north and south directions was obtained in the period 1988-1992, the highest value in the east direction was obtained in the period 1998-2002 and the highest value in the west direction was obtained in the period 1983-1987. According to the average values, the highest value was observed in the 1983-1987 period. Apart from this, the change in B concentration in the eastern direction in the periods 1983-1987 and 2018-2022 was below the determinable limits.

4. Discussion

The results of the study show that boron concentration varies significantly on the basis of species, organ, direction and year range. The lowest values were obtained in *P. menziesii* and the highest values in *C. arizonica*. Especially in some directions, differences close to five times between species were obtained. These results show that there is a great difference between the boron utilization and accumulation capacities of the species. Previous studies have shown that heavy metal accumulation potential varies greatly on species basis [27-32].

The results of the study show that the change in boron concentration in the wood is not statistically significant according to the average values. However, the change in boron concentration in the bark differs on a species basis, and even differences exceeding 3 times between species occur. This situation can be interpreted as the accumulation of boron in the bark is mainly airborne. Heavy metals in the air adhere to particulate matter, contaminating particulate matter with heavy metals, and these particulate matter settle on plant bark, increasing heavy metal concentrations [33-36].

The nutrient utilization and heavy metal accumulation potential of plants is the product of a complex mechanism shaped by the interaction of many factors. The determining factors in this process are genetic structure and environmental factors [37-41] Therefore, all factors related to these factors affect the nutrient utilization and accumulation potential of plants. This is because plant habitus and development affect plant element uptake and accumulation. Therefore, all factors affecting plant habitus also affect the uptake and accumulation of elements into the plant, and plant habitus is influenced by genetic structure and environmental factors such as climatic and edaphic factors, stress factors such as drought, frost, UV-B, radiation, heavy metals. Therefore, many of these factors directly and indirectly affect the elemental uptake and accumulation potential of plants, and knowledge on this complex mechanism is still limited [42-44].

5. Conclusion

As a result of the study, no statistically significant difference was found between the directions in any organ. This can be interpreted as the absence of a significant source of boron pollution in the study area. Similarly, when the change in the process is examined, it is seen that the boron concentration varies within a narrow range. This result can be interpreted as no significant boron pollution has occurred in the last 40 years.

Conflict of Interest

The authors have no conflicts of interest to declare.

Ethics Committee Approval

Not applicable

Author Contribution

Conceptization: BA, ŞK; methodology and laboratory analyzes: BA, ŞK; writing draft: BA, ŞK; proof reading and editing: BA, ŞK. Other: All authors have read and agreed to the published version of manuscript.

Funding

The author declared that this study has received no financial support.

Acknowledgements

Not applicable

6 References

[1] Koç, İ., & Nzokou, P. (2022). Gas exchange parameters of 8-year-old Abies fraseri (Pursh) Poir. seedlings under different irrigation regimes. Turkish Journal of Agriculture-Food Science and Technology, 10(12), 2421-2429.

[2] Isinkaralar, O. (2023). Discovery of spatial climate parameters and bioclimatic comfort change simulation in Türkiye under socioeconomic pathway scenarios: a basin-scale case study for urban environments. Natural Hazards, 1-11.

[3] Goyal, D., Yadav, A., Prasad, M., Singh, T. B., Shrivastav, P., Ali, A., ... & Mishra, S. (2020). Effect of heavy metals on plant growth: an overview. Contaminants in agriculture: sources, impacts and management, 79-101.

[4] Noman, A., & Aqeel, M. (2017). miRNA-based heavy metal homeostasis and plant growth. Environmental Science and Pollution Research, 24, 10068-10082.
[5] Ullah, A., Heng, S., Munis, M. F. H., Fahad, S., & Yang, X. (2015). Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environmental and Experimental Botany, 117, 28-40.
[6] Angelova, V., Ivanova, R., Todorov, G., & Ivanov, K. (2008). Heavy metal uptake by rape. Communications in Soil Science and Plant Analysis, 39(3-4), 344-357.
[7] Shults, P., Nzokou, P., & Koc, I. (2020). Nitrogen contributions of alley cropped Trifolium pratense may sustain short rotation woody crop yields on marginal lands. Nutrient Cycling in Agroecosystems, 117(2), 261-272.
[8] Khan, A. H. A., Kiyani, A., Mirza, C. R., Butt, T. A., Barros, R., Ali, B., ... & Yousaf, S. (2021). Ornamental plants for the phytoremediation of heavy metals: Present knowledge and future perspectives. Environmental Research, 195, 110780.
[9] Wani, P. A., Khan, M. S., & Zaidi, A. (2008). Effects of heavy metal toxicity on growth, symbiosis, seed yield and metal uptake in pea grown in metal amended soil. Bulletin of environmental contamination and toxicology, 81, 152-158.
[10] Işınkaralar, K., & Erdem, R. (2021). Changes of calcium content on some trees in Kocaeli. Kastamonu University Journal of Engineering and Sciences, 7(2), 148-154.
[11] Pilipović, A., Zalesny Jr, R. S., Rončević, S., Nikolić, N., Orlović, S., Beljin, J., & Katanić, M. (2019). Growth, physiology, and phytoextraction potential of poplar and willow established in soils amended with heavy-metal contaminated, dredged river sediments. Journal of environmental management, 239, 352-365.
[12] Cooper, A. M., Felix, D., Alcantara, F., Zaslavsky, I., Work, A., Watson, P. L., ... & Schroeder, J. I. (2020). Monitoring and mitigation of toxic heavy metals and arsenic accumulation in food crops: A case study of an urban community garden. Plant Direct, 4(1), e00198.
[13] Işinkaralar, Ö., & Bayraktar, E. P. (2022). Urban Public Spaces, Public Health, and Heavy Metal Pollution Threatening in Ankara City Center: Strategies for Urban Planning. Kastamonu University Journal of Engineering and Sciences, 8(2), 116-121.
[14] Ateya, T. A. A., Bayraktar, O. Y., & Koç, İ. (2023). Havadaki metal kirliliğinin (Ca, M, Mn) tespitinde kent merkezindeki Mavi ladin (Picea pungens) ağacının yaprak ve dallarının biyomonitör olarak kullanılabilirliği. Bartın Orman Fakültesi Dergisi, 25(2), 255-264.
[15] Işınkaralar, Ö., & Şevik, H. (2023). A Statistical Analysis of Traffic-Related Pollution and City Center Dynamics in Ankara: The Cases of Ulus and Kızılay. Journal of Ankara Studies, 11(1), 25-35.
[16] Şahin, Ü.A., Onat, B., Akın, Ö., Ayvaz, Ç., Uzun, B., Mangır, N., Doğan, M., & Harrison, R.M. (2020). Temporal variations of atmospheric black carbon and its relation to other pollutants and meteorological factors at an urban traffic site in Istanbul. Atmospheric Pollution Research, 11, 1051–1062
[17] Ghoma, W., Sevik, H. & Isinkaralar, K. (2022). Using indoor plants as biomonitors for detection of toxic metals by tobacco smoke. Air Qual Atmos Health 15, 415-424
[18] Zhuang, P., Zou, B., Li, N. Y., & Li, Z. A. (2009). Heavy metal contamination in soils and food crops around Dabaoshan mine in Guangdong, China: implication for human health. Environmental geochemistry and health, 31, 707-715.
[19] Singh, A., Sharma, R. K., Agrawal, M., & Marshall, F. M. (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. Food and chemical toxicology, 48(2), 611-619.
[20] Khan, S., Rehman, S., Khan, A. Z., Khan, M. A., & Shah, M. T. (2010). Soil and vegetables enrichment with heavy metals from geological sources in Gilgit, northern Pakistan. Ecotoxicology and environmental safety, 73(7), 1820-1827.
[21] Isinkaralar, O., Isinkaralar, K., & Yilmaz, D. (2023). Climate-related spatial reduction risk of agricultural lands on the Mediterranean coast in Türkiye and scenario-based modelling of urban growth. Environment, Development and Sustainability, 25(11), 13199-13217.
[22] Isinkaralar, O. (2023). A climate-sensitive approach for determining the urban growth boundaries: Towards a spatial exploration for Bursa, Türkiye. Journal of Urban Planning and Development, 149(4), 04023046.
[23] Varol, T., Canturk, U., Cetin, M., Ozel, H. B., Sevik, H., & Zeren Cetin, I. (2022). Identifying the suitable habitats for Anatolian boxwood (Buxus sempervirens L.) for the future regarding the climate change. Theoretical and Applied Climatology, 150(1-2), 637-647.
[24] Işınkaralar, K., & Erdem, R. (2022). The effect of atmospheric deposition on potassium accumulation in several tree species as a biomonitor. Environmental Research and Technology, 5(1), 94-100.
[25] Yadav, S. K. (2010). Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African journal of botany, 76(2), 167-179.

[26] Zeren Cetin, I., Varol, T., Ozel, H. B., & Sevik, H. (2023). The effects of climate on land use/cover: a case study in Turkey by using remote sensing data. Environmental Science and Pollution Research, 30(3), 5688-5699.
[27] Yayla, E. E., Sevik, H., & Isinkaralar, K. (2022). Detection of landscape species as a low-cost biomonitoring study: Cr, Mn, and Zn pollution in an urban air quality. Environmental Monitoring and Assessment, 194(10), 1-10.
[28] Istanbullu, S. N., Sevik, H., Isinkaralar, K., & Isinkaralar, O. (2023). Spatial distribution of heavy metal contamination in road dust samples from an urban environment in Samsun, Türkiye. Bulletin of Environmental Contamination and Toxicology, 110(4), 78.
[29] Ghoma, W. E. O., Sevik, H., & Isinkaralar, K. (2023). Comparison of the rate of certain trace metals accumulation in indoor plants for smoking and non-smoking areas. Environmental Science and Pollution Research, 30, 75768–75776.
[30] Kuzmina, N., Menshchikov, S., Mohnachev, P., Zavyalov, K., Petrova, I., Ozel, H. B., Aricak, B., Onat, S. M., & Sevik, H. (2023). Change of aluminum concentrations in specific plants by species, organ, washing, and traffic density, BioResources 18(1), 792-803.
[31] Sevik, H., Isinkaralar, K., & Isinkaralar, O. (2018). Indoor air quality in hospitals: the case of Kastamonu Turkey. J Chem Biol Phys Sci Sect D, 9(1), 67-73.
[32] Kurz, M., Koelz, A., Gorges, J., Carmona, B. P., Brang, P., Vitasse, Y., ... & Csillery, K. (2023). Tracing the origin of Oriental beech stands across Western Europe and reporting hybridization with European beech–Implications for assisted gene flow. Forest Ecology and Management, 531, 120801.
[33] Isinkaralar, K. (2022). Some atmospheric trace metals deposition in selected trees as a possible biomonitor. Romanian Biotechnological Letters, 27(1), 3227-3236.
[34] QAir (2021) 2021 World Air Quality Report. Region & City PM2.5 Ranking, Report (1-43). https://www.iqair.com/us/world-most-polluted-cities
[35] Isinkaralar, K., Isinkaralar, O., Koç, İ., Özel, H.B., & Sevik, H. (2023). Assessing the possibility of airborne bismuth accumulation and spatial distribution in an urban area by tree bark: A case study in Düzce, Türkiye. Biomass Conversion and Biorefinery, 1-12.
[36] Onat, B., Şahin, Ü.A., Uzun, B., Akın, Ö., Özkaya, F., & Ayvaz, Ç. (2019) Determinants of exposure to ultrafine particulate matter, black carbon, and PM2.5 in common travel modes in Istanbul. Atmospheric Environment, 206, 258–270.
[37] Sulhan, O. F., Sevik, H., & Isinkaralar, K. (2023). Assessment of Cr and Zn deposition on Picea pungens Engelm. in urban air of Ankara, Türkiye. Environment, Development and Sustainability, 25(5), 4365-4384.
[38] Bhaduri, A. M., & Fulekar, M. H. (2012). Antioxidant enzyme responses of plants to heavy metal stress. Reviews in Environmental Science and Bio/Technology, 11, 55-69.
[39] Bayraktar, E. P., Isinkaralar, O., & Isinkaralar, K. (2022). Usability of several species for monitoring and reducing the heavy metal pollution threatening the public health in urban environment of Ankara. World Journal of Advanced Research and Reviews, 14(3), 276-283.
[40] Isinkaralar, O., Isinkaralar, K., Sevik, H., & Küçük, Ö. (2023). Spatial modeling the climate change risk of river basins via climate classification: a scenario-based prediction approach for Türkiye. Natural Hazards, 1-18.
[41] Kumar, A., Kumar, P., Singh, H., & Kumar, N. (2021). Adaptation and mitigation potential of roadside trees with bio-extraction of heavy metals under vehicular emissions and their impact on physiological traits during seasonal regimes. Urban Forestry & Urban Greening, 58, 126900.
[42] Liu, Y., Zhao, X., Liu, R., Zhou, J., & Jiang, Z. (2022). Biomonitoring and phytoremediation potential of the leaves, bark, and branch bark of street trees for heavy metal pollution in urban areas. Environmental Monitoring and Assessment, 194(5), 344.
[43] Hussain, J., Wei, X., Xue-Gang, L., Shah, S. R. U., Aslam, M., Ahmed, I., ... & Azam, T. (2021). Garlic (Allium sativum) based interplanting alters the heavy metals absorption and bacterial diversity in neighboring plants. Scientific reports, 11(1), 5833.
[44] Ajmal, A. W., Saroosh, S., Mulk, S., Hassan, M. N., Yasmin, H., Jabeen, Z., ... & Mumtaz, S. (2021). Bacteria isolated from wastewater irrigated agricultural soils adapt to heavy metal toxicity while maintaining their plant growth promoting traits. Sustainability, 13(14), 7792.

doi:10.55385/kastamonujes.1374901 KUJES 9(2):83-89, 2023

Kastamonu University
Journal of Engineering and Sciences
e-ISSN 2667-8209
http://dergipark.gov.tr/kastamonujes

Accumulation and Transfer of P and K, Macronutrient Elements for Plants, in *Corylus colurna* L. Stem Sections

Kubra Key[a, *], **Şemsettin Kulaç**[b]

[a] Institute of Science, Düzce University, 81620, Düzce, Türkiye
[b] Faculty of Forestry, Department of Forest Engineering, Düzce University Düzce, Türkiye
***Corresponding Author:** semsettin61@msn.com

Received: **October 13, 2023** ◆ Accepted: **December 11, 2023** ◆ Published Online: **December 26, 2023**

Abstract: One of the most essential factors in plant development is the nutrient content in the soil. Phosphorus (P) and potassium (K), essential macronutrients in plant nutrition, are vital in plant growth and development. Therefore, although many studies have been conducted on these elements in agricultural plants, the number of studies on forest trees could be much higher. In particular, there needs to be more information about the accumulation and transfer of these elements in different plant tissues. This study examined the change and transfer of P and K concentrations in the *Corylus colurna* tree's trunk parts. Within the scope of the study, the differences in P and K concentrations in the *Corylus colurna* trunk were examined based on organ, direction, and period. As a result, the most elevated concentrations of both elements were in the barks; the difference in direction was not evident in the wood, and both elements could be transferred within the wood.

Keywords: Annual tree ring, Phosphorus, Potassium, Turkish Hazelnut

Öz: Bitki gelişiminde en önemli faktörlerden biri topraktaki besin içeriğidir. Bitki beslenmesinde temel makro besin elementleri olan fosfor (P) ve potasyum (K), bitki büyümesi ve gelişimi için hayati öneme sahiptir. Bu nedenle, tarımsal bitkilerde bu elementler üzerine birçok çalışma yapılmış olmasına rağmen, orman ağaçları üzerine yapılan çalışmaların sayısı çok daha fazla olabilir. Özellikle, bu elementlerin farklı bitki dokularındaki birikimi ve transferi hakkında daha fazla bilgiye ihtiyaç vardır. Bu çalışmada, *Corylus colurna* ağacının gövde kısımlarındaki P ve K konsantrasyonlarının değişimi ve transferi incelenmiştir. Çalışma kapsamında, *Corylus colurna* gövdesindeki P ve K konsantrasyonlarındaki farklılıklar organ, yön ve dönem bazında incelenmiştir. Sonuç olarak, her iki elementin de en yüksek konsantrasyonlarının kabuklarda olduğu; yön farklılığının odunda belirgin olmadığı ve her iki elementin de odun içinde transfer edilebildiği görülmüştür.

Anahtar Kelimeler: Yıllık ağaç halkası, Fosfor, Potasyum, Türk Fındığı

1. Introduction

Since plants are living things that can produce food from CO_2 in the air, all other living things on Earth are directly or indirectly dependent on plants [1]. In addition to these essential and vital functions, plants also fulfill many ecological, economic, and social functions [2-3]. Plants can fulfill these functions only if they grow and develop healthily. Plant development is shaped by the interaction of genetic structure [4] and environmental factors [1, 5-7]. Among the environmental factors, soil and especially the nutrient content in the soil are among the most critical factors that directly affect plant development [8-10].

Nutrient elements needed by plants are classified as macro and micronutrients. Potassium (K), one of the elements plants need most, has fundamental roles in stomatal opening, plant growth, and cell growth. It is also necessary for the transportation of carbohydrates produced as a result of photosynthesis to the fruit or roots via the phloem. K, which is effective in the transport of anions in the xylem and phloem, is critical for adaptation to various stress conditions, photosynthesis, protein synthesis, energy transfer, osmoregulation, enzyme activation, stomatal movement, phloem transfer and cation-anion balance, and plant water relations [11]. Phosphorus (P), another macronutrient, is one of the essential plant nutrients needed by plants, especially for root development and flowering [12]. Various studies have determined that P is essential, especially in developing agricultural plants, and that P fertilizers affect plant growth [13].

Nutrient elements are the basic building blocks of plants and are found at different levels in different organs of plants after being taken from the soil. Speciation of nutrients within the plant after they are taken from the soil, determining their contribution to plant development, and knowing their transfer between organs are of great importance in terms of fully understanding the factors affecting plant development [14-16]. This study aims to contribute to eliminating the knowledge gaps in this field. Within the scope of the study, it was tried to determine to what extent P and K concentrations of

macronutrient elements accumulate in the trunk organs of *Corylus colurna* (Turkish Hazelnut) and to what extent they are transferred within the wood.

2. Material and Method

Study area and sampling

This study used samples taken from the main trunk of a *Corylus colurna* tree growing within the borders of Müsellimler Village of Ağlı District of Kastamonu province. The sample log was taken at the end of 2020, and the north direction was marked before cutting. The approximately 10 cm thick log surface, which was cut from the trunk at a height of approximately 50 cm from the ground, was smoothed in the laboratory. Annual rings were grouped into ten years, and samples were taken from the outer bark, inner bark, and wood of all ages with the help of a steel drill.

Wood samples were turned into sawdust, labeled, and placed in glass petri dishes. They were left open for 15 days to air dry, then dried in an oven at 45 °C for a week. 0.5 g of dried samples were taken, 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 were added and placed in the microwave oven designed for the analysis. The program of the microwave device was set to rise to 200 °C in 15 minutes and remain at 200 °C for 15 minutes. After the samples were burned in the microwave, the liquid solution samples were taken into flasks and diluted to 50 mL with ultrapure water. Heavy metal analyses were performed with the ICP-OES device. This method has been frequently used for heavy metal analysis in recent years [17]. All analyses in the study were performed three times.

Statistical analyzes

Variance analysis was applied to the obtained data with the help of the SPSS package program. Homogeneous groups were obtained by applying the Duncan test for the factors that were found to have statistically significant differences at at least 95% confidence level (p<0.05), and the data were simplified and interpreted.

3. Findings

The results of the analysis of variance regarding the changes in K concentrations by organ and direction are given in Table 1.

