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RESEARCH ARTICLE

Effects of Proline Applications on Plant Growth and Enzyme Activities in Forage Pea (*Pisum sativum* ssp. *arvense* L.) under Different Water Limit Conditions

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ABSTRACT

This study was conducted in 2024 in the greenhouses of Atatürk University plant production center in order to determine the effects of proline applications during the seedling period on plant development and some physiological and biochemical properties in forage pea grown under drought stress. The research was conducted in the form of a pot experiment with 3 irrigation levels [full irrigation (%100) (d0), 70% of field capacity (d1) and 40% of field capacity (d2)] and four proline applications (0, 5, 10, 20 mM) in 3 replications according to the completely randomized design. At the end of the experimental period, plant development parameters and some physiological and biochemical measurements and analyses were made in forage pea plants and the differences between the applications were evaluated. According to the research findings, significant differences emerged between the applications and levels. The effect of proline applications on plant development (plant height, stem diameter, fresh, dry weight, etc.) and some plant physiological and biochemical parameters [tissue electrical conductivity (mp), tissue relative water content (rwc), hydrogen peroxide (H_2O_2), malondialdehyde (mda), proline] was significant. At the end of the study, it was determined that drought conditions negatively affected plant development and decreased rwc and stomatal conductance. However, proline application improved plant development in forage pea under drought conditions and decreased rwc content compared to the control. As a result; it can be said that proline application affected the plant more positively in non-drought conditions.

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1. Introduction

Peas (*Pisum sativum* L.) are an important agricultural crop with a history dating back approximately 9,000 years, alongside wheat and barley (McPhee, 2003). They were first cultivated in Western Asia and have been widely grown in Europe for thousands of years. The subspecies *Pisum sativum* ssp. *sativum* is used for culinary purposes, while *Pisum sativum* ssp. *arvense* is used as forage. In Türkiye, forage pea (*Pisum arvense* L.) is grown as a spring crop in cold regions and as a winter crop in temperate regions, serving as both roughage and grain feed. In the Eastern Anatolia Region, it is primarily cultivated for seed and used as grain feed. The crude protein content of forage pea

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hay ranges between 16-18%, with a dry hay yield of 700-800 kg/ha (Tan et al., 2013).

Forage pea is a significant crop for both roughage and grain feed production due to its non-toxic nature, high fodder and seed yield, adaptability to various climate and soil conditions, and nitrogen-fixing ability that enriches the soil for subsequent crops. However, in places with a vegetation period such as Erzurum, grain feed crops that meet the energy needs of animals are largely limited to barley and vetch. Despite these advantages, the most significant factors negatively affecting the yield of forage pea are abiotic stresses such as drought, salinity and cold.

Abiotic stresses (drought, salinity, cold) are known to cause up to 50% yield losses in agricultural products globally. Approximately 26% of arable land is adversely affected by drought stress, 20% by mineral stress, and 15% by cold-frost stress (Blum & Jordan, 1985; Erdoğan Bayram, 2018). Drought is one of the most critical factors limiting plant production on a large portion of global agricultural land. Increasing population and global warming have rapidly depleted surface and groundwater resources. The lack of sufficient quality water has led to the use of low-quality water in agriculture, affecting soil structure negatively and causing issues such as salinization (Kutlar Yaylalı & Çiftçi, 2008).

Plants can be exposed to drought at different periods from germination to harvest. However, plants growing in arid environments throughout the entire development period are quite small in volume compared to plants growing in environments where water is not limited (F. Liu & Stützel, 2004; Tiryaki, 2016). The effects of drought stress on plants are classified at physiological, biochemical and molecular levels (Blum & Jordan, 1985). The first response of plants to water deficiency is to slow down cell growth (Taiz et al., 2015). The decrease in turgor pressure disrupts the water balance between plant tissues and negatively affects the amount of chlorophyll due to damage to photosynthetic pigments (Levitt, 1980). In addition, it has been reported that plant root structure elongates and top organs do not develop in water deficiency (Özel et al., 2016). In recent years, researchers have turned to various exogenous applications to alleviate the negative effects of drought on plants and to develop drought-resistant varieties (He et al., 2009). One of these applications is the use of amino acids. In addition to nourishing plants, amino acids improve biochemical processes and act as phytoregulators. This dual role makes them particularly valuable in stress reduction strategies. When plants encounter osmotic stresses such as water deficit, salinity, extreme temperatures, or heavy metal exposure, they exhibit initial physiological responses such as proline accumulation in cell vacuoles. Increased proline levels act as an osmoprotectant, stabilizing cellular structures and protecting enzymes under stress conditions. This increase in proline concentration within the cell is a critical indicator of how well the plant can tolerate stress and triggers a series of metabolic reactions that initiate the plant's defense mechanisms (Ünal, 2019). Numerous studies have shown that exogenous proline application significantly promotes plant growth under drought stress. Semida et al. (2020) reported that proline application significantly increased the growth and physiological performance of plants under water deficit conditions. Similarly, Kayak et al. (2022) observed that foliar application of proline improved drought tolerance by increasing osmotic regulation and reducing oxidative damage. Yamada et al. (2005) and Moustakas et al. (2011) also emphasized that proline supplementation provided better stress adaptation and recovery, and increased plant resistance. In addition, Ghaffari et al. (2019) found that proline application alleviated the negative effects of drought by increasing water holding capacity and maintaining higher photosynthesis rates. The aim of this study was to evaluate the effect of foliar application of proline on forage pea (*Pisum sativum*) grown under water-deficit conditions in greenhouse conditions. The study aimed to reveal the potential benefits of proline application in increasing drought tolerance in forage pea by evaluating various growth parameters, physiological traits and biochemical responses.