Table 1. Difference of K (ppm) concentrations by organ, period and direction

Age Range	South	West	North	East	F-value
2011-2020	753.3^{Ci}	577.9^{Aj}	743.5^{Ck}	661.1^{Bl}	224.12***
2001-2010	711.4^{Ch}	503.4^{Ag}	697.4^{Bj}	757.0^{Dn}	1604.41***
1991-2000	784.0^{Cj}	623.0^{Ak}	639.9^{Ai}	675.4^{Bm}	123.86***
1981-1990	658.4^{Bg}	343.8^{Aa}	804.6^{Cl}	655.6^{Bl}	1442.21***
1971-1980	764.8^{Di}	391.5^{Ab}	573.6^{Ch}	527.4^{Bh}	2926.69***
1961-1970	791.4^{Cj}	396.8^{Bbc}	392.9^{Ba}	325.1^{Aa}	847.81***
1951-1960	442.6^{Cd}	419.1^{Bd}	392.1^{Aa}	384.4^{Ab}	37.26***
1941-1950	363.2^{Aa}	394.5^{Bbc}	413.8^{Cb}	448.6^{De}	131.97***
1931-1940	384.6^{Abc}	413.8^{Bd}	866.8^{Cm}	417.1^{Bc}	2176.45***
1921-1930	396.5^{Ac}	410.2^{Acd}	503.5^{Cf}	436.1^{Bd}	65.01***
1911-1920	376.4^{Aab}	442.4^{Be}	447.6^{Bc}	513.8^{Cg}	165.06***
1901-1910	379.1^{Aabc}	464.0^{Cf}	444.9^{Bc}	557.3^{Dj}	550.86***
1891-1900	393.9^{Abc}	452.6^{Bef}	463.5^{Bd}	546.8^{Cij}	337.57***
1881-1890	433.6^{Ad}	511.7^{Cg}	484.4^{Be}	579.8^{Dk}	186.56***
1871-1880	433.9^{Ad}	581.0^{Dj}	494.2^{Bef}	538.7^{Chi}	98.16***
1861-1870	460.6^{Ae}	533.2^{BCh}	515.5^{Bg}	549.7^{Cij}	32.59***
1851-1860	487.2^{Af}	553.4^{Bi}	482.0^{Be}	486.0^{Bf}	38.33***
1841-1850	470.2^{Bef}	561.2^{Ci}	451.3^{Ac}	481.9^{Bf}	161.66***
F-value	743.664***	232.579***	1268.422***	651.111***	

Wood	526.9^{Ba}	476.3^{Aa}	545.1^{Ba}	530.1^{Ba}	3.02*
Outer Bark	866.6^{Ab}	1048.6^{Cb}	1551.6^{Dc}	932.3^{Bb}	6382.67***
Inner Bark	1062.1^{Bb}	1316.2^{Dc}	1264.8^{Cb}	897.2^{Ab}	1774.34***
F-value	22.70***	234.29***	110.81***	36.90***	

According to the results of Duncan's test, values followed by the different letters (a, b, A, and B) refer to significant differences among species within each direction. ***=p<0.001; **=p<0.01; *=p<0.05; ns = not significant. Capital letters indicate the difference between horizontally, while lowercase letters indicate vertically. These explanations are also valid for Table 2.

As a result, K concentrations differed significantly (p<0.001) between periods in all directions and between directions in all periods. When the change of K concentration in wood was examined, two issues attracted attention. The first of these is that the values remained in a narrow range. The second is that there was no significant K concentration difference in direction and period. When the change on an organ basis is examined, it is seen that the lowest values were obtained in wood. The highest values in the organs were obtained in the outer bark in the west direction and the inner bark in the north direction. In other aspects, the values obtained on the barks were in the same group due to the Duncan test. Again, there was no significant change in the direction of the barks. Variance analysis results regarding the differences in P concentrations by organ and direction are given in Table 2.

Table 2. Difference of P (ppm) concentrations by organ, period and direction

Age Range	South	West	North	Doğu	F-value
2011-2020	123.2^{Dn}	67.2^{Bm}	86.0^{Cm}	57.6^{An}	10862.13***
2001-2010	60.1^{Ck}	57.4^{Bk}	80.5^{Dl}	56.5^{Am}	2596.99***
1991-2000	67.9^{Cm}	59.2^{Bl}	68.5^{Ck}	54.4^{Al}	902.86***
1981-1990	54.9^{Cj}	13.7^{Ah}	66.6^{Dj}	49.3^{Bk}	15785.71***
1971-1980	66.3^{Dl}	12.4^{Ag}	30.2^{Bi}	38.9^{Cj}	78604.56***
1961-1970	60.3^{Dk}	15.2^{Ci}	9.9^{Af}	11.4^{Bh}	19784.01***
1951-1960	13.7^{Di}	12.1^{Cf}	8.9^{Ad}	10.5^{Bf}	10109.71***
1941-1950	9.5^{Af}	10.8^{Ce}	10.1^{Bf}	12.1^{Di}	881.57***
1931-1940	14.0^{Bi}	10.2^{Ad}	20.3^{Ch}	10.4^{Af}	2581.68***
1921-1930	13.0^{Ch}	10.5^{Ae}	11.4^{Bg}	10.6^{Afg}	337.05***
1911-1920	11.8^{Dg}	8.3^{Ab}	9.4^{Be}	11.4^{Ch}	1378.59***
1901-1910	11.8^{Cg}	9.9^{Bc}	7.2^{Ac}	10.0^{Be}	2822.62***
1891-1900	8.2^{Cd}	6.9^{Aa}	7.4^{Bc}	10.8^{Dg}	792.57***
1881-1890	8.7^{Ce}	8.0^{Bb}	7.0^{Ac}	8.6^{Cc}	154.54***
1871-1880	6.8^{Bb}	8.1^{Cb}	6.2^{Ab}	8.3^{Dc}	744.59***
1861-1870	7.3^{Cc}	6.8^{Ba}	5.6^{Aa}	7.6^{Db}	829.22***
1851-1860	5.9^{Aa}	7.0^{Da}	6.1^{Bab}	6.4^{Ca}	214.57***
1841-1850	8.0^{Be}	15.6^{Dj}	5.6^{Aa}	8.9^{Cd}	1891.48***
F-value	62828.562***	33579.183***	30203.655***	53073.403***	
Wood	30.6^{a}	18.9^{a}	24.8^{a}	21.3^{a}	2.164 ns
Outer Bark	65.9^{Aa}	245.0^{Dc}	90.0^{Bb}	166.8^{Cc}	22082.21***
Inner Bark	153.8^{Bb}	218.6^{Db}	168.6^{Cc}	125.2^{Ab}	4314.58***
F-value	22.96***	351.22***	46.17***	126.43***	

As a result, P concentrations in wood differed significantly (p<0.001) in all directions on a period basis and in all periods on a direction basis. However, according to the average values, the directional change of P concentration was not statistically significant. When the values are examined, the highest P concentration in wood was obtained in wood formed in recent years, while it remained in a narrow range in the past years. Apart from this, it is noteworthy that the concentrations obtained in bark are much higher than those obtained in wood.

4. Discussion

In *Corylus colurna*, the change in the concentration of P and K elements, which are essential nutrients for plants, in the outer bark, inner bark and wood, and annual rings of the 180-year-old plant, based on organ, period, and direction, was determined in this study. As a result of the study, the elements subject to the study accumulated within determinable limits in all directions of all organs. Many studies have shown that some species have different levels of potential to accumulate some elements [18]. Therefore, it is necessary to determine which annual rings of trees are suitable for monitoring the change of which elements in the process. To date, many studies have been conducted on the usability of annual rings, especially in monitoring the change in the concentration of heavy metals in the air over time [19-20]. In the studies, *Corylus colurna* woods also contain Tl [21], Pb, Cr, Zn [22], Co, Mn, Ni [23], Cd, Fe, Al [24] and Sr [25] elements were used to determine the change in the process.

The elements evaluated within the study's scope are macronutrients and absolutely necessary for plants [12, 16]. Therefore, it is necessary to determine how the absorption and use of these elements by trees occur. With the development and diversification of industry in the last century, air pollution threatens human health, especially in cities with high population density [26-29]. Heavy metals have an important place among air pollutants. The elements subject to study are not only nutrients but also heavy metals when they exceed their thresholds (become toxic) for plants. Heavy metals are elements that can be highly harmful to humans, other living things, and the ecosystem [30-32]. It is known that heavy metals, many of which can be toxic, carcinogenic, and fatal to humans even at low concentrations, are harmful at high concentrations, even those required as nutritional elements [33-35]. Heavy metals can enter the plant body from the soil through the roots, the air through the leaves, or the trunk parts [23]. However, in the region where the study was conducted, no source would require a high concentration of the elements subject to study in the air. Therefore, almost all plant elements are thought to be absorbed from the soil.

As a result, the highest concentrations were obtained in barks as organs in this study. In many studies, the highest heavy metal concentrations are obtained in barks [20, 36]. This is related to the rough structure of the bark. After heavy metals are separated from their source, they can be transported hundreds of kilometers away from their source with the help of wind [37]. Particulate matter contaminated with heavy metals can be transported far away with the help of wind [18]. The rough structure of the bark makes it easier for particulate matter contaminated with heavy metals to adhere to the bark surface [38]. Many studies on this subject have determined that heavy metal concentrations in the outer bark are very high, especially in areas with high levels of heavy metal pollution [39]. However, there were no sources of the elements subject to study around the sample tree in this study. Therefore, it is thought that the high concentrations determined in the barks are related to the structure of the organs.

As a result, the concentrations of both elements in the wood of *Corylus colurna* were in a narrow range, and there was no significant change in direction. This result shows that the elements subject to the study can be transferred between tissues within wood. The transport of elements within the wood part of plants is primarily related to the cell structure, particularly the cell wall. The cell wall–plasma membrane (CWPM) interface illustrates an apoplastic mechanical barrier and a flexible form involved in stress sensing, perception, and signaling for the metal/metalloid stress. The CWPs responding to various abiotic strains have been extensively identified and represented among crop plants [23]. Valuable information has been obtained in a few studies on this subject, and it has been determined that the movement of heavy metals within the plant varies depending on the plant variety and heavy metal. In studies conducted on annual rings, while Zn and Pb elements were replaced in *Cedrus deodora*, the Cu element was not displaced [40-42], the Ni element was limited [43, 44], and Co was displaced in *Cedrus atlantica* [38], the Bi element is replaced in *Cupressus arizonica*, while the Cd and Ni elements are not [39], and Cd, Fe, and Al were not replaced in *Corylus colurna* [24] were determined by several research [44-48]. As a result, the accumulation of P element in wood formed in recent years was at different levels in this study. Many studies have determined that the concentrations of many elements vary depending on many factors, such as species, organs, and environmental conditions [49-51].

5. Conclusion

The accumulation of elements in the plant depends on the effects of many factors. Most of these factors are factors that shape plant development. Because plant development is shaped by the interaction of genetic structure and environmental conditions. Therefore, all these factors affect plants' nutrient utilization and accumulation potential because plant habitus and development affect the plant's element uptake and accumulation. In addition, all factors that affect plant habitus also affect the entry and accumulation of elements into the plant, which can be shaped by the mutual interaction of many factors such as genetic structure, environmental factors such as climatic and edaphic factors, and stress factors such as drought, frost, UV-B and heavy metals. Therefore, many of these factors, directly and indirectly, affect plants' element uptake and accumulation potential, and information about this complex mechanism is still limited. Therefore, studies such as this study are important. As a result of this study, the most elevated concentrations of P and K elements were in the barks; the difference in direction was not evident in the wood, and both elements could be transferred within the wood.

Conflict of Interest

The authors have no conflicts of interest to declare.

Ethics Committee Approval

Not applicable

Author Contribution

Conceptization: KK, ŞK; methodology and laboratory analyzes: KK, ŞK; writing draft: KK, ŞK; proof reading and editing: KK, ŞK. Other: All authors have read and agreed to the published version of manuscript.

Funding

The author declared that this study has received no financial support.

Acknowledgements

Not applicable

6. References

[1] Peralta-Videa, J. R., Lopez, M. L., Narayan, M., Saupe, G., & Gardea-Torresdey, J. (2009). The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. The international journal of biochemistry & cell biology, 41(8-9), 1665-1677.

[2] Wang, Q. R., Cui, Y. S., Liu, X. M., Dong, Y. T., & Christie, P. (2003). Soil contamination and plant uptake of heavy metals at polluted sites in China. Journal of Environmental Science and Health, Part A, 38(5), 823-838.

[3] Dalvi, A. A., & Bhalerao, S. A. (2013). Response of plants towards heavy metal toxicity: an overview of avoidance, tolerance and uptake mechanism. Ann. Plant Sci, 2(9), 362-8.

[4] Kurz, M., Koelz, A., Gorges, J., Carmona, B. P., Brang, P., Vitasse, Y., ... & Csillery, K. (2023). Tracing the origin of Oriental beech stands across Western Europe and reporting hybridization with European beech–Implications for assisted gene flow. Forest Ecology and Management, 531, 120801.

[5] Isinkaralar, O. (2023). Discovery of spatial climate parameters and bioclimatic comfort change simulation in Türkiye under socioeconomic pathway scenarios: a basin-scale case study for urban environments. Natural Hazards, 1-11.

[6] Verma, P., George, K. V., Singh, H. V., Singh, S. K., Juwarkar, A., & Singh, R. N. (2006). Modeling rhizofiltration: heavy-metal uptake by plant roots. Environmental Modeling & Assessment, 11, 387-394.

[7] Isinkaralar, K. (2022). Some atmospheric trace metals deposition in selected trees as a possible biomonitor. Romanian Biotechnological Letters, 27(1), 3227-3236.

[8] Shults, P., Nzokou, P., & Koc, I. (2020). Nitrogen contributions of alley cropped Trifolium pratense may sustain short rotation woody crop yields on marginal lands. Nutrient Cycling in Agroecosystems, 117(2), 261-272.

[9] Yıldırım, N., Bayraktar, A., Atar, F., Güney, D., Öztürk, M., & Turna, I. (2020). Effects of different genders and hormones on stem cuttings of Salix anatolica. Journal of Sustainable Forestry, 39(3), 300-308.

[10] Kuffner, M., Puschenreiter, M., Wieshammer, G., Gorfer, M., & Sessitsch, A. (2008). Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. Plant and Soil, 304, 35-44.

[11] Işınkaralar, K., & Erdem, R. (2021). Changes of calcium content on some trees in Kocaeli. Kastamonu University Journal of Engineering and Sciences, 7(2), 148-154.

[12] Athar, R., & Ahmad, M. (2002). Heavy metal toxicity: effect on plant growth and metal uptake by wheat, and on free living Azotobacter. Water, Air, and Soil Pollution, 138, 165-180.

[13] Mardamootoo, T., Du Preez, C. C., & Barnard, J. H. (2021). Phosphorus management issues for crop production: A review. African Journal of Agricultural Research, 17(7), 939-952.

[14] Koç, İ., Cantürk, U., & Çobanoğlu, H. (2022). Changes of plant nutrients K and Mg in several plants based on traffic density and organs. Kastamonu University Journal of Engineering and Sciences, 8(1), 54-59.

[15] Isinkaralar, O., Isinkaralar, K., & Yilmaz, D. (2023). Climate-related spatial reduction risk of agricultural lands on the Mediterranean coast in Türkiye and scenario-based modelling of urban growth. Environment, Development and Sustainability, 25(11), 13199-13217.

[16] Al-Wabel, M. I., Usman, A. R., El-Naggar, A. H., Aly, A. A., Ibrahim, H. M., Elmaghraby, S., & Al-Omran, A. (2015). Conocarpus biochar as a soil amendment for reducing heavy metal availability and uptake by maize plants. Saudi journal of biological sciences, 22(4), 503-511.

[17] Ghoma, W. E. O., Sevik, H., & Isinkaralar, K. (2023). Comparison of the rate of certain trace metals accumulation in indoor plants for smoking and non-smoking areas. Environmental Science and Pollution Research, 30, 75768–75776.

[18] Mourato, M. P., Moreira, I. N., Leitão, I., Pinto, F. R., Sales, J. R., & Martins, L. L. (2015). Effect of heavy metals in plants of the genus Brassica. International journal of molecular sciences, 16(8), 17975-17998.
[19] Yayla, E. E., Sevik, H., & Isinkaralar, K. (2022). Detection of landscape species as a low-cost biomonitoring study: Cr, Mn, and Zn pollution in an urban air quality. Environmental monitoring and assessment, 194(10), 687.
[20] Schäfer, J., Hannker, D., Eckhardt, J. D., & Stüben, D. (1998). Uptake of traffic-related heavy metals and platinum group elements (PGE) by plants. Science of the Total Environment, 215(1-2), 59-67.
[21] Bayraktar, E. P., Isinkaralar, O., & Isinkaralar, K. (2022). Usability of several species for monitoring and reducing the heavy metal pollution threatening the public health in urban environment of Ankara. World Journal of Advanced Research and Reviews, 14(3), 276-283.
[22] Abou-Shanab, R. A., Ghanem, K., Ghanem, N., & Al-Kolaibe, A. (2008). The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. World Journal of Microbiology and Biotechnology, 24, 253-262.
[23] Isinkaralar, O., & Isinkaralar, K. (2023). Projection of bioclimatic patterns via CMIP6 in the Southeast Region of Türkiye: A guidance for adaptation strategies for climate policy. Environmental Monitoring and Assessment, 195(12), 1448.
[24] Key, K., Kulaç, Ş., Koç, İ., & Sevik, H. (2022). Determining the 180-year change of Cd, Fe, and Al concentrations in the air by using annual rings of *Corylus colurna* L. Water, Air, & Soil Pollution, 233(7), 1-13,
[25] Khan, A. G., Kuek, C., Chaudhry, T. M., Khoo, C. S., & Hayes, W. J. (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere, 41(1-2), 197-207.
[26] Işınkaralar, K., & Erdem, R. (2022). The effect of atmospheric deposition on potassium accumulation in several tree species as a biomonitor. Environmental Research and Technology, 5(1), 94-100.
[27] Wei, S., Zhou, Q., & Wang, X. (2005). Identification of weed plants excluding the uptake of heavy metals. Environment International, 31(6), 829-834.
[28] Meers, E., Tack, F. M., Van Slycken, S., Ruttens, A., Du Laing, G., Vangronsveld, J., & Verloo, M. G. (2008). Chemically assisted phytoextraction: a review of potential soil amendments for increasing plant uptake of heavy metals. International Journal of Phytoremediation, 10(5), 390-414.
[29] Shahid, M., Khalid, S., Abbas, G., Shahid, N., Nadeem, M., Sabir, M., ... & Dumat, C. (2015). Heavy metal stress and crop productivity. Crop production and global environmental issues, 1-25.
[30] de la Fuente, C., Clemente, R., Martínez-Alcalá, I., Tortosa, G., & Bernal, M. P. (2011). Impact of fresh and composted solid olive husk and their water-soluble fractions on soil heavy metal fractionation; microbial biomass and plant uptake. Journal of Hazardous Materials, 186(2-3), 1283-1289.
[31] Işınkaralar, K. (2021). Changes in Cadmium (Cd) concentrations in some plants depending on traffic density. New Trends and Issues Proceedings on Advances in Pure and Applied Sciences,(14), 63-70.
[32] Guney, D., Koc, I., Isinkaralar, K., & Erdem, R. (2023). Change in Pb and Zn concentrations in some trees by the plant species, organ, and traffic density. Baltic Forestry (In press).
[33] Kuzmina, N., Menshchikov, S., Mohnachev, P., Zavyalov, K., Petrova, I., Ozel, H. B., Aricak, B., Onat, S. M., & Sevik, H. (2023). Change of aluminum concentrations in specific plants by species, organ, washing, and traffic density, BioResources 18(1), 792-803.
[34] Sulhan, O. F., Sevik, H., & Isinkaralar, K. (2023). Assessment of Cr and Zn deposition on Picea pungens Engelm. in urban air of Ankara, Türkiye. Environment, Development and Sustainability, 25(5), 4365-4384.
[35] Isinkaralar, K., Isinkaralar, O., Koç, İ., Özel, H.B., & Sevik, H. (2023). Assessing the possibility of airborne bismuth accumulation and spatial distribution in an urban area by tree bark: A case study in Düzce, Türkiye. Biomass Conversion and Biorefinery, 1-12.
[36] Koc, I., Sevik, H., Kulaç, Ş., Cantürk, U., Çobanoğlu, H., & Key, K. (2023). Change of Cr concentration from past to present in areas with elevated air pollution. International Journal of Environmental Science and Technology, 1-12.
[37] Işınkaralar, K., Işınkaralar, Ö., & Şevik, H. (2022). Usability of some landscape plants in biomonitoring technique: an anaysis with special regard to heavy metals. Kent Akademisi, 15(3), 1413-1421.
[38] Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A. H., & Yousefi, N. (2009). Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential. Environmental Earth Sciences, 59, 315-323.
[39] Khan, A., Khan, S., Khan, M. A., Qamar, Z., & Waqas, M. (2015). The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. Environmental science and pollution research, 22, 13772-13799.
[40] Zhang, X. (2019). The history of pollution elements in Zhengzhou, China recorded by tree rings. Dendrochronologia, 54:71-77.