2. Materials and Methods

The study was conducted in pots in the greenhouses of Atatürk University Plant Production Application and Research Center. Taşkent forage pea variety was used as plant material in the study.

2.1. Establishment of the Experiment

The seedling study was conducted in a temperature-controlled greenhouse (45-50% humidity and 25±2°C). The seeds were planted in 2-liter pots filled with garden soil, peat, and sand mixture. Five seeds were sown in each pot at a depth of 1-1.5 cm. Standard fertilization was performed during planting with 3-5 kg/da N and 6-12 kg P2O5 kg/da (Tan, 2018). While calculating the fertilizer, it was assumed that there was 250 tons of soil in one decare area, the amount of fertilizer per pot (2 liters) was determined and applied by dissolving it in water (Bayhan et al., 2022). After seedling formation, thinning was performed so that 4 plants with a homogeneous appearance remained in each pot. Proline and water restriction applications were started 11 and 16 days after seed planting (three-leaf seedling period), respectively.

Throughout the experiment, the ambient temperature was kept constant at 25-30°C and irrigation was applied according to the amount of evaporation in the environment. In the experiment, three different irrigation subjects (completing the amount of evaporated water obtained from the evaporation pan (mm) to 100% (control), 70% and 40% of the usable water holding capacity depending on the relationship with the pot volume) were created. Before the experiment, the pot capacity (field capacity) and the usable water holding capacity (WWC) of each pot were determined accordingly (Çamoğlu, 2013). The pots were brought to pot capacity with planting. In the later stages of the pots that were brought to field capacity, water consumption was calculated according to evaporation and the study was carried out by restricting 100% water to the control group and 70-40% to the other pot groups. The water to be given in the study was Atatürk University drinking water and it was stated that it was suitable for irrigation. The trial was carried out with 4 proline applications (0 (P0), 5 (P1), 10 (P2), 20 mM (P3)), 3 water restriction applications (100% (D0), 70% (D1), 40% (D2)), 3 replications and 3 pots from each replication, in total 108 pots (4*3*3*3=108) (Table 1).

Table 1. Trial groups created in the study.

Total number of pots	Proline applications	Water restriction applications	Groups
108 pots	0 mM proline (P0)	100% (D0)	P0D0 (9 pots)
		70% (D1)	P0D1 (9 pots)
		40% (D2)	P0D2 (9 pots)
	5 mM proline (P1)	100% (D0)	P1D0 (9 pots)
		70% (D1)	P1D1 (9 pots)
		40% (D2)	P1D2 (9 pots)
	10 mM proline (P2)	100% (D0)	P2D0 (9 pots)
		70% (D1)	P2D1 (9 pots)
		40% (D2)	P2D2 (9 pots)
	20 mM proline (P3)	100% (D0)	P3D0 (9 pots)
		70% (D1)	P3D1 (9 pots)
		40% (D2)	P3D2 (9 pots)

Approximately 30 days after the trial was established, various parameters were examined from the seedlings in the greenhouse and in the laboratory. Measurements of each parameter were performed on 5 plants.

Seedling length (cm): In cm with a ruler. stem diameter (mm): In mm with a caliper. Plant fresh weight (g/plant): In grams (g) on a precision scale. Plant dry weight (g/plant): In grams (g) by drying in an oven at 68 0C until it reaches a constant weight. Root fresh weight (g/plant): In grams (g) on a precision scale. Root dry weight (g/plant): In grams by drying in an oven at 68 °C until it reaches a constant weight. Number of leaves (number/plant): Counted as pieces. Stomatal conductance (m^2s/mol): Stomatal conductance in the leaf was measured with a leaf porometer device at 10:00-11:00 hours while the plants were in pots and determined as m^2s/mol. Measurements were taken 3 days after the plants were irrigated in three different periods.

Leaf area ($cm^2/plant$): Leaf areas of the plants in each application were determined using a leaf area meter (LICOR, Model: LI-3100, Lincoln, NE, USA).

Chlorophyll content (SPAD): Chlorophyll content in plant leaves was measured with a chlorophyll meter (SPAD–502, Konica Minolta Sensing, Inc., Japan).

2.2. Tissue Electrical Conductivity (MP)

An indication of the damage caused by stress in the leaf tissue and especially in the cell membranes is the electrical conductivity measurements made on the fresh leaf tissues. For this purpose, the disks (1 cm in diameter) taken from the last developed real leaves of 2 randomly selected plants from each replication were placed in glass bottles containing 20 ml of distilled water and shaken in a shaker for 24 hours, and then the electrical conductivity of the soaking water was measured according to the method specified in Kaya et al. (2003) and the permeability (damage rate) of the cell membranes was determined (EC1). The samples were kept in an autoclave at 121°C for 20 minutes to ensure complete lysis of the cells and tissues, and then the second measurement was made (EC2). The ratio between EC1/EC2 and the relative electrical conductivity values were calculated.

2.3. Tissue Relative Water Content (RWC)

Leaf discs (1 cm in diameter) taken from 2 plants randomly selected among the surviving plants were weighed immediately and their fresh weights were determined (FW). After weighing, the discs were placed in petri dishes containing some distilled water and kept for 5 hours, then the excess water on the discs was wiped off with the help of blotting paper and weighed again and their turgor weights were determined (TW). Then, these discs were placed in petri dishes and dried in an oven set at 72°C for 48 hours and weighed again and their dry weights were determined (DW). Tissue water content was calculated according to the following formula stated in Kaya et al. (2003):

$$RWC = [(FW - DW) / (TW - DW)] \times 100 \quad (1)$$

Hydrogen Peroxide (H_2O_2): It was determined based on the method given in Özden et al. (2009). Lipid Peroxidation (Malondialdehyde-MDA): It was determined based on the method given in S. Liu et al. (2014). Catalase (CAT - EC:

1.11.1.6), Peroxidase (POD - EC: 1.11.1.7), Superoxide dismutase (SOD – EC: 1.15.1.1) enzyme activities were determined based on the method given in S. Liu et al. (2014).

2.4. Statistical Analysis

In the experiment, a completely randomized design was used. The data were subjected to analysis of variance (ANOVA) using the SPSS 20 software and means were separated by Duncan's multiple comparison test.

3. Results and Discussion

In this study, which was conducted to observe the effects of proline applications against drought stress in forage pea, the differences in plant height (cm), stem diameter (mm), plant fresh and dry weight (g), root fresh and dry weight (g) are presented in Table 2. Plant heights decreased with increased water restriction levels in all applications. In each application, the control group irrigation (D0) had the highest plant height. Proline applications generally affected plant height positively. P2 application gave better results than other applications even under water deficit conditions (D1, D2). The effects of the applications on stem diameter showed statistical differences. While stem diameters of control applications varied between 0.88-0.99 mm, proline applications varied between 0.77-1.29 mm. The highest stem diameter was obtained from P3D0 application. When the aboveground fresh mass results in forage pea were examined, it was seen that the highest values were obtained from full irrigation applications (D0). It is known that yield decreases under water deficit conditions. Accordingly, it is estimated that water scarcity is responsible for 17-70% of production losses (Ahmad et al., 2022). Among proline applications, P2D0 had the highest fresh mass value (4.16 g). In D1 applications, P1 and P2 applications reached higher fresh weight than the control (P0D1). In D2 values, which is the highest water restriction application (40%), all proline applications (P1, P2, P3) had higher fresh mass compared to the control applications. Butt et al. (2016) applied proline externally to plants under abiotic stress in their study (0.4 mM, 0.6 mM, 0.8 mM, 1 mM and 1.2 mM) and found that 0.8 mM proline concentration had the best effect and provided biomass increase. In fact, in our study, the best results were obtained from the proline application at a dose of 10 mM (P2). Plant dry weight decreased in each application depending on the decrease in water content. The highest plant dry matter weights were obtained from P2 applications among proline applications. While root fresh weights varied between 1.40-2.15 g in control applications, they varied between 0.70-1.44 g in proline applications. The first parts affected by water restriction in the plant are fresh and dry weights (Shao et al., 2008). When a significant water loss occurs from plant cells, the decrease in turgor pressure, which is the driving force for growth, and the negative effects on transpiration cause a decrease in mineral uptake, a decrease in photosynthesis and a decrease in growth rate (Capell et al., 2004; Eriş, 1990; McKersie & Leshem, 1994). With drought, the uptake of nutrients (Garg, 2003), mineralization (Bloem et al., 1992), transportation and the availability of nutrients on the root surface decrease. In addition, photosynthesis slows down in dry conditions and as a result, shoot development is weakened and root development is accelerated (Öztürk & Seçmen, 1992). In our study, it can be said that the root development of the control group was generally better compared to proline applications (Table 2). Studies have shown that in unstressed Arabidopsis seedlings, external proline supplementation at micromolar concentrations induced root elongation and branching, but when external proline was given at millimolar concentrations, root growth was inhibited with symptoms resembling cell death (Hellmann et al., 2000; Mattioli et al., 2009).

Table 2. Effect of applications on plant development in forage pea (*Pisum sativum* ssp. *arvense* L.).

Applications	**Seedling height (cm)**	**Stem diameter (mm)**	**Plant fresh weight (g)**	**Plant dry weight (g)**	**Root fresh weight (g)**	**Root dry weight (g)**
Proline (P)						
P0	31.67 b	0.94 a	2.49 a	0.36 a	1.60 a	0.51 a
P1	34.56 ab	0.96 a	2.62 a	0.34 a	1.24 b	0.31 b
P2	40.33 a	0.96 a	2.94 a	0.38 a	1.22 b	0.31 b
P3	35.00 ab	1.02 a	2.48 a	0.30 a	0.93 b	0.22 b
Water restriction (D)						
D0	40.92 a	1.12 a	3.58 a	0.45 a	1.50 a	0.41 a
D1	37.00 b	0.96 b	2.68 b	0.35 b	1.08 b	0.25 b
D2	28.25 c	0.83 c	1.64 c	0.25 c	1.16 b	0.35 ab
P x D						
P0D0	35.67 c	0.99 cd	3.43 abc	0.45 ab	2.15 a	0.72 a
P0D1	35.67 c	0.95 cde	2.55 cd	0.39 b	1.25 bc	0.29 cd

Table 2. (continued)

Applications	Seedling height (cm)	Stem diameter (mm)	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)
P0D2	23.67 e	0.88 cdef	1.50 e	0.24 d	1.40 b	0.51 b
P1D0	38.67 bc	1.04 bc	3.06 bc	0.38 bc	1.17 bc	0.28 cd
P1D1	35.33 c	1.00 cd	2.99 bc	0.37 bc	1.11 bcd	0.25 cd
P1D2	29.67 d	0.85 def	1.80 de	0.28 cd	1.44 b	0.40 bc
P2D0	49.33 a	1.18 ab	4.16 a	0.52 a	1.40 b	0.36 bcd
P2D1	41.67 b	0.90 cdef	3.13 bc	0.39 b	1.16 bc	0.28 cd
P2D2	30.00 d	0.81 ef	1.53 e	0.23 d	1.10 bcd	0.30 cd
P3D0	40.00 bc	1.29 a	3.66 ab	0.43 ab	1.28 b	0.29 cd
P3D1	35.33 c	1.00 cd	2.07 de	0.25 d	0.82 cd	0.18 d
P3D2	29.67 d	0.77 f	1.72 de	0.24 d	0.70 d	0.19 cd

Means marked with different letters are statistically different.