[41] Isinkaralar, K., Koç, İ., Kuzmina, N. A., Menshchikov, S. L., Erdem, R., & Aricak, B. (2022). Determination of heavy metal levels using Betula pendula Roth. under various soil contamination in Southern Urals, Russia. International Journal of Environmental Science and Technology, 19(12), 12593-12604.

[42] Cetin, M., Sevik, H., & Cobanoglu, O. (2020). Ca, Cu, and Li in washed and unwashed specimens of needles, bark, and branches of the blue spruce (Picea pungens) in the city of Ankara. Environmental Science and Pollution Research, 27, 21816-21825.

[43] Saleh, E. A. A., & Işınkaralar, Ö. (2022). Analysis of trace elements accumulation in some landscape plants as an indicator of pollution in an urban environment: Case of Ankara. Kastamonu University Journal of Engineering and Sciences, 8(1), 1-5..

[44] Shruti, M., & Dubey, R. S. (2010). Heavy metal uptake and detoxification mechanisms in plants. International Journal of Agricultural Research, 5(7), 482-501.

[45] Istanbullu, S. N., Sevik, H., Isinkaralar, K., & Isinkaralar, O. (2023). Spatial distribution of heavy metal contamination in road dust samples from an urban environment in Samsun, Türkiye. Bulletin of Environmental Contamination and Toxicology, 110(4), 78.

[46] Atar, F., Güney, D., Bayraktar, A., Yıldırım, N., & Turna, İ. (2020). Seasonal Change Of Chlorophyll Content (Spad Value) In Some Tree And Shrub Species. Turkish Journal of Forest Science, 4(2), 245-256.

[47] Çobanoğlu, H., Cantürk, U., Koç, İ., Kulaç, Ş., & Sevik, H. (2023). Climate change effect on potential distribution of Anatolian chestnut (Castanea sativa mill.) in the upcoming century in Türkiye. Forestist, 73(3).

[48] Isinkaralar, O., Isinkaralar, K., & Bayraktar, E. P. (2023). Monitoring the spatial distribution pattern according to urban land use and health risk assessment on potential toxic metal contamination via street dust in Ankara, Türkiye. Environmental Monitoring and Assessment, 195(9), 1085.]

[49] Koç, İ., Nzokou, P., & Cregg, B. (2022). Biomass allocation and nutrient use efficiency in response to water stress: insight from experimental manipulation of balsam fir, concolor fir and white pine transplants. New Forests, 53, 915-933.

[50] Yilmaz, Ç., Kulaç, Ş., & Beyazyüz, F. (2022). Kuraklık stresi uygulanan kayacık (Ostrya carpinifolia Scop.) fidanlarında morfolojik, fizyolojik ve biyokimyasal değişimlerin araştırılması. Düzce Üniversitesi Orman Fakültesi Ormancılık Dergisi, 18(2), 169-190.

[51] Hrivnák, M., Paule, L., Krajmerová, D., Kulaç, Ş., Şevik, H., Turna, İ., ... & Gömöry, D. (2017). Genetic variation in Tertiary relics: The case of eastern-Mediterranean Abies (Pinaceae). Ecology and evolution, 7(23), 10018-10030.

Kastamonu University
Journal of Engineering and Sciences
e-ISSN 2667-8209
http://dergipark.gov.tr/kastamonujes

Determination of the Changes in Airborne Ba and Mn Concentrations in Düzce City Center in the Last 40 Years with the Help of *Cedrus atlantica* Annual Rings

Sümeyya Uslu [a, *], Nurcan Yiğit [b]

[a] Kastamonu University, Faculty of Forestry, Kastamonu-Türkiye
[b] Kastamonu University, Faculty of Forestry, Kastamonu-Türkiye
***Corresponding Author:** sumeyyauslu3@gmail.com

Received: **October 16, 2023** ◆ Accepted: **December 13, 2023** ◆ Published Online: **December 26, 2023**

Abstract: Heavy metals are pollutants that pose a great threat to humans, other living things, and the ecosystem. All barium (Ba) compounds, one of the most dangerous and harmful heavy metals, are poisonous. Manganese (Mn) is a harmful heavy metal that can cause hallucinations, fatigue, insomnia, weakness, forgetfulness, nerve damage, Parkinson's, and lung embolism. Therefore, observing the changes in Ba and Mn concentrations in the air is essential. This study aimed to determine the change of Ba and Mn heavy metals in the Cedrus atlantica tree based on direction, period, and organ. As a result, the annual rings of *Cedrus atlantica* show that the transfer of both heavy metals into the wood of *Cedrus atlantica* was limited. Therefore, the species is a suitable biomonitor that can be used to monitor the changes in Ba and Mn concentrations in the air. In addition, the study results revealed that the concentrations of both elements in the outer barks are quite high, and Ba pollution is primarily caused by traffic.

Keywords: Atlas cedar, Annual ring, Biomonitor, Barium, Manganese

Öz: Ağır metaller insanlar yanında hem diğer canlılar hem de ekosistem için büyük tehdit oluşturan kirleticilerdir. Ağır metaller içerisinde en tehlikeli ve zararlılarından olan Ba'un bütün bileşikleri zehirlidir. Mn ise halusinasyonlar, bitkinlik, uykusuzluk, güçsüzlük, unutkanlık, sinir hasarları, parkinson, akciğer ambolisi gibi etkilere sebep olabilen zararlı ağır metallerden birisidir. Bundan dolayı havadaki Ba ve Mn konsantrasyonunun değişiminin izlenmesi büyük önem taşımaktadır. Bu çalışmada Ba ve Mn ağır metallerinin *Cedrus atlantica* ağacında yön, dönem ve organ bazında değişiminin belirlenmesi amaçlanmıştır. Çalışma sonucunda *Cedrus atlantica* yıllık halkalarının her iki ağır metalin, *Cedrus atlantica*'da odun içerisindeki transferinin sınırlı düzeyde olduğu, dolayısıyla türün havadaki Ba ve Mn konsantrasyonlarının değişiminin izlenmesi amacıyla kullanılabilecek uygun bir biyomonitor olduğunu göstermektedir. Ayrıca çalışma sonuçları her iki elementin dış kabuklardaki konsantrasyonlarının oldukça yüksek olduğunu ve Ba kirliliğinin büyük oranda trafik kaynaklı olduğunu ortaya koymaktadır.

Anahtar Kelimeler: Atlas sediri, Yıllık halka, Biyomonitor, Baryum, Mangan

1. Introduction

While the proportion of the population living in urban areas worldwide was 9% in the 1900s, it is around 50% today and is estimated to reach 90% in 2030 [1, 2]. Urban areas have many problems, and the most critical problems in these areas are environmental pollution, especially air pollution [3-6], which causes more than 7 million people to die worldwide yearly [7].

Among the air pollution components, heavy metal pollution is the most harmful and dangerous to human health [8, 9]. Due to factors such as the high number of people living per unit area in urban areas, activities carried out to meet the demands and needs of these people and traffic density, some heavy metal concentrations in the air increase significantly, and this increase threatens human health [10].

While some of the heavy metals, many of which are the primary raw materials of industrial and agricultural activities, can be toxic, carcinogenic, and fatal to living creatures even at low concentrations [11], it is stated that even some of them, which are the basic building blocks of living organisms, are harmful at high concentrations [12, 13]. Heavy metals, which do not easily deteriorate through natural processes after their release into nature, pose a great threat to living organisms and the ecosystem [14, 15].

Barium (Ba) and manganese (Mn) are among the most dangerous and harmful heavy metals for living organisms and the ecosystem, especially humans [16]. Ba, which plays a crucial role in the production of many products in the industry, produces many products such as rubber, brake pads, ink, paint, radio vacuum tubes, medicine, machine oil, photo paper, optical glass, plastic and textile products, detergents, batteries, and petroleum industry. Although it is used in production,

it is one of the most dangerous heavy metals, and all Ba compounds are poisonous [17]. Mn, one of the heavy metals that is dangerous and harmful to human health, affects mainly the respiratory system and brain when it reaches humans through the food chain. Mn may cause effects such as hallucinations, fatigue, insomnia, weakness, nerve damage, bronchitis, forgetfulness, lung embolism, Parkinson's, and impotence in men [18, 19]. Due to these dangers, it is of great importance to observe the change in heavy metal pollution in urban centers where the population is dense [20].

Monitoring heavy metal pollution in the atmosphere can be done directly or indirectly. However, direct determination of pollution is not preferred because it is pricy, and the direct effect of atmospheric contamination on the ecosystem cannot be determined [21, 22]. The most effectively used method for monitoring the indirect effect of heavy metal contamination is biomonitors. Plants, especially those used as biomonitors, accumulate heavy metals in different plant parts, and determining the metal concentration in these organs provides essential evidence about heavy metals concentration in the airborne [23]. In regions where trees enter dormancy, the development of plants varies depending on the season, and thus, annual tree rings are made in the wood. By comparing the amounts of heavy metals accumulated in the annual rings of trees over a long period, unequal environmental factors and genetic structure can be equalized, and the variation in the long term can be evaluated. Though studies on the usability of annual rings of trees as biomonitors have been carried out for a while, the amount of evidence on the speciation of heavy metals within the plant species and their alteration between organs after their entry into the plant body is relatively restricted [24].

By evaluating the studies on using plants as biomonitors, deficiencies in the literature were identified, and this study was planned. This study determined changes in Ba and Mn concentrations, two of the most hazardous heavy metals for human and environmental health, in the annual rings of a *Cedrus atlantica* tree growing in Düzce, based on organ, period, and direction.

2. Material and Method

The study was carried out on a *Cedrus atlantica* tree growing in Düzce. The log samples used in the study were obtained by marking the north direction on the log and taking its coordinates during the cutting in a park in the center of Düzce province in 2022. The log sample was examined and determined to be 40 years old. The upper surface of the log was smoothed in the laboratory to make the annual rings more clearly visible. Considering the annual ring widths, they are grouped between 1 and 8 age clusters, from outside to inside, for 5 years. After the wood surface was divided into groups and age ranges were determined, samples of wood in each age range were taken with the help of a steel-tipped drill and placed in glass petri dishes. The samples taken into glass containers were kept open in laboratory conditions until they became room dry, and the samples that became room dry were taken to the oven and dried at 45 °C for two weeks. 0.5 g of the dried samples was taken, 6 ml of 65% HNO3 and 2 mL of 30% H2O2 were added and placed in the microwave oven. The program of the microwave device was set to rise to 200 °C for 15 minutes and remain at 200 °C for 15 minutes. After the samples were burned in the microwave oven, the samples in solution were taken into scaled balloon test tubes diluted to 50 mL with ultrapure water. Ba and Mn heavy metal analyses were performed with the ICP-OES device, and the results were calculated by multiplying by the dilution factor. The raw data were evaluated with the help of variance analysis (ANOVA) and the Duncan test using the SPSS 22.0 package program. This method has been frequently used in studies on heavy metal analysis in recent years and is used in both soil [25, 26] and plant parts [27, 28] is frequently used for analysis.

3. Result

The change of Ba concentration based on organ and direction is given in Table 1.

Table 1. Change of Ba concentration based on organ and direction

Organ	North	East	South	West	Average
Outer bark	14232.4^{b}	273.3	5472.9^{b}	23015.0^{c}	10748.4^{b}
Inner bark	553.8^{a}	50.9	78.2^{a}	115.0^{a}	199.4^{a}
Wood	643.0^{a}	181.6	956.8^{a}	1509.4^{b}	822.7^{a}
F value	2104.4***	0.8 ns	65.4***	976.0***	66.1***
Average	1993.0^{AB}	177.7^{A}	1320.5^{A}	3520.5^{B}	3.6*

According to the outcomes of Duncan's test, concentrations followed by the different letters (a, b, and A, B) refer to significant differences among organs within each direction. ns = not significant; * = $p<0.05$; *** = $p<0.001$. Capital letters indicate a horizontal direction, while lowercase letters refer to the vertical direction.

According to the results of ANOVA, the change in Ba concentration was statistically noteworthy on an organ basis in all directions except the East. The highest Ba values were obtained in the outer bark. Considering the average values, the

highest value was obtained in the West direction, while the lowest was in the East and South direction. The change of Ba concentration based on period and direction is given in Table 2.

Table 2. Change of Ba concentration based on period and direction

Age cluster	North	East	South	West	Average
2018-2022	589.4^{c}	70.5^{a}	2354.4^{g}	1785.5^{e}	1199.9
2013-2017	772.4^{e}	58.3^{a}	1635.6^{f}	1506.8^{d}	993.2
2008-2012	560.6^{c}	45.9^{a}	788.6^{cd}	2125.7^{f}	880.2
2003-2007	659.5^{d}	52.8^{a}	918.4^{d}	1064.1^{c}	673.7
1998-2002	543.8^{c}	648.0^{c}	17.6^{a}	2679.7^{g}	972.3
1993-1997	1142.9^{f}	41.7^{a}	727.3^{c}	1621.8^{d}	883.4
1988-1992	396.4^{a}	33.9^{a}	1062.1^{e}	549.0^{a}	510.3
1983-1987	479.4^{b}	502.0^{b}	150.4^{b}	742.5^{b}	468.5
F value	165.8***	293.5***	306.3***	210.6***	1.5 ns

According to the outcomes of Duncan's test, concentrations followed by the different letters (a and b) refer to significant differences among periods within each direction. ns = not significant; *** = $p<0.001$.

Ba concentration change was statistically significant in all directions on a period basis. There was no statistically significant difference when evaluating the average Ba values according to the periods. The change of Mn concentration based on organ and direction is given in Table 3.

Table 3. Change of Mn concentration (ppb) based on organ and direction

Organ	North	East	South	West	Average
Outer bark	46820.5^{b}	25772.6^{b}	32826.0^{c}	51295.9^{c}	39178.7^{b}
Inner bark	45325.2^{b}	61515.3^{c}	23162.9^{b}	39837.4^{b}	42460.2^{b}
Wood	8637.0^{a}	7158.0^{a}	7150.9^{a}	8493.9^{a}	7860.0^{a}
F value	161.8***	248.1***	47.0***	107.2***	201.2***
Average	16124.2	14455.2	11319.6	15908.4	0.6 ns

According to the outcomes of Duncan's test, concentrations followed by the different letters (a and b) refer to significant differences among organs within each direction. ns = not significant; *** = $p<0.001$.

When the Mn concentrations were examined, Mn concentration change was statistically significant in all directions on an organ basis. When evaluating the average values, the highest values were observed in wood, while the lowest values were obtained in barks. There was no statistically noteworthy alteration between directions when the directions were examined according to the average values. The change of Mn concentration based on period and direction is given in Table 4.

Table 4. Change of Mn concentration (ppb) based on period and direction

Age cluster	North	East	South	West	Average
2018-2022	5708.9^{c}	4103.7^{b}	12529.4^{c}	6801.7^{b}	7285.9^{bc}
2013-2017	16951.5^{g}	8213.8^{c}	16401.9^{d}	6657.4^{b}	12056.1^{ef}
2008-2012	13098.1^{f}	14426.4^{f}	11483.4^{c}	16451.7^{d}	13864.9^{f}
2003-2007	5474.4^{c}	12387.0^{e}	4939.7^{b}	18283.3^{e}	10271.1^{de}
1998-2002	9429.3^{d}	9064.5^{d}	3752.6^{a}	11268.9^{c}	8378.8^{cd}
1993-1997	11484.5^{e}	2812.5^{a}	2853.1^{a}	2566.7^{a}	4929.2^{ab}
1988-1992	4017.2^{b}	2593.7^{a}	2594.7^{a}	2765.1^{a}	2992.7^{a}
1983-1987	2932.4^{a}	3662.7^{b}	2652.4^{a}	3156.3^{a}	3100.9^{a}
F value	304.1***	370.1***	226.0***	555.0***	16.2***

According to the outcomes of Duncan's test, concentrations followed by the different letters (a and b) refer to significant differences among periods within each direction. *** = $p<0.001$.

Considering the results, it was found that the Mn concentration change was statistically significant in all directions on a period basis. While the highest value in the North and South was observed in 2013-2017, the highest value in the East and West was obtained in 2003-2007. According to the average values, it was seen that the Mn concentration is inversely proportional to age, and the concentrations are higher in wood formed in recent years.

4. Discussion

This study examined the usability of *Cedrus atlantica* annual rings in observing the alterations in Ba and Mn concentrations in the air. Since the effects of heavy metals on human and environmental health are known, many studies

have been conducted on the usability of tree annual rings in monitoring the change in heavy metal concentrations in the air over time [23, 28, 29]. These studies mainly examined elements such as Pb, Cr, Ni, Co, and Cd [30, 31]. However, elements such as Sr, Pd, As, Be, Pu, Sb, Sr, Th, Tl, and V, which are not subject to much of these studies, are also included in the list of ATSDR's priority pollutants due to their dangers to human and environmental health [15]. Mn and Ba are among the priority pollutants in this list regarding human and environmental health, but the number of studies on these elements is relatively low.

As a result of the study, the highest values were generally obtained in the barks, especially the outer bark. Studies have determined that the maximum concentrations of many heavy metals are obtained in the outer barks. This situation is generally due to the structure of the outer bark and the adhesion of particulate matter contaminated with heavy metals to the outer bark. Particulate matter in the air is contaminated with heavy metals, and since the outer shell surface is rough and cracked, these particles can adhere to the shell surface [11, 29]. As a result of the study, it was determined that Mn concentrations in the inner bark were relatively high. This situation is related to the entry of heavy metals into the plant body. Heavy metals enter the plant body mainly through roots, leaves, and stem parts [32]. It can be said that Mn in the inner bark enters through the trunk parts, and therefore, the concentrations in the inner bark, which is not in contact with air, are higher than in the wood.

The element concentrations subject to the study were quite variable, and there were significant alterations between their concentrations in different directions in the same period or different periods in the same direction in this study. For example, while the concentration of Mn in the western direction in 1993-1997 was 2566.7 ppb, the concentration obtained in 1998-2002 was 11268.9 ppb. Similarly, the concentration obtained in the south direction in the same period was 3752.6 ppb. Similar results were obtained for Ba. These results show that the transfer of Ba and Mn into wood is limited. This result is significant because it emphasizes that the essential need for more information about the usability of biomonitors in monitoring heavy metal pollution is about the transfer of elements in wood. As a matter of fact, studies have shown that the displacement potential of different elements in the wood of different species is at different levels [33-35].

The elements transport within the plants wood is mainly related to the cell structure, especially the cell wall (apoplastic pathway) and stress factors [36]. Plants frequently face abiotic stress factors throughout their life cycle. Among the stress factors that plants encounter most frequently are those related to climatic parameters such as drought [37] and frost [38]. Because plant development depends on the interaction of genetic structure [39] and environmental conditions [40]. Rigid changes in the climate constitute the most important source of stress factors in plant development, and this effect may even result in individual, species, and population losses in large areas [41]. Factors that cause significant and permanent changes in climatic parameters, such as global climate change [42], trigger the stress mechanisms of plants [63]. In addition, increasing UV-B stress due to climate change [43], anthropogenic radiation [27], and heavy metal pollution [18] are important sources of stress for plants. These stress sources inevitably affect plant metabolism and, therefore, plants' heavy metal accumulation potential [36].

The current study obtained the highest Ba concentrations in the western direction. There is a highway located on the west side where the study was conducted. It is stated that Ba is spread into the atmosphere mainly due to anthropogenic sources [69]. This situation is actual for many heavy metals. Studies show that the primary sources of heavy metals are human activities such as traffic, urbanization, industry, and mining activities [44, 45].

5. Conclusion and Suggestions

The study examined the usability of Cedrus atlantica annual tree rings in observing the alterations in Ba and Mn concentrations in the air. The study revealed that Cedrus atlantica is a suitable biomonitor that can monitor the change of both elements. However, reducing heavy metal pollution in the air is as important as monitoring it, and the most suitable instruments that can be used for this purpose are plants. In order to determine the plants that can be used to reduce Ba and Mn concentrations in the air, studies comparing many plants should be carried out. Heavy metals are one of the most critical environmental problems regarding human and environmental health. However, studies have been conducted to monitor and reduce heavy metal pollution, focusing on specific elements. It is recommended that elements such as Be, Sr, Th, Tl, and V should be included in studies on the subject, especially in addition to Ba.

Conflict of Interest

The authors have no conflicts of interest to declare.

Ethics Committee Approval

Not applicable

Author Contribution

Conceptization: SU, NY; methodology and laboratory analyzes: SU, NY; writing draft: SU, NY; proof reading and editing: SU, NY. Other: All authors have read and agreed to the published version of manuscript.

Funding

The author declared that this study has received no financial support.

Acknowledgements

We thanks to TUBİTAK

5. References

[1] Yu, K. F., Kamber, B. S., Lawrence, M. G., Greig, A., & Zhao, J. X. (2007). High-precision analysis on annual variations of heavy metals, lead isotopes and rare earth elements in mangrove tree rings by inductively coupled plasma mass spectrometry. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 255(2), 399-408.

[2] Beramendi-Orosco, L. E., Rodriguez-Estrada, M. L., Morton-Bermea, O., Romero, F. M., Gonzalez-Hernandez, G., & Hernandez-Alvarez, E. (2013). Correlations between metals in tree-rings of Prosopis julifora as indicators of sources of heavy metal contamination. Applied geochemistry, 39, 78-84.

[3] Isinkaralar, O. (2023). Spatio-temporal patterns of climate parameter changes in Western Mediterranean basin of Türkiye and implications for urban planning. Air Quality, Atmosphere & Health, 16(11), 2351-2363.

[4] Serbula, S. M., Kalinovic, T. S., Ilic, A. A., Kalinovic, J. V., & Steharnik, M. M. (2013). Assessment of airborne heavy metal pollution using Pinus spp. and Tilia spp. Aerosol and Air Quality Research, 13(2), 563-573.

[5] Sawidis, T., Breuste, J., Mitrovic, M., Pavlovic, P., & Tsigaridas, K. (2011). Trees as bioindicator of heavy metal pollution in three European cities. Environmental pollution, 159(12), 3560-3570.

[6] Işınkaralar, Ö., & Şevik, H. (2023). A Statistical Analysis of Traffic-Related Pollution and City Center Dynamics in Ankara: The Cases of Ulus and Kızılay. Journal of Ankara Studies, 11(1), 25-35.

[7] Ghoma, W. E. O., Sevik, H., & Isinkaralar, K. (2022). Using indoor plants as biomonitors for detection of toxic metals by tobacco smoke. Air Quality, Atmosphere & Health, 15(3), 415-424.

[8] Doucet, A., Savard, M. M., Bégin, C., Marion, J., Smirnoff, A., & Ouarda, T. B. (2012). Combining tree-ring metal concentrations and lead, carbon and oxygen isotopes to reconstruct peri-urban atmospheric pollution. Tellus B: Chemical and Physical Meteorology, 64(1), 19005.

[9] Işınkaralar, K., & Erdem, R. (2022). The effect of atmospheric deposition on potassium accumulation in several tree species as a biomonitor. Environmental Research and Technology, 5(1), 94-100.

[10] Isinkaralar, O., Isinkaralar, K., & Bayraktar, E. P. (2023). Monitoring the spatial distribution pattern according to urban land use and health risk assessment on potential toxic metal contamination via street dust in Ankara, Türkiye. Environmental Monitoring and Assessment, 195(9), 1085.

[11] Sawidis, T., Krystallidis, P., Veros, D., & Chettri, M. (2012). A study of air pollution with heavy metals in Athens city and Attica basin using evergreen trees as biological indicators. Biological trace element research, 148, 396-408.

[12] Kord, B. E. H. Z. A. D., Mataji, A., & Babaie, S. (2010). Pine (Pinus Eldarica Medw.) needles as indicator for heavy metals pollution. International Journal of Environmental Science & Technology, 7, 79-84.

[13] Samecka-Cymerman, A., Kolon, K., & Kempers, A. J. (2009). Short shoots of Betula pendula Roth. as bioindicators of urban environmental pollution in Wrocław (Poland). Trees, 23, 923-929.

[14] Koç, İ., Cantürk, U., Çobanoğlu, H. (2022). Changes of plant nutrients K and Mg in several plants based on traffic density and organs. Kastamonu University Journal of Engineering and Sciences, 8(1), 54-59.

[15] Saleh, E. A. A., & Işinkaralar, Ö. (2022). Analysis of trace elements accumulation in some landscape plants as an indicator of pollution in an urban environment: Case of Ankara. Kastamonu University Journal of Engineering and Sciences, 8(1), 1-5.

[16] Vaculík, M., Konlechner, C., Langer, I., Adlassnig, W., Puschenreiter, M., Lux, A., & Hauser, M. T. (2012). Root anatomy and element distribution vary between two Salix caprea isolates with different Cd accumulation capacities. Environmental Pollution, 163, 117-126.

[17] Işınkaralar, K. (2021). Changes in Cadmium (Cd) concentrations in some plants depending on traffic density. New Trends and Issues Proceedings on Advances in Pure and Applied Sciences,(14), 63-70.

[18] Hristovski, S., & Melovski, L. (2010). Radial patterns of 13 elements in the tree rings of beech trees from Mavrovo National Park, FYROM. Archives of Biological Sciences, 62(2), 351-361.

[19] Alves, E. S., Moura, B. B., & Domingos, M. (2008). Structural analysis of Tillandsia usneoides L. exposed to air pollutants in São Paulo City–Brazil. Water, air, and soil pollution, 189, 61-68.

[20] Sulhan, O. F., Sevik, H., & Isinkaralar, K. (2023). Assessment of Cr and Zn deposition on Picea pungens Engelm. in urban air of Ankara, Türkiye. Environment, Development and Sustainability, 25(5), 4365-4384.

[21] Isinkaralar, O., Isinkaralar, K., Sevik, H., & Küçük, Ö. (2023). Spatial modeling the climate change risk of river basins via climate classification: a scenario-based prediction approach for Türkiye. Natural Hazards, 1-18.

[22] Gallagher, F. J., Pechmann, I., Bogden, J. D., Grabosky, J., & Weis, P. (2008). Soil metal concentrations and vegetative assemblage structure in an urban brownfield. Environmental pollution, 153(2), 351-361.

[23] Işınkaralar, K., Işınkaralar, Ö., & Şevik, H. (2022). Usability of some landscape plants in biomonitoring technique: an anaysis with special regard to heavy metals. Kent Akademisi, 15(3), 1413-1421.
[24] Key, K., Kulaç, Ş., Koç, İ., & Sevik, H. (2023). Proof of concept to characterize historical heavy-metal concentrations in atmosphere in North Turkey: determining the variations of Ni, Co, and Mn concentrations in 180-year-old Corylus colurna L.(Turkish hazelnut) annual rings. Acta Physiologiae Plantarum, 45(10), 1-13.
[25] Işınkaralar, K., & Erdem, R. (2021). Changes of calcium content on some trees in Kocaeli. Kastamonu University Journal of Engineering and Sciences, 7(2), 148-154.
[26] Hanć, A., Barałkiewicz, D., Piechalak, A., Tomaszewska, B., Wagner, B., & Bulska, E. (2009). An analysis of long-distance root to leaf transport of lead in Pisum sativum plants by laser ablation–ICP–MS. International Journal of Environmental and Analytical Chemistry, 89(8-12), 651-659.
[27] Ghoma, W. E. O., Sevik, H., & Isinkaralar, K. (2023). Comparison of the rate of certain trace metals accumulation in indoor plants for smoking and non-smoking areas. Environmental Science and Pollution Research, 30, 75768–75776 (2023).
[28] Ristić, M., Perić-Grujić, A., Antanasijević, D., Ristić, M., Urošević, M. A., & Tomašević, M. (2013). Plants as monitors of lead air pollution. Pollutant Diseases, Remediation and Recycling, 387-431.
[29] Yayla, E. E., Sevik, H., & Isinkaralar, K. (2022). Detection of landscape species as a low-cost biomonitoring study: Cr, Mn, and Zn pollution in an urban air quality. Environmental Monitoring and Assessment, 194(10), 1-10.
[30] Istanbullu, S. N., Sevik, H., Isinkaralar, K., & Isinkaralar, O. (2023). Spatial distribution of heavy metal contamination in road dust samples from an urban environment in Samsun, Türkiye. Bulletin of Environmental Contamination and Toxicology, 110(4), 78.
[31] Siwik, E. I., Campbell, L. M., & Mierle, G. (2010). Distribution and trends of mercury in deciduous tree cores. Environmental Pollution, 158(6), 2067-2073.
[32] Leonelli, G., Battipaglia, G., Siegwolf, R. T., Saurer, M., di Cella, U. M., Cherubini, P., & Pelfini, M. (2012). Climatic isotope signals in tree rings masked by air pollution: A case study conducted along the Mont Blanc Tunnel access road (Western Alps, Italy). Atmospheric Environment, 61, 169-179.
[33] Isinkaralar, K., Isinkaralar, O., Koç, İ., Özel, H. B., & Şevik, H. (2023). Assessing the possibility of airborne bismuth accumulation and spatial distribution in an urban area by tree bark: A case study in Düzce, Türkiye. Biomass Conversion and Biorefinery, 1-12.
[34] Sun, F., Wen, D., Kuang, Y., Li, J., Li, J., & Zuo, W. (2010). Concentrations of heavy metals and polycyclic aromatic hydrocarbons in needles of Masson pine (Pinus massoniana L.) growing nearby different industrial sources. Journal of Environmental Sciences, 22(7), 1006-1013.
[35] Işınkaralar, K. (2022). Evaluation of environmental barium concentration biomonitoring in tree rings. Turkish Journal of Agriculture-Food Science and Technology, 10(4), 754-759.
[36] Al-Khashman, O. A., Ala'a, H., & Ibrahim, K. A. (2011). Date palm (Phoenix dactylifera L.) leaves as biomonitors of atmospheric metal pollution in arid and semi-arid environments. Environmental pollution, 159(6), 1635-1640.
[37] Kulac, S. Nzokou, P., D. Guney, B.M. Cregg, I. Turna. (2013).Growth and physiological response of Fraser fir [Abies fraseri (Pursh) Poir.] seedlings to water stress: Seasonal and diurnal variations in photosynthetic pigments and carbohydrate concentration. HortScience. 47(10):1512–1519.
[38] Koç, İ., Nzokou, P. (2022). Gas exchange parameters of 8-year-old Abies fraseri (Pursh) Poir. seedlings under different irrigation regimes. Turkish Journal of Agriculture-Food Science and Technology, 10(12), 2421-2429.
[39] Beyazyüz, F., & Kulaç, Ş. (2023). klık Stresinin Kestane (Castanea Sativa Mill.) Fidanlarında Yaprak Gaz Değişkenlerine ve Prolin Miktarlarına Etkisi. Turkish Journal of Agriculture-Food Science and Technology, 11(7), 1231-1237.
[40] Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. Environmental chemistry letters, 8, 199-216.
[41] Isinkaralar, O., Isinkaralar, K., & Yilmaz, D. (2023). Climate-related spatial reduction risk of agricultural lands on the Mediterranean coast in Türkiye and scenario-based modelling of urban growth. Environment, Development and Sustainability, 25(11), 13199-13217.
[42] Cui, M., He, X., Davi, N., Chen, Z., Zhang, X., Peng, J., & Chen, W. (2013). Evidence of century-scale environmental changes: Trace element in tree-ring from Fuling Mausoleum Shenyang, China. Dendrochronologia, 31(1), 1-8.
[43] Isinkaralar, O., & Isinkaralar, K. (2023). Projection of bioclimatic patterns via CMIP6 in the Southeast Region of Türkiye: A guidance for adaptation strategies for climate policy. Environmental Monitoring and Assessment, 195(12), 1448.
[44] Meyers, D. E., Auchterlonie, G. J., Webb, R. I., & Wood, B. (2008). Uptake and localisation of lead in the root system of Brassica juncea. Environmental Pollution, 153(2), 323-332.
[45] Zhang, C., Huang, B., Piper, J. D., & Luo, R. (2008). Biomonitoring of atmospheric particulate matter using magnetic properties of Salix matsudana tree ring cores. Science of the Total Environment, 393(1), 177-190.

doi:10.55385/kastamonujes.1395608 KUJES 9(2):96-103, 2023

Kastamonu University
Journal of Engineering and Sciences
e-ISSN 2667-8209
http://dergipark.gov.tr/kastamonujes

A Low-Power 30MHz,6th Order Bandpass Differential Gm-C Filter on Chip Utilizing Floating Current Source

Hüseyin Demirel [a,*], Arsen Ahmed Mohammed[b]

[a] Ankara Yıldırım Beyazıt University, Department of Managament Information Systems, Ankara, Türkiye
[b] Kirkuk University, Engineering Faculty, Electrical Department, Kirkuk, Iraq
***Corresponding Author:** huseyindemirel@aybu.edu.tr

Received: November 24, 2023 ◆ Accepted: December 17, 2023 ◆ Published Online: December 26, 2023

Abstract: This work employs a leap-frog Gm-C structure to design a sixth-order Butterworth and elliptic bandpass filter that the cutoff frequency (Fo = 30MHz). A continuous-time differential Gm-C biquad and its corresponding voltage-mode version are elucidated, utilizing F.C.S (Floating Current Source) circuits as fundamental components. The enhanced current source architecture exhibits a streamlined configuration. It incorporates a reduced number of transistors, which facilitates optimal utilization of the chip's area and introduces a streamlined approach to circuit design. The suggested filter topology lacks a crucial resistor component, vital in achieving integration functionality. The suggested filter incorporates a grounding configuration for all capacitors, thereby mitigating the detrimental impact of parasitic effects. The filter design has been effectively realized utilizing TSMC's 0.18µm CMOS process. The findings from simulations have been provided to validate the theoretical analysis.

Keywords: Continuous-Time, Gm-C Bandpass Filter, Floating Current Source (F.C.S), TSMC's 0.18µm CMOS

Öz: Bu çalışma, bir altıncı derece Butterworth ve eliptik bant geçiren filtre tasarlamak için kesim frekansı (Fo = 30MHz) olacak şekilde bir sıçrama (leap-frog) Gm-C yapısını kullanır. Sürekli zamanlı bir diferansiyel Gm-C biquad ve buna karşılık gelen voltaj modlu versiyonu, temel bileşen olarak F.C.S (Floating Current Source - Yüzen Akım Kaynağı) devrelerini kullanarak açıklanmıştır. Geliştirilmiş akım kaynağı mimarisi, basitleştirilmiş bir yapı sergiler. Bu, transistör sayısını azaltır ve çip alanının optimal kullanımını kolaylaştırarak devre tasarımına akıcı bir yaklaşım getirir. Önerilen filtre topolojisi, entegrasyon işlevini başarmada önemli olan bir direnç bileşenini içermez. Önerilen filtre, tüm kapasitörler için bir topraklama konfigürasyonu içerir, bu da parazit etkilerin olumsuz etkilerini azaltır. Filtre tasarımı, TSMC'nin 018µm CMOS süreci kullanılarak etkili bir şekilde gerçekleştirilmiştir. Simülasyonlardan elde edilen bulgular, teorik analizi doğrulamak için sunulmuştur.

Anahtar Kelimeler: Sürekli Zamanlı, Gm-C Band Geçiren Filtre, Yüzen Akım Kaynağı (F.C.S), TSMC'nin 0.18µm CMOS'u

1. Introduction

Gm-C filters have been widely utilized, from consumer products to computer peripherals, owing to their exceptional high-frequency performance and straightforward design [1-5]. With a reduced power supply voltage, the significance of this factor becomes more pronounced, particularly as the demand for portable systems continues to increase. The high-frequency operation of Gm-C filters poses challenges due to the rapid degradation of F.C.S performance [1]. The integrator serves as the primary constituent in the Gm-C filter topology, wherein it may be implemented through a transconductor component coupled with a capacitor [2]. One of the various techniques employed in the design of active filters [4]. It has the capability to instantiate low-voltage filters based on the trans conductor design methodology for the purpose of designing low-voltage signal processing filters. A transconductor's configuration typically resembles an operational amplifier, albeit with a distinct variation in output impedance. Novel techniques were devised and implemented to enhance the performance of trans conductors.

2. Method

Improved Floating Current Source (F.C.S)

The schematic representation of the generic (F.C.S) and its corresponding electrical symbol are depicted in (Figure 1) [8]. The system primarily comprises two interconnected complementary Long-Tailed Pairs (LTPs) denoted as M7-M8 and M5-M6. These LTPs generate tail currents supplied by the output stages of the two current mirrors, M3-M4 and M1-M2. Assuming that the input terminals INP and INM are subjected to a relatively small differential signal, it can be observed that the nodes ComoutP and ComoutM exhibit characteristics of small signal virtual grounds, which implies that the small signal voltages at these nodes are effectively zero, and the currents delivered by M1-M2 and M3-M4 remain constant despite finite output impedance. It can be deduced that the small signal output currents I_{out} - = I_{out} + are equivalent, and

the floating current source (F.C.S) remains unaffected despite the presence of a standard mode (CM) DC offset between them, given that I (M1, M2) is not equal to I (M3, M4). The (F.C.S) has demonstrated its versatility as a fundamental component, well-suited for utilization as a standard cell, exhibiting a performance that surpasses the Long-Term Potentiation (LTP) [3]. The (F.C.S) is designed to deliver two symmetrical output currents, as depicted in the block diagram illustrated in Figure 1. The currents flowing through these two output terminals are determined by

$$I_{O1} = -I_{O2} = -\frac{1}{2}.vd\left[\sqrt{Kn}.\sqrt{2I_B - \frac{Kn.vd^2}{4}} + \sqrt{Kp}.\sqrt{2I_B - \frac{Kn.vd^2}{4}}\right] \tag{1}$$

$$Vd = V_1 - V_2 \tag{2}$$

V2 and V1 refer to the applied voltages to Y2 and Y1, respectively. 2IB refers to the F.C.S bias current. Kp and Kn are technology factors of the pMOS and nMOS transistors, respectively, and specified by:

$$Kn = \mu_n Cox \frac{W_1}{L_1}$$
$$Kp = \mu_p Cox \frac{W_4}{L_4} \tag{3}$$

Where μ_p and μ_n are the PMOS and NMOS transistors mobility, respectively. Cox refers to the oxide capacitance/unit area. $\frac{W_4}{L_4}$ and $\frac{W_1}{L_1}$ refer to the aspect proportions of transistors M4 and M1, respectively [4].