The number of leaves has an average of 18.25 pcs/plant in control applications. It was determined as 17.62 pcs/plant in P1 applications, 20.03 pcs/plant in P2 applications and 16.47 pcs/plant in P3 applications (Table 3). As in plant fresh weight, the number of leaves in P2 application was more effective than other applications. Chlorophyll content is an important indicator of the growth status of a plant (Pavlović et al., 2014). All applications except POD2, PID0, P2D2 were statistically in the same group. In general, the chlorophyll content increased as water restriction increased in all applications. The researchers attributed this increase to the decrease in the unit area of the leaf and the increase in leaf thickness (Küçükkömürcü, 2011). Under control conditions, the leaf area value was determined as 178.25-205.20 cm2/plant (P0D2-P0D0) (Table 3). In the irrigation regimes where proline applications affected the leaf area, the highest value was calculated as 211.37 cm^2/plant in P3D0, while the lowest value was calculated as 189.48 cm2/plant in P1D2 level. It is numerically seen that the average value of control plants (P0D0, P0D1, P0D2) is 191.97 cm^2/plant, and the average value of proline application is 200.90 cm^2/plant with high leaf area. It is seen that proline applications generally increase the leaf area compared to control and have a positive effect. The best effect was obtained from P3 applications with the highest proline dose. RWC measurement was performed to determine the effect of proline application on water status in the plant against drought stress (Table 3). Drought stress caused a statistical increase in RWC values in the plant. It was determined that P2 application among proline applications regulates plant water status better than other groups. The fact that RWC values are consistent in P2 applications, as in plant root fresh and dry weights, shows that proline regulates the development of response to drought stress at a certain concentration (10 mM). This shows that the healing effect of proline varies according to the concentration. Electrical conductivity values increased with increasing stress in all applications (Table 3). Membrane permeability, which is considered as an indicator of damage occurring in plant cells under drought conditions, is measured as EC and is expressed as an ion imbalance that develops due to intracellular and extracellular osmotic incompatibility, especially in plants under salt and water stress (Kuşvuran, 2010). Ors et al. (2016) found that water restriction applied during the seedling period in squash increased EC in the plant. Stomatal conductance decreased with increasing stress in the control group. An increase was observed in the proline group. Studies have reported that stomatal conductance decreased under stress conditions (Yıldız, 2017). Stomatal conductance in proline applications increased with increased application dose. This can be attributed to the effect of proline in reducing plant stress.

Table 3. Effect of applications on leaf number, chlorophyll value (SPAD), leaf area, tissue proportional water content, electrical conductivity, stomatal conductivity in forage pea (*Pisum sativum* ssp. *arvense* L.).

Applications	**Number of leaves (pcs/plant)**	**Chlorophyll SPAD**	**Leaf area (cm^2/plant)**	**RWC (%)**	**MP**	**Stoma**
Proline (P)						
P0	18.25 a	35.10 a	191.98 b	79.18 a	38.52 a	21.52 a
P1	17.62 a	32.63 a	193.60 b	68.95 a	47.51 a	19.92 b
P2	20.03 a	34.22 a	201.53 ab	71.60 a	42.63 a	22.30 b
P3	16.48 a	34.65 a	207.59 a	69.87 a	38.97 a	27.69 b
Water restriction (D)						
D0	19.47 a	32.76 b	203.75 a	0.51 a	31.97 b	21.82 a
D1	20.38 a	34.21 ab	201.30 a	0.65 b	38.47 b	23.16 a
D2	14.44 b	35.48 a	190.97 b	0.67 c	55.27 a	23.60 a
P x D						
P0D0	19.55 abcd	33.66 ab	205.20 abc	86.37 a	32.45 cd	22.50 cd
P0D1	22.44 ab	34.73 ab	192.48 bcd	81.05 ab	36.44 cd	21.86 cd
P0D2	12.77 e	36.09 a	178.25 abcd	70.12 bcdef	46.66 abc	20.22 cd
P1D0	20.00 abcd	31.50 b	191.43 bcd	76.55 abcd	40.06 bcd	18.71 d
P1D1	17.77 abcde	33.40 ab	199.88 abc	67.17 cdef	43.19 abcd	20.14 cd
P1D2	15.11 cde	33.01 ab	189.48 cd	63.13 def	59.27 a	20.91 cd
P2D0	20.55 abc	32.13 ab	207.02 ab	83.34 ab	29.29 cd	21.42 cd
P2D1	23.77 a	33.57 ab	202.57 abc	70.80 bcdef	42.22 abcd	22.16 cd
P2D2	15.77 cde	36.97 a	195.02 abc	60.67 ef	56.38 ab	23.34 cd
P3D0	17.77 abcde	33.76 ab	211.37 a	77.83 abc	26.10 d	24.64 bc
P3D1	17.55 bcde	35.14 ab	210.28 a	73.90 abcde	32.04 cd	28.49 ab

Means marked with different letters are statistically different.