The enhanced (F.C.S) exhibits a highly streamlined architecture, as depicted in Figure 1. Utilizing two distinct transconductance magnitudes, Gm, and its frequent application in contemporary research render the Field-Effect Current Source (F.C.S) a valuable tool. The transconductance (Gm) of the system can be dynamically adjusted by utilizing bias current in the operational transconductance amplifier (OTA) configurations. The operational principle of developmental Field-Effect Transistor (FET) Current Source (F.C.S) bears resemblance to that of Operational Transconductance Amplifier (OTA). However, it is essential to note that the operational transconductance amplifier (OTA) typically has a single transconductance magnitude, denoted as Gm. On the other hand, in the case of the fully differential cascode stage (F.C.S), there are two distinct transconductance magnitudes, referred to as Gm1 and Gm2. The Improved Floating Current Source's output impedance exhibits a higher magnitude than the floating current source originally suggested by Arbel and Goldminz [5]. The conventional Floating Current Source is enhanced by incorporating M5, M6, M7, and M8 transistors to achieve elevated output resistance magnitudes. The presence of a high output resistance is imperative in voltage-mode configurations, as depicted in the formula. Consequently, the conventional Enhanced Floating Current Source is favoured per established electrical engineering principles. A high output resistance yields superior performance in the low-frequency domain. The output current of the Enhanced Floating Current Source can be determined by performing the multiplication operation between the voltage disparity across the P and N terminals and the transconductance (Gm). The transconductance (Gm) for the P terminal can be expressed as the ratio of g3 to g4 divided by 2. On the other hand, the transconductance for the n terminal can be calculated as the negative sum of g1 and g2 divided by 2. The capacitors are electrically coupled to the structure to avoid including resistive elements [11-12]. In this research, a suggested Gm-C filter application is presented. The output impedance of the system will be reduced as the circuit's output voltage is enhanced, eventually leading to VDSsat oscillations. The output resistance is specified in Farads per Coulomb-second (F.C.S) [6].

$$r_{out+} = r_{out-} \cong \left[\left(\frac{gm4gds3gds8}{gm8(gm3+gm4)}\right) + \left(\frac{gm1gds2gds6}{gm6(gm2+gm1)}\right)\right] \tag{4}$$

The advantage of the structure of the F.C.S's simplicity and use for high-frequency applications, while being suitable for connecting the four or eight transistors utilized in a row due to low supply voltage for buildings, constitutes the disadvantage.

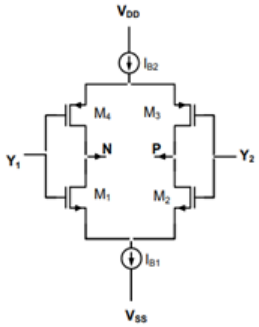

Figure 1: Diagram showing the enhanced floating current source [7]

Table 1 provides the dimensions of the transistors, while Table 2 presents the circuit's direct current (DC) magnitudes. The operational characteristics of the suggested circuit are exemplified on a 6th-order bandpass ladder filter configuration, as depicted in Figure 1.

Table 1: Transistor dimensions

Device	W(μm)	L(μm)
M_1,M_2	18	0.18
M_3,M_4	72	0.18

Table 2: DC magnitudes of enhanced Floating Current Source

Parameters	Magnitude
V_{DD}=-V_{SS}	0.9V
I_{B1} = $I_{B\,2}$	300μA

A 6th-order bandpass ladder passive filter

A 6th-order leap-frog filter has been implemented utilizing the transconductor depicted in Figure 2. The filter is obtained through the utilization of the passive LC ladder filter. [6].

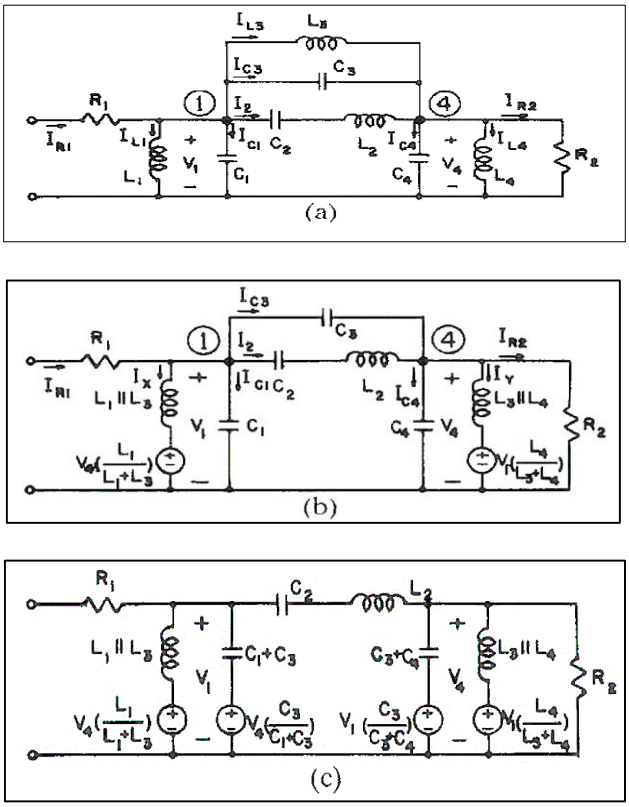

Figure. 2. (a) Doubly terminated sixth-order elliptic bandpass LC ladder, (b) Inductor L, represented by a voltage-controlled voltage source, (c) Final Thevenin equivalent circuit of Figure 2(a) [4]

As depicted in Figure 2, a doubly terminated sixth-order elliptic bandpass filter is illustrated. It has been needed to determine the Final Thevenin equivalent circuit to transform this circuit into a Butterworth bandpass filter. By formulating the nodal formula at node (l), it can derive the following expression.

$$I_{R1} - \frac{V_1}{SL_1} - I_{C1} - I_2 - I_{C3} - \frac{V_1 - V_4}{SL_3} = 0 \tag{5}$$

Rewriting (4), to be;

$$V_1 = (I_{R1} - I_{C1} - I_2 - I_{C3}) \frac{SL_1L_3}{L_1 + L_3} + V_4 \frac{L_1}{L_1 + L_3} \tag{6}$$

In a similar vein, formulating the nodal formula at node (4) will yield:

$$V_4 = (I_{C3} + I_2 - I_{C4} - I_{R2}) \frac{SL_3L_4}{L_3 + L_4} + V_1 \frac{L_4}{L_3 + L_4} \tag{7}$$

Based on equations (5) and (6), it can be observed that the inductor L3 can be equivalently modelled utilizing two voltage-controlled voltage sources, each associated with a specific alteration in the inductor characteristics, as depicted in Figure 2(b). Similarly, to eliminate capacitor C_3, it has been formulated the nodal formula at node (1) as depicted in the diagram. 2 (b) can be expressed as an electrical engineering notation.

$$I_{R1} - I_X - I_2 - V_1(SC_1) - (V_1 - V_4)SC_3 = 0 \tag{8}$$

Or

$$V_1 = (I_{R1} - I_X - I_2) \frac{1}{S(C_1 + C_3)} + V_4 \frac{C_3}{C_1 + C_3} \tag{9}$$

Likewise, for node (4)

$$V_4 = (I_2 - I_Y - I_{R2}) \frac{1}{S(C_3 + C_4)} + V1 \frac{C_3}{C_3 + C_4} \tag{10}$$

Equations (9) and (10) resulting in the final circuit shown in Figure. 2(c).

3. Result

Simulations

The simulations use the LTSPICE software, employing the TSMC CMOS 0.18 μm process variables. The block diagram depicting the suggested 6th-order Elliptic and Butterworth Gm-C bandpass filters is illustrated in Figure 3 and Figure 4 correspondingly. The proposed filter block diagram represents the filter equivalent to the ladder filter depicted in [Figure 1a] and [Figure 1c]. The Butterworth and Elliptic ladder filter component magnitudes are provided in Table 3, showcasing the crucial electrical engineering aspects of these filters, which comprise passive elements. The Gm-C filter component magnitudes and performance variables are presented in Table 4, as per the electrical engineering specifications.

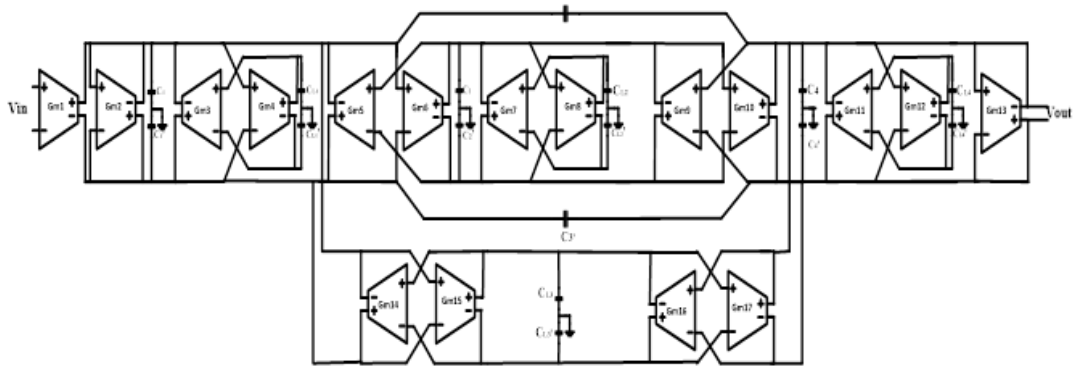

Figure 3: Sixth-order elliptic differential Gm-C bandpass filter block diagram

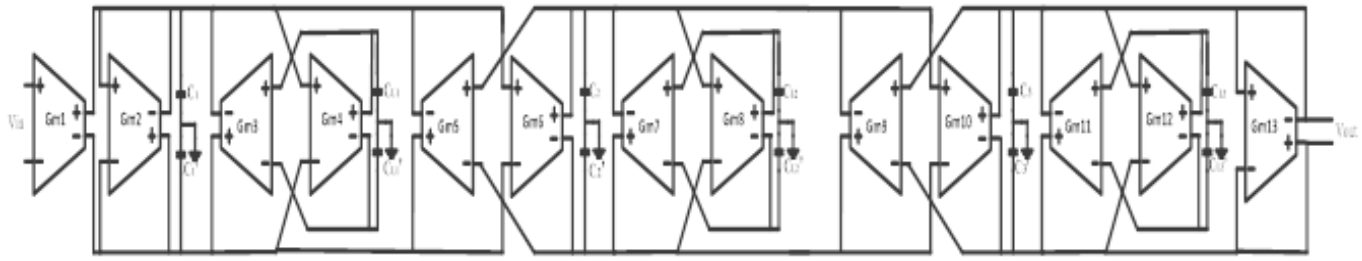

Figure 4: The sixth-order Butterworth differential Gm-C bandpass filter block diagram

Table 3. Ladder filter component magnitudes

Component	Magnitude Elliptic filter	Magnitude Butterworth filter
R_1, R_2	100Ω	100Ω
L_1	125nH	125nH
L_2	2.251µH	2.251µH
L_3	2.251µH	125nH
L_4	125nH	-
C_1	225pF	225pF
C_2	12.5pF	12.5pF
C_3	12.5pF	225pF
C_4	225pF	-

Table 4: Gm-c filter component magnitudes and performance parameters

Component	Magnitude Elliptic filter	Magnitude Butterworth filter
$C._{L1}$	0.4pF	0.4pF
$C._{L2}$	12.3pF	12.3pF
$C._{L3}$	0.4pF	0.4pF
$C._{L4}$	12.2pF	-
I_L	400µA	150µA
I_R	100µA	400µA
G_M Parameter		
$g_{m1}==g_{m3}==g_{m4}==g_{m5}==g_{m6}==g_{m7}==g_{m8}==g_{m9}$ $==g_{m10}==g_{m11}==g_{m12}==g_{m14}==g_{m15}==g_{m16}==g_{m17}$	2.817mA/V	-
$g_{m1}==g_{m3}==g_{m4}==g_{m5}==g_{m6}==g_{m7}==g_{m8}==g_{m9}==g_{m10}==g_{m11}==g_{m12}$	-	1.389mA/V
$g_{m2}==g_{m13}$	0.98mA/V	2.816mA/V

The current outputs at the n and p terminals of the enhanced Floating Current Source in response to the input voltage (VY1-VY2) can be observed in Figure 5. The output currents exhibit a variation of ± 300µA. Figure 6 demonstrates the Gm (transconductance) performance of the enhanced Floating Current Source, showcasing its commendable operation in the vicinity of 100 MHz frequencies. Furthermore, Figure 7 provides an insightful depiction of the alternating current (AC) features of the Floating Current Source (F.C.S). The ladder circuit depicted in Figure 3 and the suggested design illustrated in Figure 4 have been simulated utilizing the component magnitudes specified in (Table 3, 4). The outcome is depicted in Figures 8 and 9.

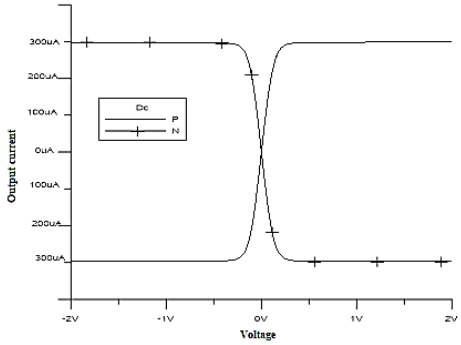

Figure 5: The enhanced floating current source's DC transfer feature.

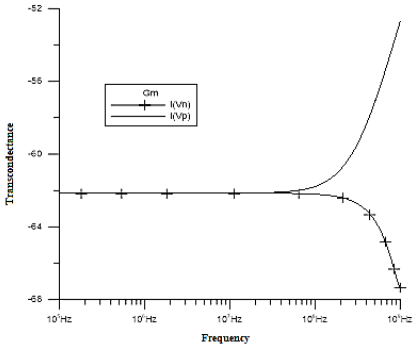

Figure 6: The enhanced floating current source G_m AC transfer feature

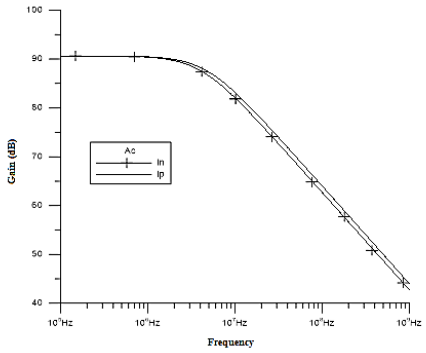

Figure 7: An enhanced floating current source with AC features

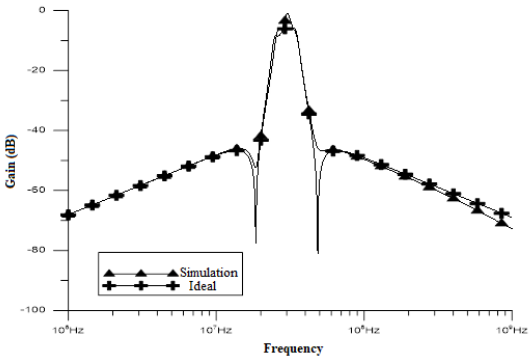

Figure 8: Ideal and simulated Elliptic the sixth order differential Gm-C bandpass filter frequency responses.

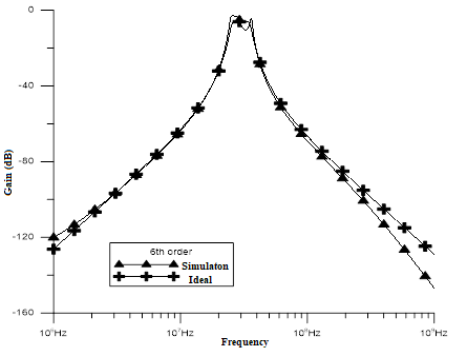

Figure 9: Ideal and simulated Butterworth the sixth order differential Gm-C bandpass filter frequency responses.

The simulation outcomes of the ideal and suggested sixth-order differential Gm-C bandpass filter are presented. The simulated filter responses in LTSPICE exhibit a center frequency of 30MHz. The theoretical center frequency was determined by evaluating $\omega 0 = 2\pi \times 30.05$ MHz, utilizing the data provided in Table 4. The simulation findings obtained from our experiments exhibit a satisfactory correspondence with the theoretical outcomes. Figures 8 and 9 exhibit the

potential applicability of the suggested filters within contemporary RF communication circuits, given that the filters function at intermediate frequencies within a superheterodyne (superhet) receiver. The suggested bandpass filter was subjected to significant signal analysis, wherein a sinusoidal signal with varying amplitudes at a frequency of 10 MHz was applied to the input. Simulations conducted on the filters have demonstrated a Total Harmonic Distortion (T.H.D) below 4% at a frequency of 10MHz. The cumulative harmonic distortion gradually increases proportionately to the input voltage, which remains below 400m Vpp [8-10].

4. Conclusion

This work describes a Gm-C filter implemented in a standard CMOS process, operating at high voltage and low power. Utilizing (F.C.S) made it feasible to achieve high-frequency operation while maintaining a low supply voltage. The (F.C.S) exhibits similarities to the Operational Transconductance Amplifier (OTA). However, the Fully Differential Cascode Structure exhibits superior performance compared to the Operational Transconductance Amplifier (OTA) due to its inclusion of two distinct transconductance (Gm). In contrast, OTA only possesses a single Gm. The (F.C.S) exhibits a straightforward and uncomplicated architecture. The transconductance (Gm) of the device can be dynamically adjusted by utilizing bias current control techniques. The center frequency of the filter is approximately 30 megahertz (MHz). The simulation findings exhibit a favorable correspondence with the theoretical outcomes. The sixth-order bandpass filter (BPF) was realized utilizing standard CMOS TSMC 0.18μm technology, incorporating capacitors. Figure 10 exhibits a die photograph of the filter, wherein the circuit's overall size, encompassing the pads, amounts to 2.4mm^2 for the elliptic filter.

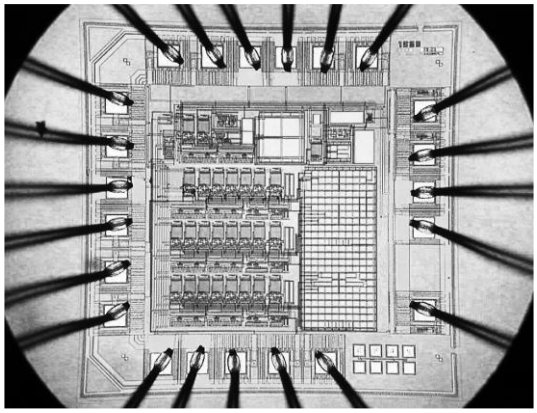

Figure 12: Die photograph of elliptic BP Gm-c filter.

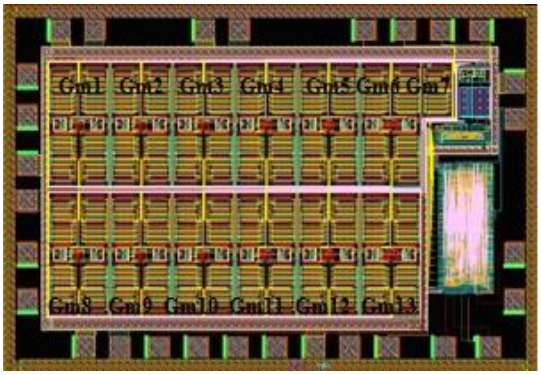

Figure 13: Die photograph of Butterworth BP Gm-c filter

Furthermore, Figure 12 portrays the layout of the receiver. The die, encompassing the pads, occupies a compact area measuring 2.66mm x 2.22mm. Figure 11 illustrates the schematic representation of the (13 part) Gm and capacitor Butterworth filter. The proposed filter functions at intermediate frequencies and applies to contemporary RF communication circuits. The LTSPICE simulations demonstrate satisfactory circuit performance with minimal total harmonic distortion. The enhanced current source architecture exhibits a streamlined configuration with fewer transistors. Hence, it can achieve enhanced circuit design simplicity by effectively utilizing the chip area and mitigating investment costs. The filter lacks the presence of a resistor, and all capacitors are effectively connected to the ground. Hence, it is well-suited for seamless integration and exhibits reduced susceptibility to parasitic influences.

Conflict of Interest

The authors have no conflicts of interest to declare.

Ethics Committee Approval

Not applicable

Author Contribution

Conceptization: HD, AAM; methodology and laboratory analyzes: HD, AAM; writing draft: HD, AAM; proof reading and editing: HD, AAM. Other: All authors have read and agreed to the published version of manuscript.

Funding

The author declared that this study has received no financial support.

Acknowledgements

Not applicable

5. References

[1] Feng, J., Wang, C., Zang, M., & Ren, Y. (2011, January). Realization of current-mode general nth-order filter based on current mirrors. In 2011 3rd International Conference on Advanced Computer Control. IEEE, 367-370.

[2] Bozomitu, R.G., & Cehan, V. A. (2009). Vlsi Implementation of a New Low Voltage 4th Order Differential Gm-C Bandpass Filters for Different Approximation In CMOS Technology. Acta Technica Napocensis, Electronics and Telecommunications, 50, 5-12.

[3] Ranjan, A., & Paul, S. K. (2011). Voltage mode universal biquad using CCCII. Active and Passive Electronic Components, 1-5.

[4] Gaspar, J., Chen, S. F., Gordillo, A., Hepp, M., Ferreyra, P., & Marqués, C. (2004). Digital lock in amplifier: study, design and development with a digital signal processor. Microprocessors and Microsystems, 28(4), 157-162.

[5] Hiroshi, Y. (1999). A 450kHz CMOS Gm-C Band-pass Filter with±0.5% Center Frequency Accuracy for On-Chip PDC IF Receivers. In IEEE International Solid-State Circuits Conference, February.

[6] Song, H. J., & Kim, C. K. (1990). An MOS four-quadrant analog multiplier using simple two-input squaring circuits with source followers. IEEE Journal of Solid-State Circuits, 25(3), 841-848.

[7] Khorramabadi, H. (1992). A CMOS line driver with 80-dB linearity for ISDN applications. IEEE journal of solid-state circuits, 27(4), 539-544.

[8] Edwards, R. T., Strohbehn, K., & Jaskulek, S. E. (2000, May). A field-programmable mixed-signal array architecture using antifuse interconnects. In 2000 IEEE International Symposium on Circuits and Systems (ISCAS). IEEE (3), 319-322.

[9] Ahmed, M. A., Khalaf, M. Z., & Demirel, H. (2023). Study of finfet transistor. Critical and literature review in finfet transistor in the active filter. 3 c TIC: cuadernos de desarrollo aplicados a las TIC, 12(1), 65-81.

[10] Ahmed, A., & Demirel, H. (2023). DESIGN Third order Sinusoidal Oscillator Employing Current Differencing Cascaded Trans conductance Amplifiers. Gazi University Journal of Science Part C: Design and Technology, 11(3), 735-743.

doi:10.55385/kastamonujes.1399589 KUJES 9(2):104-116, 2023

Kastamonu University
Journal of Engineering and Sciences
e-ISSN 2667-8209
http://dergipark.gov.tr/kastamonujes

Investigation of the Use of *Arthrospira (Spirulina) platensis* and *Cladophora glomerata* Algae in *Agaricus bisporus* (white button mushroom) Cultivation to Increase Growth and Yield

Göksal Sezen[a, *], Mustafa Turunçoğlu[a]

[a]Harran University, Faculty of Arts and Sciences, Department of Biology, Şanlıurfa, Türkiye
***Corresponding Author:** sezen@harran.edu.tr

Received: **December 03, 2023** ◆ Accepted: **December 18, 2023** ◆ Published Online: **December 26, 2023**

Abstract: This study was conducted with three replicates using three different doses of *Cladophora glomerata* and *Arthrospira (Spirulina) platensis* algae (*C. glomerata* dry weight dose 10 g, 25 g, and 50 g; *A. platensis* dry weight dose 5 g, 12.5 g, and 25 g) in order to develop and determine the most suitable growing media for *Agaricus bisporus* species. Between 28.04.2021 and 10.07.2021, research trials were conducted in a private mushroom production enterprise in Korkuteli District of Antalya Province. The composts in which the seed mushroom mycelia of "Amycel Company" were planted and inoculated were obtained from "SMS Ersanlar Compost Company" in this district. During the research, some distinguishing characteristics of mushroom quality such as mushroom yield, average mushroom weight, mushroom cap and stem weights, mushroom cap diameter, and height, mushroom stem diameter and length, total mushroom length, dry weight, ash weight, and pH were analyzed. It was observed that Algae treatments to composts generally gave better results than the control group. Cld 250 and Spr 250 application doses increased the total mushroom yield by 7% and 15%, respectively.

Keywords: *Agaricus bisporus*, *Arthrospira (Spirulina) platensis*., *Cladophora glomerata*, Culture mushroom cultivation , Edible mushroom

Öz: Bu çalışma, *Agaricus bisporus* türünün en uygun yetiştirme ortamlarının geliştirilmesi ve belirlenmesi amacıyla, *Cladophora glomerata* ve *Arthrospira (Spirulina) platensis* alglerinin üç farklı dozu (*C. glomerata* kuru ağırlık dozu 10 gr, 25 gr ve 50 gr; *A. platensis* kuru ağırlık dozu 5 gr, 12,5 gr ve 25 gr) kullanılarak üç tekerrürlü olarak yapılmıştır. 28.04.2021 ile 10.07.2021 tarihleri arasında Antalya İli'nin Korkuteli İlçesi'nde özel bir mantar üretim işletmesinde araştırma denemeleri yapılmıştır. "Amycel Firması"'nın tohumluk mantar miselleri içinde ekilmiş ve aşılı olan kompostlar, "SMS Ersanlar Kompost Firmasın'dan yine bu ilçe içinden temin edilmiştir. Araştırmada sırasında mantar kalitesinin bazı ayırt edici özelliklerinden olan; mantar verimi, ortalama mantar ağırlığı, mantar şapka ve sap ağırlıkları, mantar şapka çapı ve yüksekliği, mantar sap çapı ve uzunluğu, toplam mantar uzunluğu, kuru ağırlık, kül ağırlığı ve pH gibi kıstaslar incelenmiştir. Mantar kompostlarına alg uygulamalarının genelde kontrol grubuna göre daha iyi sonuçlar verdiği görülmüştür. Cld 250 ve Spr 250 uygulama dozlarının toplam mantar verimini sırasıyla % 7 ve % 15 oranda arttırdıkları tespit edilmiştir.

Anahtar Kelimeler: *Agaricus bisporus*, *Arthrospira (Spirulina) platensis*, *Cladophora glomerata*, Kültür mantarı yetiştiriciliği, Yenilebilir mantar

1. Introduction

Since ancient times and due to their intriguing nature, fungi have attracted great interest from scientists. These researchers are discovering new species of fungi, with a discovery rate reaching 1200 species per year in the last decade. Today, there are more than 3000 species considered edible mushrooms, including economically and commercially cultivated species and species produced on an industrial scale. However, the white button mushroom (Agaricus bisporus) is still one of the world's most produced mushroom species and today is the fourth most cultivated mushroom species worldwide, accounting for 15% of global mushroom production. By 2025, it is expected to dominate the global market [1]. World mushroom production is 50 million tons and 80% of this production is met by Asian countries, 10% by European countries and 5% by the USA. Turkey has a share of 0.0012% with a production value of 61460 tons [2]. According to the data of Turkish Statistical Institute (TurkStat), mushroom cultivation area reached 895 decares and mushroom production reached 61460 tons in 2021 and Korkuteli district of Antalya ranked first with 280 decares of cultivated area and 28000 tons of product and 45.55% on a national basis [3, 4].

It is especially preferred by consumers interested in vegan and 'clean' diets due to its high nutritional value and many health benefits. In addition to identifying the main growth directions and requirements, this fungus has gained a large

share of scientific studies investigating methods to optimize each of the key steps of the mushroom production cycle, such as composting, spawning, sheathing, pinning, trimming and harvesting, and to develop new species with higher yield capacity and resistance to specific diseases [1].

Bacteria and algae in particular are the main component of culture beds, some of which have the ability to increase mushroom yield and quality [5]. Not only in *Agaricus bisporus*, but also in other mushrooms such as *Pleurotus ostreatus*, the addition of bacterial cultures to the mushroom growing medium has led to faster growth of mushroom mycelia [6]. These effective bacteria produce specific metabolites that can initiate sporophore formation. Phytohormones such as indole 3-acetic acid are the most important factors proposed for the growth stimulating effect of these microorganisms. Cyanobacteria and some other algae are a group of microorganisms that can influence the mycelial growth of fungi. Cyanobacteria and some eukaryotic algae produce a wide range of secondary metabolites, including antibiotics, algicides, toxins, pharmaceuticals and plant growth regulators. Among the growth regulators, giberellin, auxin, cytokinin, ethylene, abscisic acid and jasmonic acid have been identified in cyanobacteria and some eukaryotic algae [7-11].

In this study, it was aimed to investigate the effects of the addition of *Spirulina plantesis* and *Cladophora glomerata*, which are among the micro and macro algae with high nutritional values and content used in plant nutrition, animal feeds, human food supplements, on the yield and quality of *Agaricus bisporus* mushroom cultivation in the mycelial development stage.

2. Material and Method

Arthrospora (*Spirulina*) platensis

Arthrospora (Spirulina) platensis was obtained from Çukurova University, Faculty of Fisheries. The culture was produced in 20 l plastic bottles in Harran University, Faculty of Arts and Sciences, Department of Biology, Department of Hydrobiology - Algology Laboratory, at a constant temperature of 30 ± 1 ºC, pH 9.8-10.3, 24 hours of white LED lamp illumination adjusted to 4000 ± 100 lux light intensity and continuously mixed with fresh air by aquarium pump. The culture reached maximum density every 8-9 days and was harvested by filtering through a 20 µ plankton mesh. The harvested *Artrospora platensis* was dried in the shade, pulverized with a 46 thousand speed blender (Tefal Ultra High Speed Blender - shredder) and stored in a -20 ºC deep freezer. *A. platensis* was cultured with "Spirulina medium" [12].

Cladophora glomerata

Cladophora glomerata required for the experiment was collected from Karkamış Dam in Birecik, Şanlıurfa. The collected algae were cleaned from stones, sand and invertebrates (mussels and snails), dried in the shade, pulverized with a 46 thousand speed blender (Tefal Ultra High Speed Blender - shredder) and stored in -20 oC deep freezer.

Table 1. The systematics of *Agaricus bisporus*, *A. platensis* ve *C. glomerata* are given in Table 1.

Empire	Prokaryota	*Eukaryota*	Eukaryota
Kingdom	Eubacteria	*Plantae*	Fungi
Subkingdom	Negibacteria	*Viridiplantae*	
Phylum	Cyanobacteria	*Chlorophyta*	Basidiomycota
Class	Cyanophyceae	*Chlorophytina*	Homobasidiomycetes
Subclass	Oscillatoriophycidae	*Ulvophyceae*	Homobasidiomycetidae
Order	Oscillatoriales	*Cladophorales*	Agaricales
Family	Microcoleaceae	*Cladophoraceae*	Agaricaceae
Genus	*Arthrospira platensis* Gomont 1892	*Cladophora glomerata* (Linnaeus) Kützing 1843	*Agaricus bisporus (J.E.Lange) Imbach*

Mushroom compost

The experiment was carried out in a private mushroom production enterprise in Korkuteli District of Antalya Province. The compost used in the experiment was obtained from "SMS Ersanlar Kompost" company operating in Antalya / Korkuteli district. Seed mycelia were obtained from "Amycel Company" as planted in the compost. The peat used as cover material of mushroom compost was obtained from "Keskin Torf Company" from Çebiçli District of Burdur.

Establishment of the Experiment and Application Method

The compost to be used in the experiment was placed in the mushroom house warehouse where the experiment would be carried out. After the internal temperature of the bag was determined, the necessary air conditioning conditions were provided. The composts placed in the warehouse in the form of presses were placed in bags of 10 kilograms each, weighed and placed on the bunks. The study setup was prepared according to factorial experimental design with 4 replications. In

the experiment, 21 bags of 10 kg each were used. The total amount of compost used was 210 kg. The daily procedures performed in the experiment are given in Table 2 and the changes in the temperature and humidity of the growing room and compost are given in Table 2.

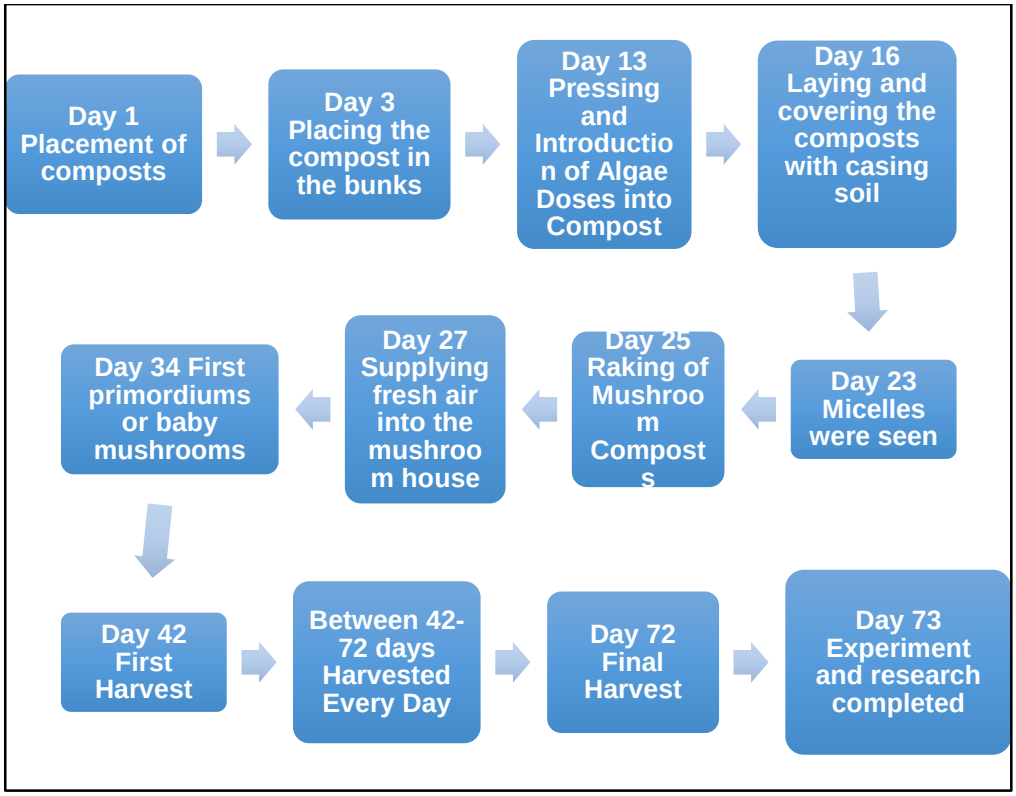

Figure 1. Daily treatments in the experiment

Table 2. Variation of temperature and humidity of the growing room and compost

Process Periods Room	Temperature Bag	Internal Temperature	In Room Humidity
Mycelial pre-growth period	18-22 ºC	20-25 ºC	75-80
Application of cover soil	20-22 ºC	22-28 ºC	80-85
Aeration after raking	15-17 ºC	17-19 ºC	75-80
Harvest time	15-17 ºC	15-17 ºC	70-75

Powdered *Cladophora glomerata* was diluted to 10%. The powdered *Arthrospira (Spirulina) platensis* used in the study was diluted with water to 5%. After disinfection of the prepared mixtures, applications were made. After the completion of the mycelial pre-development period, the printing process was carried out. After the printing process was completed, labeling process representing the application doses and groups was performed in the test bags. Three groups were formed as Spirulina, Cladophora and control group and each group was applied 100 ml, 250 ml and 500 ml mixture homogeneously with 3 repeated injections. The depth of application was 3-4 cm in the compost. In the control group, 100 ml, 250 ml and 500 ml of water were applied. Table 3. shows the dry weight of algae applied to the experimental groups in grams.

Table 3. Dry weights of algae applied to the experimental groups

Application Groups	Cld 100	Cld 250	Cld 500	Spr 100	Spr 250	Spr 500	Cont
In dry weight amounts of algae (gr)	10	25	50	5	12,5	25	0

Analysis and Measurements in the Experiment

The mushrooms were harvested daily from each trial bag and the necessary measurements were made to determine the total yield during the one-month harvest period. In order to determine the characteristics of each group, 10 samples with the characteristics that reflect the general characteristics of the group were separated. If there were not enough samples, the necessary measurements were made on the samples obtained. At the same time, the samples required for analysis in the groups were taken for freezing and drying.

Physical measurements and analyses to determine mushroom quality were performed according to total carpophore weight, average carpophore weight, total number of carpophores, cap diameter (mm), cap height (mm), cap weight (g), stem length (mm), stem diameter (mm), stem weight (g), average carpophore height (mushroom full height), ash content, dry matter percentage and pH [13, 14].

3. Conclusion and Discussion

Total number of carpophores (mushrooms)

The lowest and highest number of carpophores was 182 in the Spr 500 group and 246.33 in the Cld 250 group, which is about 12% higher than the control groups. The data obtained are shown in Figure 4.1. It was found that the increased activity of nitrifying bacteria was related to the high level of extractable nitrate in Agaricus compost, which increased slowly before the completion of the cropping process, and that the total number of carpophores (fungi) could be increased by adding cyanobacteria such as *Nostoc sp*. to the compost [15]. In a similar study conducted by various researchers, it was observed that various bacteria, algae and organic matter added to the compost increased mushroom yield, quality and total carpophore (mushroom) count compared to the control group [16-20].

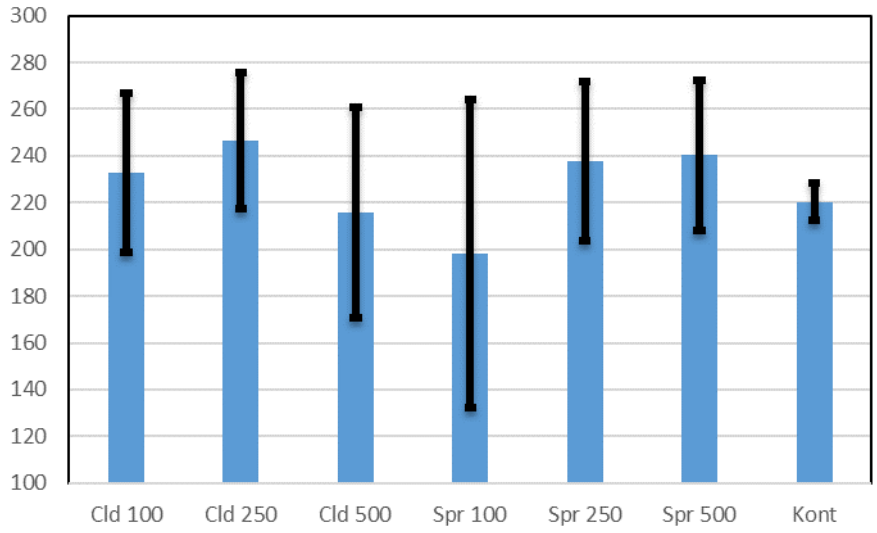

Figure 2. Effect of different dose applications on total carpophore number

Mushroom full length (mm)

The lowest and highest mushroom full length was 42.21 mm in Spr 100 group and 45.72 mm in Spr 500 group, 7% more than the control group. The data obtained are shown in Figure 4.2. In similar studies, it was observed that various bacteria and organic substances added to the mushroom compost increased the mushroom full length compared to the control group [17, 19, 21].

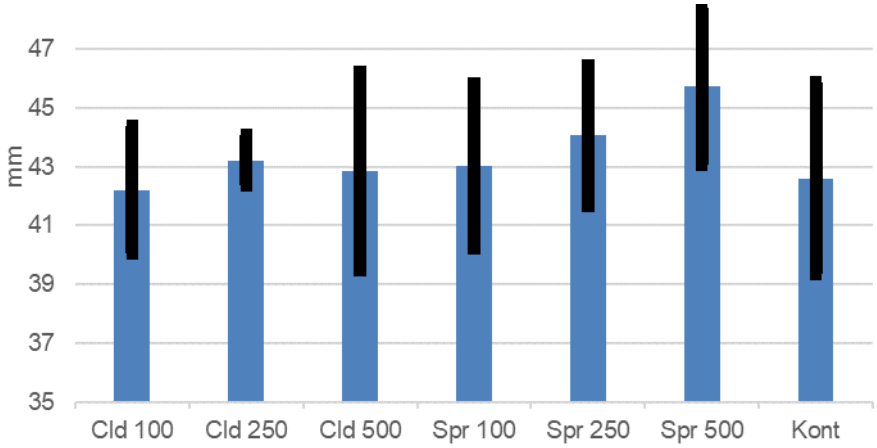

Figure 3. Effect of different dose applications on mushroom full length

Mushroom cap diameter (mm)

The lowest and highest mean hat diameter was 38.11 mm in the Cld 250 group and 43.16 mm in the Spr 500 group, which were 13% higher than the control group. The data obtained are shown in Figure 4.3. In the experiments carried out by various researchers, it was observed that various bacteria and organic substances added to the compost increased the mushroom cap diameter [17, 21-25].