Proline has a role that can be naturally synthesized in the plant and its amount can change according to the stress experienced by the plant. In the scope of the study, 3 different doses (5 mM, 10 mM and 20 mM) were selected in order to determine the best proline dose to be applied to the leaves. The selected doses were applied to the leaves in the form of external pulverization in forage peas grown under full and restricted irrigation conditions. At the end of the seedling development period, Catalase (CAT), Superoxide dismutase (SOD), peroxidase (POD), H_2O_2 (mmol/kg), MDA (nmol/g) values in the plants were measured and the obtained data are given in Table 4. Restricted irrigation and proline applications did not play a decisive role in CAT antioxidant enzyme activity. All applications except P2D1, P3D2 applications were statistically included in the same group. When the POD values were examined, it was seen that the control applications had the lowest values among all applications. The highest POD values were determined in P3 applications. SOD antioxidant enzyme was positively affected by external applications and decreased in all proline applications compared to the control under water limited conditions (D1, D2). All proline D1 applications (P1D1, P2D1, P3D1) were found to be lower than the control D1 applications (P0D1). This situation is valid for D2 and D3 applications. Anjum et al. (2012) applied a total of 4 different water stress subjects as 80%, 60%, 40% and 20% in their study to determine the physiological responses of two pepper (*Capsicum annuum* L.) varieties under drought stress. It has been reported that with the onset of drought conditions, the antioxidant enzyme activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) increased, then decreased with the severity of drought and reached lower levels than the relevant control levels. However, they stated that the growth, development and yield values of the variety with high activities of SOD, POD and CAT were higher than the other variety. As a result of the study, they stated that due to the presence of high antioxidant enzymes, reduced lipid peroxidation, better osmolyte accumulation and preservation of tissue water content in the plant, better growth and yield were recorded and drought resistance was increased. In many studies, it has been reported that the working principle of enzyme activity depends on a combination of parameters such as the type of stress conditions, its function in the plant and the plant species (Bhaduri & Fulekar, 2012; Malecka et al., 2001; Shah et al., 2001). In our study, H_2O_2 level in the control group decreased in D1 application and increased again with increasing drought at D2 level. This is the highest value among all applications (16.84 mmol/kg). In proline applications, it increased or remained the same in D1 applications from D0 process and decreased in D2 applications. However, all applications except P0D2, P3D2 applications were statistically in the same group and proline applications did not have a determining effect. When the study findings are examined, it was observed that MDA levels were the lowest in the control group. In proline applications, an increase was observed in MDA values due to the increase in water stress and proline dose. Similar studies have shown that MDA accumulation increases due to stress. It has been reported that drought stress increases the amount of MDA in wheat (Naveed et al., 2014).

Table 4. Effects of treatments on antioxidant enzyme activities (Prolin, CAT, POD, SOD, H_2O_2, MDA) in forage pea (*Pisum sativum* ssp. *arvense* L.).

Applications	**CAT-(EU/Gta)**	**POD-(EU/gTA)**	**SOD-(EU/Gta)**	**H_2O_2-(mmol/kg)**	**MDA- (nmol/g)**
Proline (P)					
P0	0.01 a	17.87 c	82.13 a	14.60 ab	0.55 a
P1	0.01 a	23.03 b	78.54 ab	15.86 a	0.58 a
P2	0.04 a	22.61 b	72.11 ab	14.11 ab	0.64 a
P3	-0.0356 a	30.52 a	70.45 b	11.02 b	0.67 a
Water restriction (D)					
D0	0.004 a	23.25 a	75.81 a	14.29 a	0.51 b
D1	0.04 a	21.21 a	77.50 a	14.17 a	0.65 a
D2	-0.02 a	26.06 a	74.11 a	13.23 a	0.67 a
P x D					
P0D0	0.013 ab	17.58 d	76.53 ab	14.37 ab	0.43 c
P0D1	0.016 ab	18.41 d	85.24 a	12.59 ab	0.67 abc
P0D2	0.003 ab	17.62 d	84.61 a	16.84 a	0.56 abc
P1D0	-0.020 ab	22.19 bcd	76.08 ab	16.08 ab	0.45 bc
P1D1	0.006 ab	22.04 cd	81.40 a	16.10 ab	0.62 abc
P1D2	0.046 ab	24.85 bc	78.14 ab	15.42 ab	0.67 abc
P2D0	0.023 ab	25.04 bc	78.33 ab	14.83 ab	0.58 abc
P2D1	0.110 a	17.59 d	77.42 ab	15.87 ab	0.63 abc
P2D2	0.006 ab	25.21 bc	60.59 b	11.65 ab	0.70 ab
P3D0	0.000 ab	28.21 b	72.29 ab	11.90 ab	0.57 abc
P3D1	0.030 ab	26.82 bc	65.96 ab	12.14 ab	0.68 abc
P3D2	0.136 b	36.54 a	73.10 ab	9.03 b	0.75a

Means marked with different letters are statistically different.

4. Conclusion

In the study investigating the effect of proline doses under different water constraint conditions, it was determined that the increase in drought negatively affected the plant in all applications. The positive effect of proline applications was detected more clearly in applications without water restriction (D0). Leaf number, leaf area, plant fresh weight are the parameters on which P2 doses are effective. In water-limited conditions, the best results were obtained from P2 dose and D1 combinations. According to the results obtained from our study, it can be said that proline applications can be an important strategy for plant growth and development in areas without water shortage. New studies are needed to test different water-limited conditions and proline doses.

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Conflict of Interest

The authors have no conflict of interest to declare.