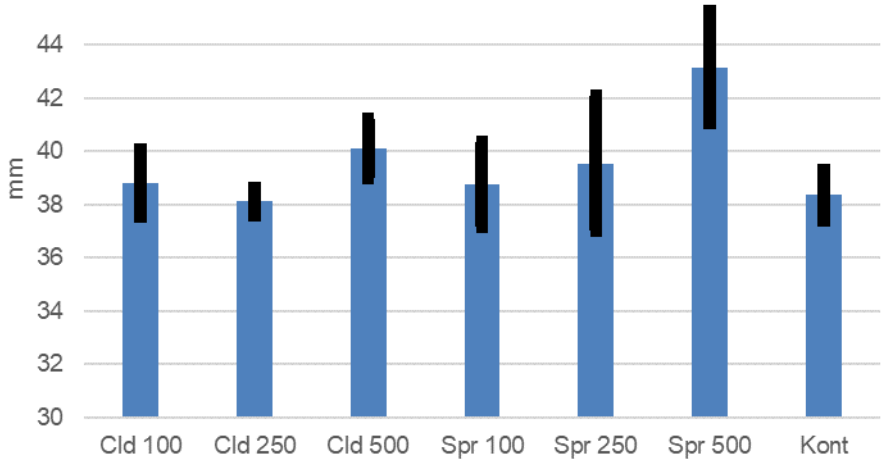

Figure 4. The effect of different dose applications on hat diameter

Height of mushroom cap (mm)

The lowest and highest average hat height was 18.53 mm in Spr 100 group and 32.14 mm in Spr 500 group, which were 70% higher than the control group. The data obtained are shown in Figure 4.4. In some studies, it was determined that various bacteria and organic substances added to mushroom compost increased the height of mushroom cap compared to the control group [17, 21].

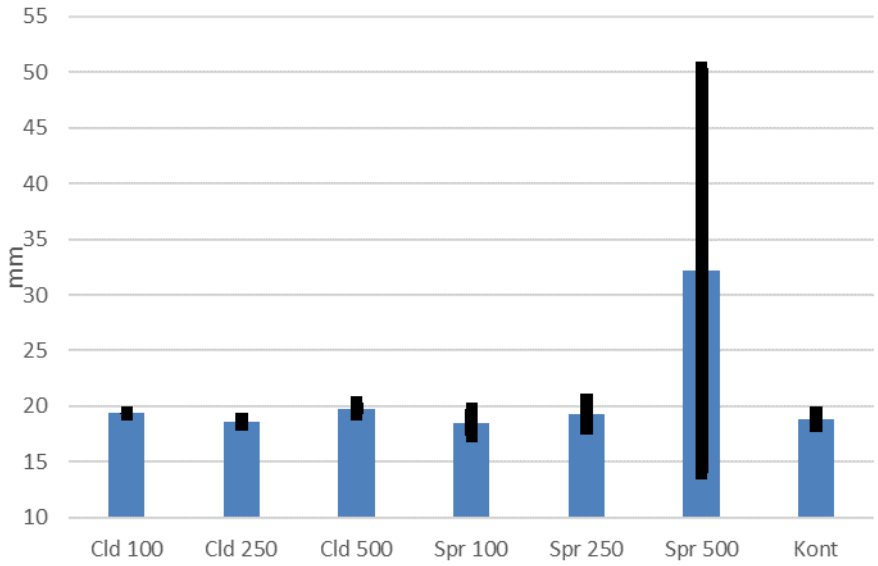

Figure 5. Effect of different dose applications on average hat height

Mushroom stem diameter (mm)

The lowest and highest mean stem diameter was 16.76 mm in the Cld 250 group and 18.72 mm in the Spr 500 group, 4% more than the control group. The data obtained are shown in Figure 4.5. In similar studies, it was found that various bacteria and organic substances added to the mushroom compost increased the mushroom stem diameter compared to the control group [17, 21].

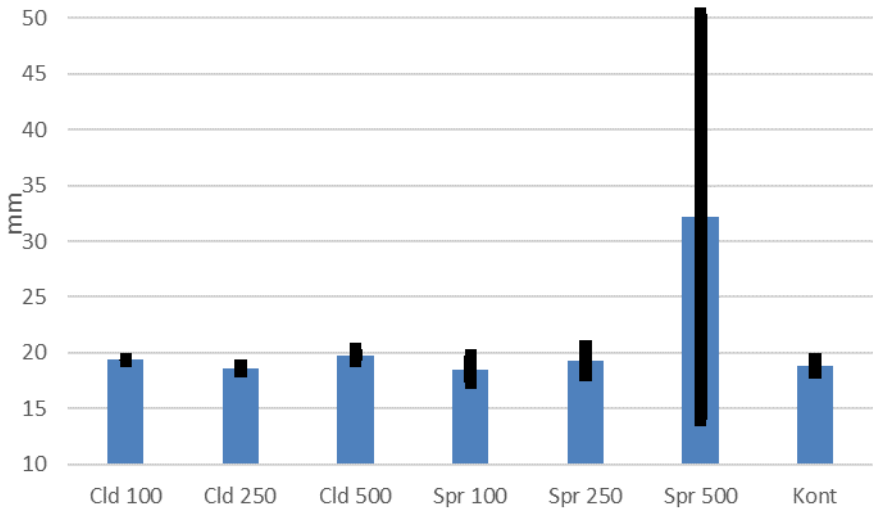

Figure 6. Effect of different dosage treatments on stem diameter

Mushroom stem height (mm)

The lowest and highest mean stem height was 30.91 mm in Cld 100 group and 34.92 mm in Spr 500 group, which were 10% higher than the control group. The data obtained are shown in Figure 4.6. In a similar study, stem length values were observed in the range of 14.45-17.21 cm, the highest value was observed in BM2 group with 17.21 cm and the lowest value was observed in SR8 group with 14.45 cm [21]. In similar studies conducted by various researchers, it was observed that various bacteria, algae and organic substances added to mushroom compost increased the stem length values compared to the control group [17, 18, 22-27].

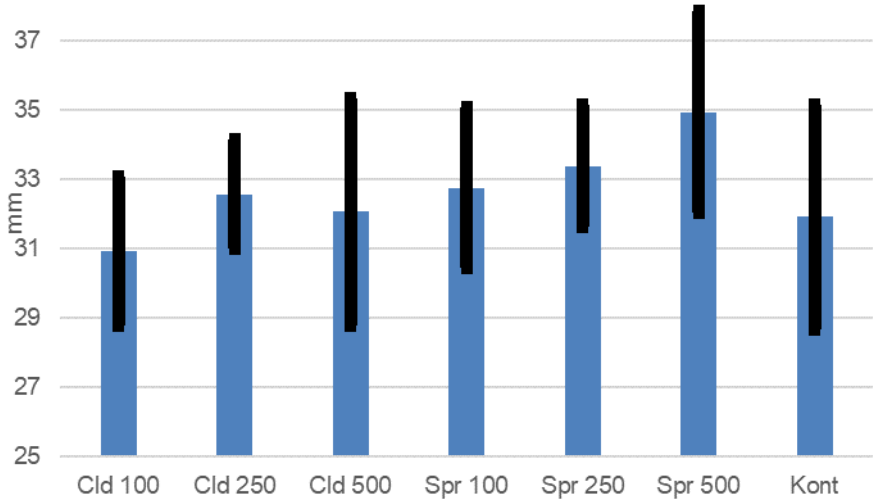

Figure 7. Effect of different doses on stem height

Total carpophore (mushroom) weight

According to the application dose, the lowest total carpophore weight was 2744.93 g and the highest was 3572.89 g, which was 15% more than the control group. The data obtained are given in Figure 4.7. In a study investigating the effect of *Scytalidium thermophilum* inoculation of mushroom composts on yield, it was determined that mushroom yield was twice higher in inoculated composts compared to pasteurised controls [28]. A large increase in the yield of *A. bisporus* mushroom was observed by spraying the photosynthetic bacteria on the cover soil [29]. They observed that the application of nitrogenobacters mixed into mushroom composts did not change the yield of *A. bitorquis* much, but *Bacillus thuringiensis, Bacillus circulans* -II and *Alcaligenes feacalis* significantly increased mushroom yield compared to non-inoculated groups [30]. *Streptomyces violaceorubidus, Microbacterium humi, Gordonia hydrophobica, Curtobacterium citreum, Agaricicola taiwanensis, Advenella incenata* and *Actinomycetales bacterium* isolates isolated from the cover soil of *Agaricus blazei* increased fresh mushroom yield from 70% to 115% [31]. In another study, it was reported that isolates containing *Pseudomonas fluorescens, P. putida* and *Bacillus mycoides* bacteria, which were isolated in *A. bisporus* cultivation and had good interaction in the cover soil, provided yield increase in the range of 8-40% compared to the

control, and P. putida bacteria provided the best result [32]. In a recent study, the highest mushroom yield values of two different commercial microbial fertilisers named BM-MegaFlu and SS-Super Root with different bacterial contents were found in the range of 1063.3 - 2768.5 g, the highest value was found in BM8 with 2768.5 g and the lowest value was found in BM8 application with 1063.3 [21]. In similar studies conducted by various researchers, it was determined that various bacteria, algae and organic substances added to mushroom composts increased the total carpophore (mushroom) weight values compared to the control group [15-17].

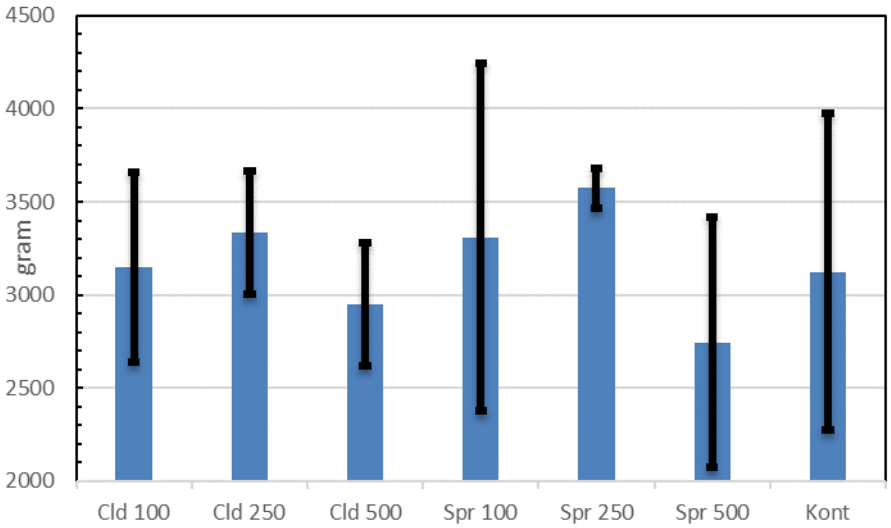

Figure 8. Effect of different dose applications on total carpophore weight

Average carpophore (mushroom) weight

The lowest and highest average carpophore weight was 16.35 g in Cld 250 group and 19.89 g and 22.57 g in Spr 500 and Cld 500 groups, respectively, which were 18% and 34% higher than the control group. The data obtained are shown in Figure 4.8. In the study of application of photosynthetic bacteria to mushroom composts, it was observed that the average mushroom weight varied between 10.71 - 11.20 g [29]. In similar studies conducted by various researchers, it was determined that various bacteria, algae and organic substances added to the composts increased the average carpophore (mushroom) weight values compared to the control group [17, 21]. It is thought that the differences observed in these studies in terms of mushroom weights may be due to the genetic structure of fungi, differences in treatments and environments.

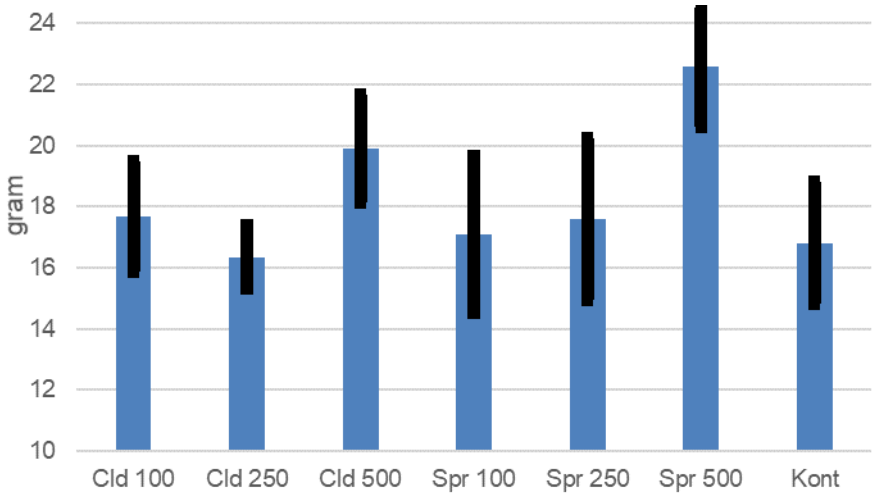

Figure 9. Effect of different dose applications on average carpophore weight

Mushroom hat weight

The lowest and highest average hat weight was found to be 10.63 g in Cld 250 group and 15.75 g in Spr 500 group, 43% more than the control group. The data obtained are shown in Figure 4.9. In similar researches and studies carried out by various researchers, it was determined that various bacteria, algae and organic substances added to mushroom composts increased mushroom cap weight values compared to the control group [17, 21].

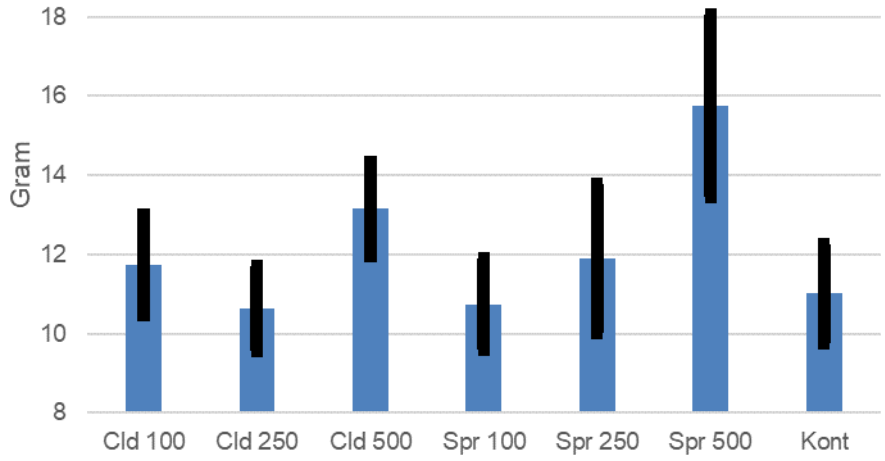

Figure 10. The effect of different dose applications on hat weight

Mushroom stem weight

The lowest and highest average stem weight was 5.74 g in Cld 250 group and 7.14 g in Spr 500 group, which were 27% higher than the control group. The data found are shown in Figure 4.10. In similar studies carried out by various researchers, it was determined that bacteria, algae and organic substances added to mushroom composts increased mushroom stem weight values compared to the control group [17, 21].

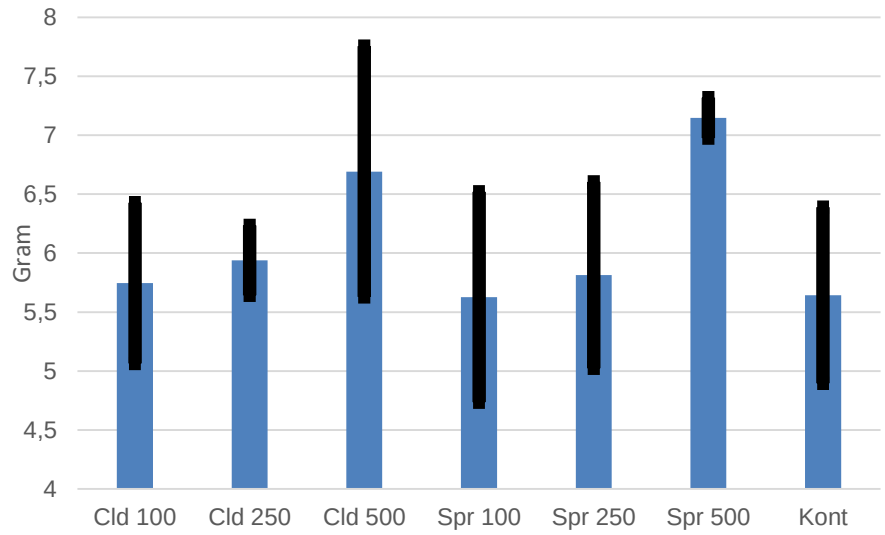

Figure 11. Effect of different dosage treatments on mushroom stem weight

Mushroom wet weight

The lowest and highest mushroom wet weight values were 24.87 g in Cld 100 group and 36.00 g in Cld 500 group, which were 77 % higher than the control group. The wet weights of the mushrooms in the experiments are given in Figure 4. 11. In similar studies carried out by various researchers, it was determined that bacteria, algae and organic materials added to mushroom composts increased mushroom wet weight values compared to the control group [16, 17, 21].

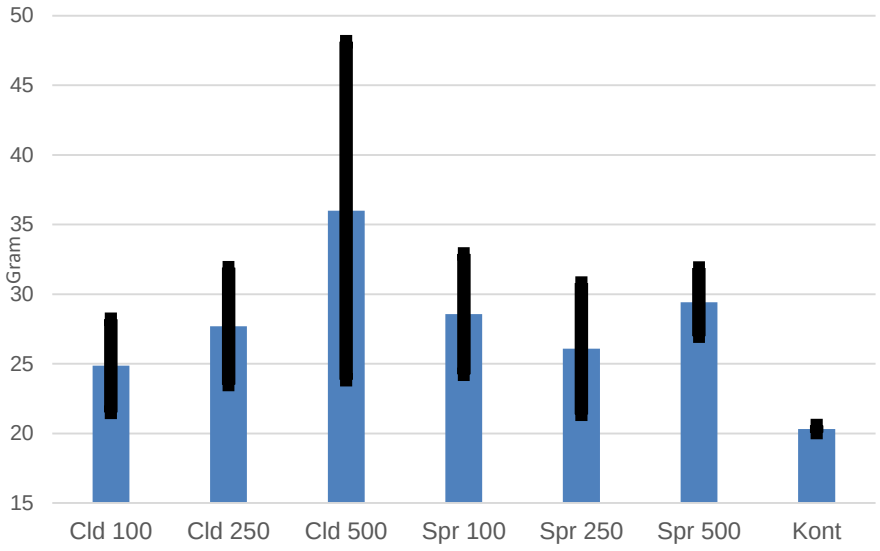

Figure 12. Effect of different dose applications on mushroom wet weight

Mushroom dry weight

Depending on the application dose, the values obtained from the experimental groups varied between 1.79 g. The lowest and highest mushroom dry weight values were 0.91 g in Cld 100 group and 1.79 g in Cld 500 group, which were 112 % higher than the control group. The data obtained are given in Figure 4.12. In a study, it was determined that 9 - 12 % of 100 g fresh mushroom was dry matter and the amount of dry matter of mushroom was influenced by the species and varieties of mushrooms, cultural processes, nutrient content of the mushroom compost medium [33]. In another study, the amount of dry matter was found to be 6.38% [27], and in another study, it was found to be in the range of 7.67 - 9.10%, however, it was also stated that the amount of dry matter decreased in the second and third harvest period compared to the first harvest period [34]. In similar studies carried out by various researchers, it was found that bacteria, algae and organic materials added to mushroom composts increased mushroom dry weight values compared to the control group [15-17, 20, 21].

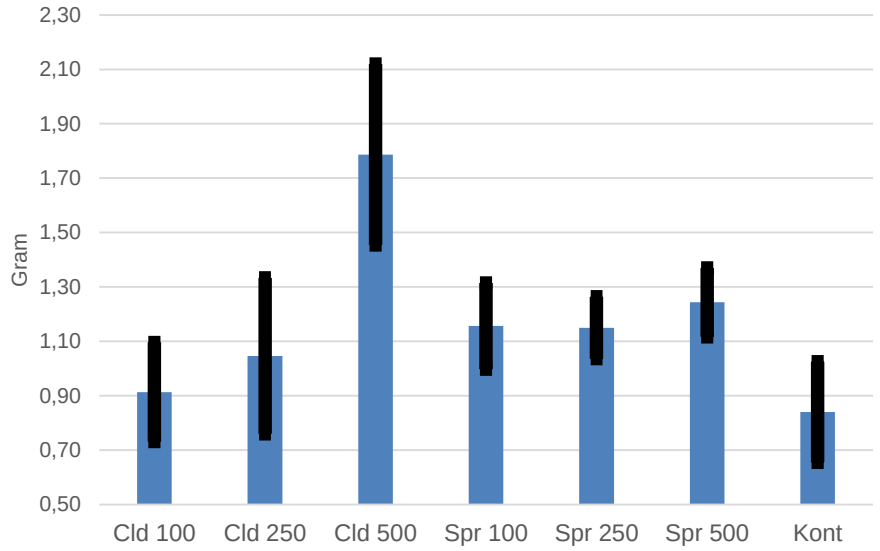

Figure 13. Effect of different dose applications on mushroom dry weight

Mushroom ash weight

The lowest and highest values were 0.33 g in Cld 100 group and 0.70 g in Cld 500 group, which were 109 % higher than the control group. The data obtained are shown in Figure 4.13. In a study, it was found that the amount of mushroom ash was higher in compost formulas and recipes with bacteria and organic fertiliser, the highest was 12.33% ST+B and the lowest was 11.26% ST [35]. In similar studies, it was found that bacteria, algae and organic materials added to mushroom composts increased mushroom ash weight compared to the control group [15, 17, 20, 21].

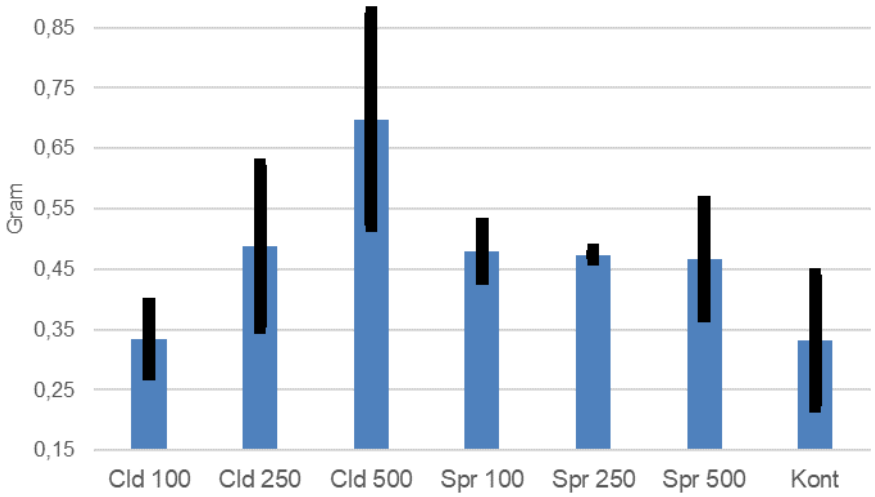

Figure 14. Effect of different doses on mushroom ash weight

Mushroom (%) moisture content

The lowest value was 94.82 % in Cld 500 group and the highest value was 96.35 % in Cld 100 group. The data obtained are shown in Figure 4. 14.

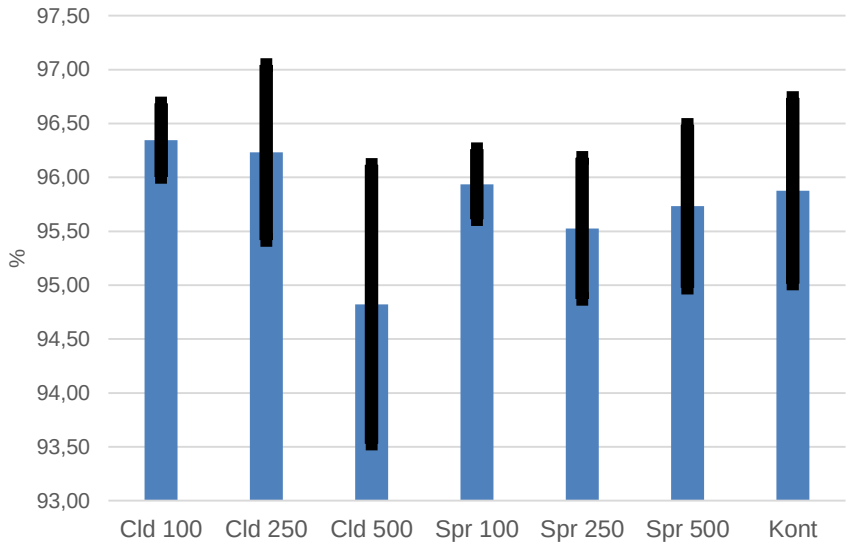

Figure 15. Effect of different dose applications on the moisture content of mushroom (%)

Mushroom pH values

The lowest value was found in Cld 500 group with 6.62 and the highest value was found in Spr 500 group with 7.31, which was 5% higher than the control group. All of the Spirulina sp. treatments were slightly higher than the control groups. It is thought that this may be due to the fact that Spirulina sp. grows in alkaline conditions with pH >10 and pH values are high. The pH result data obtained are given in Figure 4. 15. In a study [21], the pH measurements of the mushroom were measured between 6.76 (BM6) - 7.53 (BM3). These results are in parallel with the studies carried out by different researchers and they stated that different environments and applications may be effective on the pH of mushroom [17, 22, 26, 36].

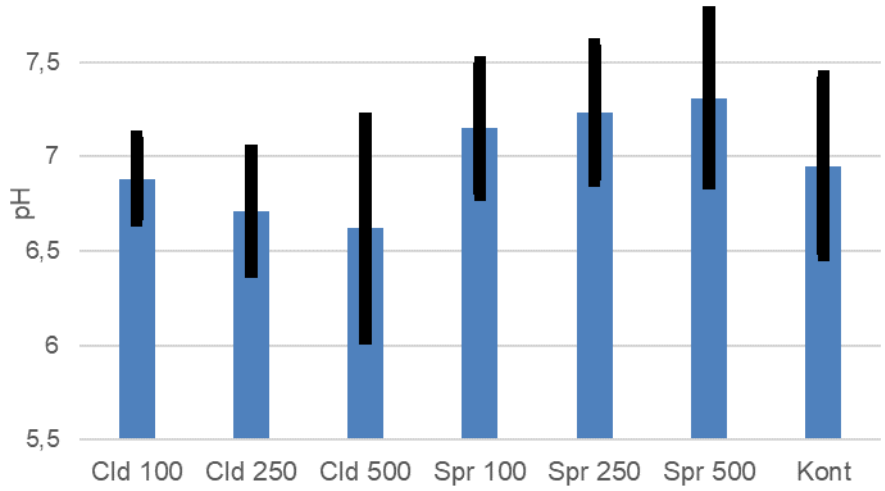

Figure 16. Effect of different dose applications on mushroom pH values

4. Conclusions and Recommendations

This study was conducted with three replicates using three different doses of *Cladophora glomerata* and *Arthrospira (Spirulina) platensis* algae (*C. glomerata* dry weight dose 10 g, 25 g and 50 g; *A. platensis* dry weight dose 5 g, 12.5 g and 25 g) in order to develop and determine the most suitable growing media for *Agaricus bisporus* species. Between 28.04.2021 and 10.07.2021, research trials were conducted in a private mushroom production enterprise in Korkuteli District of Antalya Province. The composts planted and inoculated in the seed mushroom mycelia of "Amycel Company" were obtained from "SMS Ersanlar Compost Company" in this district. During the research, some distinguishing characteristics of mushroom quality such as mushroom yield, average mushroom weight, mushroom cap and stem weights, mushroom cap diameter and height, mushroom stem diameter and length, total mushroom length, dry weight, ash weight and pH were analysed. It was observed that algae treatments to mushroom composts generally gave better results than the control group. Cld 250 and Spr 250 application doses increased the total mushroom yield by 7% and 15%, respectively.

In recent years, the demand for organic agricultural products has increased due to the negative effects of conventional agricultural techniques (pesticides, chemical fertilisers, antibiotics, hormones, etc.). These are biofertilisers of plant and animal origin. In addition, in recent years, various bacteria and algae, which have the effects of increasing plant growth, earliness and long harvest period, have started to be used. Many researchers are using macroalgae, which are abundant and cheap in nature, and a large number of bacteria and algae species and varieties isolated from different sources, inexpensively reproduced or cultivated with advanced techniques and used as biofertilisers at appropriate doses.

Soil microorganisms, especially bacteria, are the main component of mushroom compost, some of which have the ability to increase mushroom yield and quality [1]. Not only in Agaricus bisporus, but also in other mushrooms such as *Pleurotus sp.*, the addition of bacterial culture to the mushroom growing medium made the mushroom mycelia work faster [1]. These effective bacteria produce specific metabolites that can initiate sporophore formation. Phytohormones such as indole 3-acetic acid are the most important factors proposed for the growth-stimulating effect of these microorganisms. Cyanobacteria or blue-green algae (BGA) are another group of soil microorganisms that can influence the mycelial growth of fungi. Cyanobacteria produce a wide range of secondary metabolites, including antibiotics, algicides, toxins, pharmaceuticals and plant growth regulators. Among the growth regulators, giberellin, auxin, cytokinin, ethylene, abscisic acid and jasmonic acid have been identified in cyanobacteria [7- 11, 17].

In this study, the effects of macroalgae and microalgae which can be used as organic and biofertilisers on mushroom yield and quality were investigated. It was found that mycelial growth in composts treated with organic fertiliser, bacteria and algae occurred in a shorter time, covered the compost more quickly, harvesting could be done earlier and positive effects in terms of yield increase were determined. Organic and biofertiliser added compost formulas and recipes can be easily used by producers for mushroom cultivation due to these features.

In terms of the main characteristics and criteria used in the quality of mushrooms, very important effects of these applications were observed. Positive effects of biofertilisers on compost formulas and recipes were determined in terms of quality parameters such as hat, stem and whole weight of mushroom, whole length of mushroom, hat length, hat diameter, and stem length weight.

In nature or in our environment, many algae and some bacterial groups (Plant Growth Promoting Rhizobacteria or PGPRs, Cyanobacteria -are photosynthetic bacterias-, etc.) can be used as biofertilizers. In this study, only two algae species were used. Considering that there may be algae and bacterial species that will have much more effects, we think that more

research and studies should be carried out in this field and subject. Especially in the case that these biofertilisers to be obtained may be expensive, and the properties of other cheap materials that can be found are not very good, the use of these biofertilisers even at a very small rate and amount may give much better results. We think that more research, trials and studies should be carried out on these issues and we propose them to all researchers.

Conflict of Interest

The authors have no conflicts of interest to declare.

Ethics Committee Approval

Not applicable

Author Contribution

All authors have read and agreed to the published version of manuscript.

Funding

This study was supported by Harran University Scientific Research Projects Commission (HUBAP) with project number 21082.

Acknowledgements

We would like to thank SMS ERSANLAR company for providing the composts and Harran University Scientific Research Projects Commission (HUBAP) for supporting/financing our study and thesis with project number 21082.

5. References

[1] Sassine, Y.N. (Ed.). (2021). Mushrooms: Agaricus Bisporus 37. CABI.
[2] Faostat-Crops, F.A.O., (2020). http://www.fao.org/faostat/en/#data/QC/visualize Erişim tarihi 01.03.2023
[3] B. A. K. A. (2021). Antalya Korkuteli Mevcut Durum Analizi 2021, https://baka.ka.gov.tr/assets/upload/dosyalar/antalya-korkuteli-mevcut-durum-analizi.pdf Erişim Tarihi 01.03.2023
[4] TUİK, (2022). Bitkisel Üretim İstatistikleri 2021 https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2021-37249 Erişim Tarihi 01.03.2023
[5] Ebadi, A., Alikhani, H.A. & Rashtbari, M. (2012). Effect of plant growth promoting bacteria (PGPR) on the morpho-physiological properties of button mushroom Agaricus bisporus in two different culturing beds. International Research Journal of Applied and Basic Sciences. 3(1), 203-2012.
[6] Kim, M.K., Math, R.K., Cho, K.M., Shin, K.J., Kim, J.O., Ryu, J.S., Lee, Y.H. & Yun, H.D. (2008). Effect of Pseudomonas sp. P7014 on the growth of edible mushroom Pleurotus eryngii in bottle culture for commercial production. Bioresources Technology. 99(8), 3306-8.
[7] Gupta A. & Agarwal P. R. (1973). Extraction, Isolation, and Bioassay of a Gibberellin-like Substance from Phormidium foveolarum. Annals of Botany, 37(4), 737-741.
[8] Stirk, W. A. & Van Staden, J. (1996). Comparison of cytokinin-and auxin-like activity in some commercially used seaweed extracts. Journal of Applied Phycology, 8, 503-508.
[9] Ordog, V. & Pulz, O. (1996). Biologically active compounds from microalgae. Enzyme Microbia Technology. 8, 386-394.
[10] Manickavelu, A., Nadarajan, N., Ganesh, S.K., Ramalingam, R., Raguraman, S. & Gnanamalar, R.P. (2006). Organogenesis induction in rice callus by cyanobacterial extracellular product. African Journal of Biotechnology. 5 (5), 437-439.
[11] Seyed Hashtroudi, M., Ghassempour, A.R., Riahi H, Shariatmadari, Z. & Khanjir, M. (2012). Endogenous auxins in plant growth promoting cyanobacteria Anabaena vaginicola and Nostoc calcicola. Journal of Appllied Phycology.
[12] Schlösser, U.G. (1982). Sammlung von algenkulturen. Berichte der Deutschen Botanischen Gesellschaft, 95(1), 181-276.
[13] Falandysz, J., Kunito, T., Kubota, R., Gucia, M., Mazur, A., Falandysz, J. J., & Tanabe, S. (2008). Some mineral constituents of Parasol Mushroom (Macrolepiota procera). Journal of Environmental Science and Health, Part B, 43(2), 187-192.
[14] Krüger, D., Hughes, K. W., & Petersen, R. H. (2004). The tropical Polyporus tricholoma (Polyporaceae)—taxonomy, phylogeny, and the development of methods to detect cryptic species. Mycological Progress, 3(1), 65-79.
[15] Riahi, H., Shariatmadari, Z., Khangir, M., & Seyed Hashtroudi, M. (2017). Cyanobacterial culture as a liquid supplement for white button mushroom (Agaricus bisporus). Journal of Phycological Research, 1(1), 38-46.
[16] Khalili, H.R., Olfati, J. A. & Fallah, A. (2013). Plant growth promoting rhizobacteria affect button mushroom yield and quality. South Western J. Hortic. Biol. Environ, 4, 83-89.

[17] Ekinci, M. & Dursun, A. (2014). The effects of compost added bacteria, organic fertilizer and their mixtures on yield and quality of mushroom (Agaricus bisporus). CR Acad. Bulg. Sci, 67, 1441-1450.
[18] Ahlawat, O.P. & Manikandan, K. (2015). Evaluation of bacterial inoculants for yield enhancement of white button mushroom, agaricus bisporus under commercial scale cultivation. Mushroom Res. 24, 95-104
[19] Lotfi, M., Farsi, M., Mirshamsi, Kakhki, A. & Janpoorm, J. (2018). Influence of Pseudomonas putida isolateson the yield of edible white button mushroom Agaricus bisporus. Journal of Horticultural Science, 2, 273-286.
[20] Mosa, İ.A S. (2019). Farklı kayın mantarı misellerine manyetik alan ugulaması, farklı kompost çeşitlerinde yetişen mantarların verimi ve bazı fiziko-kimyasalların verimi ve bazı fiziko-kimyasal özelliklerinin belirlenmesi (Doctoral dissertation, Kastamonu Üniversitesi).
[21] Basim, V. (2022). "Yararlı bakteri uygulamalarının Agaricus bisporus yetiştiriciliğinde verim ve kaliteye etkileri" Akdeniz Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Ana Bilim Dalı, Yüksek Lisans Tezi
[22] Uzun, A. (1996). Karadeniz Bölgesinde kültür mantarı (Agaricus bisporus (Lange) Sing.) üretiminde kullanılabilecek organik materyallerin tespiti ile bunların mantarın verim ve kalitesine etkisi üzerine bir araştırma. Doktora Tezi, Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Samsun, 216 s.
[23] Aksu, Ş. & Işik, E. (2004). Besin Çözeltisinde Geliştirilen Mantar (Agaricus bisporus) Miselinin Dane Misel Üretiminde Anakültür Olarak Kullanılması ve Elde Edilen Misellerin Mantarın Verim ve Kalitesine Etkisi Üzerine Bir Araştırma. Türkiye VII. Yemeklik Mantar Kongresi, 22-24 Eylül, Korkuteli- Antalya, 43-48.
[24] Yücel, N.M., Taşkin, H. & Baktemur, G. (2010). Mantar yetiştiriciliğinde üre ve demir uygulamalarının verim ve kaliteye etkisi. VIII. Sebze Tarımı Sempozyumu.23- 26 Haziran, Van, 406-408
[25] Paksoy, M., Seymen, M. & Türkmen. Ö. (2010). Plastik kaplarda farklı kompost kalınlıklarının kültür mantarında verim ve kalite özelliklerine etkisi VIII. Sebze Tarımı Sempozyumu, 23-26 Haziran 2010, Van, 409-415.
[26] Günay, A. & Uzun, A. (1996). Buğday sapı ve tawk gübresinin esas alındığı değişik yetiştirme ortamlarının mantarın (Agaricus bisporus) verim ve kalitesine etkileri. Türkiye 5. Yemeklik Mantar Kongresi, 5-7 Kasım, Yalova, 143-153.
[27] Özşimşir, S. & Arin, L. (1996). Farklı örtü toprağı karışımlarmın mantar (Agaricus bisporus) verim, erkencilik ve kalitesine etkisi. Türkiye 5. Yemeklik Mantar Kongresi, 5-7 Kasım, Yalova, 220-225.
[28] Straatsma, G., Olijnsma, T.W., Gerrits, J.P.G., Amsing, J.G.M., Op Den Camp, H.J.O. & Van Griensven, L.J.L.D. (1994). Inoculation of Scytalidium thermophilum in Buton Mushroom Compost and Its Effect on Yield. Applied and Environmental Microbiology, 60(9), 3049-3054.
[29] Han, J. (1999). The influence of photosyntetic bacteria treatments on crop yield, dry matter content, and protein content of Mushroom Agaricus bisporus. Scientia Horticulturae 82,171-178.
[30] Zarenejad, F.; Yaklichali, B. & Rasooli, L. (2012). Evaluation of indigenous potent mushroom growth promoting bacteria (MGPB) on Agaricus bisporus production. World Journal of Microbiology and Biotechnology, 28, 99-104.
[31] Young, L.S., Chu, J.N., Hameed, A. & Young, C.C. (2013). Cultivable mushroom growth-promoting bacteria and their impact on Agaricus blazei productivity. Pesquisa Agropecuâria Brasileira, 48(6), 636-644.
[32] Çetin, M., Özaktan, H. & Boztok, K., (2016). Örtü Toprağında Bulunan Bazı Yararlı Bakterilerin Kültür Mantarı Agaricus bisporus"un Gelişimi ve Verimi Üzerine Etkileri. Türk Tarım - Gıda Bilim ve Teknoloji Dergisi, 4(3), 197-203.
[33] Günay, A., (2005). Sebze Yetiştiriciliği, Meta Basunevi Cilt II. İzmir, 425-531.
[34] Pekşen, A. & Günay, A. (2009). Kültür mantarı (Agaricıis bisporus L. Sing.) yetiştiriciliğinde çay atığı ve buğday sapı karışımından hazırlanan kompostların kullanımı. Ekoloji, 19(73), 48-54.
[35] Mattila, P., Könkö, K., Eurola, M., Pihlava, J. M., Astola, J., Vahteristo, L., ... & Piironen, V. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. Journal of agricultural and food chemistry, 49(5), 2343-2348.
[36] Dursun, A., Zülkadir, A., Alan, R. & Aydin, A. (1996). Farklı Torba Hacminin Kültür Mantarında (Agaricus bisporus) Verim ve Kalite Unsurlarına Etkisi. Türkiye 5. Yemeklik Mantar Kongresi, 5-7 Kasım. Yalova, 233-239.