References

Ahmad, H. M., Fiaz, S., Hafeez, S., Zahra, S., Shah, A.N., Gul, B., Aziz, O., Rahman, M. U., Fakhar, A., Rafique, M., Chen, Y., Yang, S. H., & Wang, X. (2022). Plant growth-promoting rhizobacteria eliminate the effect of drought stress in plants: A review. *Frontiers in Plant Science*, *13*, 875774. https://doi.org/10.3389/fpls.2022.875774

Anjum, S. A., Farooq, M., Xie, X. Y., Liu, X. J., & Ijaz, M. F. (2012). Antioxidant defense system and proline accumulation enables hot pepper to perform better under drought. *Scientia Horticulturae*, *140*, 66-73. https://doi.org/10.1016/j.scienta.2012.03.028

Bayhan, M., Özkan, R., Albayrak, Ö., Akıncı, C., & Yıldırım, M. (2022). Bazı arpa (*Hordeum vulgare* L.) genotipleri ile f1 melezlerinin kontrollü koşullarda değerlendirilmesi. *ISPEC Journal of Agricultural Sciences*, *6*(3), 563-572. https://doi.org/10.1007/BF00018060

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. https://doi.org/10.1007/s11157-011-9251-x

Bloem, J., De Ruiter, P. C., Koopman, G. J., Lebbink, G., & Brussaard, L. (1992). Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management. *Soil Biology and Biochemistry*, *24*(7), 655-665. https://doi.org/10.1016/0038-0717(92)90044-X

Blum, A., & Jordan, W. R. (1985). Breeding crop varieties for stress environments. *Critical Reviews in Plant Sciences*, *2*(3), 199-238. https://doi.org/10.1080/07352688509382196

Butt, M., Ayyub, C. M., Amjad, M., & Ahmad, R. (2016). Proline application enhances growth of chilli by improving physiological and biochemical attributes under salt stress. *Pakistan Journal of Agricultural Sciences*, *53*(1), 43-49. https://doi.org/10.21162/PAKJAS/16.4623

Çamoğlu, G. (2013). The effects of water stress on evapotranspiration and leaf temperatures of two olive (*Olea europaea* L.) cultivars. *Zemdirbyste-Agriculture*, *100*(1), 91-98. https://doi.org/10.13080/z-a.2013.100.012

Capell, T., Bassie, L., & Christou, P. (2004). Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. *PNAS*, *101*(26), 9909-9914. https://doi.org/10.1073/pnas.0306974101

Erdoğan Bayram, S. (2018). Su stresi ve bitkilerde su stresine bağlı fizyolojik değişimler. *Tralleis Elektronik Dergisi*, *3*(2), 219-228. (In Turkish)

Eriş, A. (1990). *Bahçe bitkileri fizyolojisi*. Uludağ Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Garg, B. K. (2003). Nutrient uptake and management under drought: Nutrient-moisture interaction. *Current Agriculture*, *27*(1/2), 1-8.

Ghaffari, H., Tadayon, M. R., & Nadeem, M., Cheema, M., & Razmjoo, J. (2019). Proline-mediated changes in antioxidant enzymatic activities and the physiology of sugar beet under drought stress. *Acta Physiologiae Plantarum*, *41*, 23. https://doi.org/10.1007/s11738-019-2815-z

He, L., Gao, Z., & Li, L. (2009). Pretreatment of seed with H_2O_2 enhances drought tolerance of wheat (*Triticum aestivum* L.) seedling. *African Journal of Biotechnology*, *8*(22), 6151-6157. https://doi.org/10.5897/AJB09.490

Hellmann, H., Funck, D., Rentsch, D., & Frommer, W. B. (2000). Hypersensitivity of an Arabidopsis sugar signaling mutant toward exogenous proline application. *Plant Physiology*, *123*(2), 779-789. https://doi.org/10.1104/pp.123.2.779

Kaya, C., Higgs, D., Ince, F., Amador, B. M., Cakir, A., & Sakar, E. (2003). Ameliorative effects of potassium phosphate on salt-stressed pepper and cucumber. *Journal of Plant Nutrition*, *26*(4), 807-820. https://doi.org/10.1081/PLN-120018566

Kayak, N., Kal, Ü., Dal, Y., Yavuz, D., & Seymen, M. (2022). Do proline and glycine betaine mitigate the adverse effects of water stress in spinach? *Gesunde Pflanzen*, *75*(1), 97-113. https://doi.org/10.1007/s10343-022-00675-6

Küçükkömürcü, S. (2011). *Tuzluluk ve kuraklık streslerine tolerans bakımından bamya genotiplerinin taranması* (Master's thesis, Çukurova University). (In Turkish)

Kuşvuran, Ş. (2010). *Kavunlarda kuraklık ve tuzluluğa toleransın fizyolojik mekanizmaları arasındaki bağlantılar* (Doctoral dissertation, Çukurova University). (In Turkish)

Kutlar Yaylalı, İ., & Çiftçi, N. (2008). *Tuzlu suların tarımda kullanımı ve domates yetiştiriciliğinde verime etkisi*. Konya Kapalı Havzası Yeraltısuyu ve Kuraklık Konferansı Bildiriler Kitabı. (In Turkish)

Levitt, J. (1980). *Responses of plants to environmental stresses. Volume II. Water, radiation, salt, and other stresses*. Academic Press.

Liu, F., & Stützel, H. (2004). Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Scientia Horticulturae*, *102*(1), 15-27. https://doi.org/10.1016/j.scienta.2003.11.014

Liu, S., Dong, Y., Xu, L., & Kong, J. (2014). Effects of foliarapplications of nitric oxide and salicylic acid on saltınduced changes in photosynthesis and antioxidative metabolism of cotton seedlings. *Plant Growth Regulation*, *73*(1), 67-78. https://doi.org/10.1007/s10725-013-9868-6

Malecka, A., Jarmuszkiewicz, W., & Tomaszewska, B. (2001). Antioxidative defense to lead stress in subcellular compartments of pea root cells. *Acta Biochimica Polonica*, *48*(3), 687-698.

Mattioli, R., Falasca, G., Sabatini, S., Altamura, M. M., Costantino, P., & Trovato, M. (2009) The proline biosynthetic genes *P5CS1* and *P5CS2* play overlapping roles in *Arabidopsis* flower transition but not in embryo development. *Physiol Plantarum*, *137*(1), 72-85. https://doi.org/10.1111/j.1399-3054.2009.01261.x

McKersie, B. D., & Leshem, Y. (1994). *Stress and stress coping in cultivated plants*. Kluwer Academic Publishers.

McPhee, K. (2003). Dry pea production and breeding. *Journal of Food Agriculture and Environment*, *1*(1), 64-69.

Moustakas, M., Sperdouli, I., Kouna, T., Antonopoulou, C. I., & Therios, I. (2011). Exogenous proline induces soluble sugar accumulation and alleviates drought stress effects on photosystem II functioning of *Arabidopsis thaliana* leaves. *Plant Growth Regulation*, *65*, 315-325. https://doi.org/10.1007/s10725-011-9604-z

Naveed, M., Hussain, M. B., Zahir, Z. A., Mitter, B., & Sessitsch, A. (2014). Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. *Plant Growth Regulation*, *73*, 121-131. https://doi.org/10.1007/s10725-013-9874-8

Ors, S., Ekinci, M., Yildirim, E., & Sahin, U. (2016). Changes in gas exchange capacity and selected physiological properties of squash seedlings (*Cucurbita pepo* L.) under well-watered and drought stress conditions. *Archives of Agronomy and Soil Science*, *62*(12), 1700-1710. https://doi.org/10.1080/03650340.2016.1168517

Özden, M., Demirel, U., & Kahraman, A. (2009). Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. *Scientia Horticulturae*, *119*(2), 163-168. https://doi.org/10.1016/j.scienta.2008.07.031

Özel, S. D., Gökkuş, A., & Alatürk, F. (2016). Farklı sulama seviyelerinin macar fiği (*Vicia pannonica* Crantz.) ve yem bezelyesinin (*Pisum arvense* L.) gelişimine etkileri. *Alinteri Journal of Agriculture Science*, *30*(1), 46-52. (In Turkish)

Öztürk, M. A., & Seçmen, Ö. (1992). *Bitki ekolojisi*. Ege Üniversitesi Fen Fakültesi Yayınları. (In Turkish)

Pavlovic, D., Nikolic, B., Djurović, S., Waisi, H., Anđelković, A., & Dragana, M. (2014). Chlorophyll as a measure of plant health: Agroecological aspects. *Pesticidi i Fitomedicina*, *29*(1), 21-34. https://doi.org/10.2298/PIF1401021P

Semida, W. M., Abdelkhalik, A., Rady, M. O., Marey, R. A., & Abd El-Mageed, T. A. (2020). Exogenously applied proline enhances growth and productivity of drought stressed onion by improving photosynthetic efficiency, water use efficiency and up-regulating osmoprotectants. *Scientia Horticulturae*, *272*, 109580. https://doi.org/10.1016/j.scienta.2020.109580

Shah, K., Kumar, R. G., Verma, S., & Dubey, R. S. (2001). Effect of cadmium on lipid peroxidation superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science*, *161*(6), 1135-1144. https://doi.org/10.1016/S0168-9452(01)00517-9

Shao, H. B., Chu, L. Y., Jaleel, C. A., & Zhao, C. X. (2008). Water-deficit stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies*, *331*(3), 215-225. https://doi.org/10.1016/j.crvi.2008.01.002

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development*. Sinauer Associates.

Tan, M. (2018). *Baklagil ve buğdaygil yem bitkileri*. Atatürk Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Tan, M., Koç, A., Dumlu Gül, Z., Elkoca, E., & Gül, İ. (2013). Determination of dry matter yield and yield components of local forage pea (*Pisum sativum ssp. arvense* L.) ecotypes. *Journal of Agricultural Sciences*, *19*(4), 289-296. https://doi.org/10.1501/Tarimbil_0000001254

Tiryaki, İ. (2016). Yoncada (*Medicago sativa* L.) kuraklık stresi ve tolerantlık mekanizması. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *19*(3), 296-304. (In Turkish)

Ünal, Z. (2019). *Bazı abiyotik streslerin prolinle desteklenen turuçgil anacı çöğürleri üzerine morfolojik, fizyolojik ve biyokimyasal etkileri* (Master's thesis, Akdeniz University). (In Turkish)

Yamada, M., Morishita, H., Urano, K., Shiozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K., & Yoshiba, Y. (2005). Effects of free proline accumulation in petunias under drought stress, *Journal of Experimental Botany*, *56*(417), 1975-1981, https://doi.org/10.1093/jxb/eri195

Yıldız, S. (2017). *Kuraklık ve tuz stresinin lahanada fide gelişimi ve bazı fizyolojik özellikler üzerine etkisi* (Master's thesis, Atatürk University). (In Turkish)

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RESEARCH ARTICLE

Prediction of Energy Balance Based on Milk Parameters Across Different Lactation Stages

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ABSTRACT

Following the changes observed in milk parameters throughout the lactation period will offer an easy and practical method for the evaluation of the feeding programs of animals as well as presenting opportunities to improve the quality of milk. For this purpose, the milk samples collected from 1390 Holstein cows Bursa Province in Turkey on the test day of the month (at an interval of 30 days) were examined and their fat, protein, dry matter and lactose contents were measured. The cows with boundary values for the fat/ protein accepted for the metabolic states of the cows in the study are considered to be at risk of acidosis if they are lower than 1.2, to be healthy if they are between 1.2 and 1.4 and to have a ketosis risk if the values are over 1.4. The results of the study have shown that the risk for acidosis among the animals are 39.5%, 32.4% and 33.9% respectively during the lactation periods 1, 2 and 3. The percentage of animals at risk of ketosis was determined to be 30.2%, 37.9%, 36.6% respectively. Energy balance is defined as the difference between energy intake from feed and energy required for animal performance. It is an important concept in cattle management and nutrition because it directly impacts the health, productivity, and reproductive success of cows, particularly during lactation. The percentages of the animals at the positive energy balance are 30.3, 29.7 and 29.6 respectively. Estimates for early, mid and late lactation show that in early lactation 31% of cows have acidosis, 37% have ketosis and 32% have positive energy balance, in mid lactation 31% have acidosis, 28% have ketosis and 32% have positive energy balance, while in late lactation 34% have acidosis, 34% have ketosis and 32% have positive energy balance.

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1. Introduction

In a nutritionally balanced dairy cattle ration, approximately seventy percent of the animal's protein requirement should be met by microbial protein produced as a result of the fermentation of nutrients in the feed in the rumen. Achieving this important task largely depends on the supply and adequate consumption of energy and protein in appropriate amounts and proportions in the ration. Reaching the optimum level of milk yield and composition in dairy cattle can only be achieved by ensuring good rumen fermentation. For this, the amount and degradability of energy and protein sources in the diet must be balanced to ensure the optimization of rumen functions (Beever, 1993). Metabolic disorders caused by nutrient deficiency or imbalance in the diet also cause changes in the chemical composition of milk. Therefore, changes in the protein and fat content of individual milk samples during the lactation period of cows are important parameters that directly affect the health, reproduction and productivity of the animals (Mäntysaari & Mäntysaari, 2010).

In the late lactation period, as the productivity of cows decreases, roughage can meet the energy and other nutritional

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needs of cows. In such cases, milk composition reflects forage quality. High-quality forages are rich in both energy and protein content and can provide milk yields of around 30 kg or more per day with little or no added nutrients. Negative energy balance, which occurs due to inadequate feeding of cows despite high milk yield in the early lactation period, is an important problem that reduces milk yield and threatens milk quality in the postpartum period. Monitoring the symptoms of subclinical ketosis is important in terms of milk quality, herd health and reproductive performance, and preventing economic losses in the enterprise (Arnould et al., 2013).

Both the fat and protein percentage of milk on the control day are significantly associated with the risk of subclinical ketosis, and it has been reported that high milk fat percentage and low milk protein percentage significantly increase the risk of subclinical ketosis. While an increase of 1% in milk fat increased the risk of subclinical ketosis by at least twofold, an increase of 1% in milk protein reduced the risk of subclinical ketosis by more than 50% (Duffield et al., 1997). In ketosis, which is a metabolic disorder frequently seen in early lactation, milk protein content generally decreases while milk fat content increases. However, in subclinical acidosis, milk protein content increases and milk fat content decreases (Pavlata et al., 2008). It has been reported that milk protein and especially casein levels depend on the increase in the level of starch (cereal) consumed in the diet, but this increase raises the risk of rumen acidosis (Beever, 2006; Yang & Beauchemin, 2007). Mackle et al. (2000) reported that increasing energy level in the diet affects milk protein, and this may be related to the increase in microbial protein synthesis in the rumen.

The amount of fat and protein in milk is an effective parameter for monitoring the efficiency of the ration. Changes in the concentrations or mutual ratios of these two milk components in milk can give important clues about changes or problems in health as well as nutritional deficiency or balance in the ration (Lean & Golder, 2024; Nelson & Redlus, 1989; Rathwell, 1990). This study aimed to evaluate the milk fat/milk protein ratio of cows at various stages of lactation as an indicator of metabolic disorders and to use this ratio in estimating energy balance.

2. Materials and Methods

The data of the study were obtained from individual data collected from 1390 Holstein dairy cattle in the commercial dairy cattle farm registered to the cattle breeders association in Bursa province. On the farm where the research was conducted, animals housed in free stall barns are grouped according to their milk yield level, and the number of milking is 3 for those with high milk yield and 2 for animals with low milk yield. 9,920 data consisting of fertility and milk yield records of individual animals were evaluated. This study monitored the lactation periods of cows, which were divided into three stages:

Early lactation: Days in milk (DIM) 0–100

Mid lactation: Days in milk 100–200

Late lactation: Days in milk >200

The research was conducted to determine milk fat, milk protein, milk solids and milk lactose values of milk samples collected on monthly test days during a 10-month period between August and May (30 days apart) with a Bently FTS/FCM COMBI 400 (Bentley Nexgen 400) model device. On the farm where the research was conducted, animals are fed with full rations suitable for milk yield. Dry matter, crude protein, crude fat, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude ash analyses of the ration samples taken during the morning feeding on the first control day and monthly (on test days) throughout the duration of the research were carried out in the Feed Analysis Laboratory of the Department of Animal Science, Faculty of Agriculture, Selçuk University (Table 1). NDF and ADF contents of feed materials and rations were determined with Van Soest (1994), and other nutrients were determined through methods specified by Akyıldız (1984). The average nutrient compositions of the rations used on the farm are shown in Table 1. Statistical analyses in the research were carried out using the SPSS 21 package program. Differences between groups were determined by repeated One-Way-ANOVA.

Table 1. Feed analysis information on the farm.

	Feed analysis information on the farm	
Farm Groups	MY*<35	MY≥35
DM (%)	35.22	40.85
CF (%)	4.68	6.31
CA (%)	6.70	6.39
CP (%)	13.54	14.50
ADF (%)	20.62	18.31
NDF (%)	34.92	32.19
ME (Mcal/kg KM**)	1.73	2.63
NFC***	34.41	34.24
Roughage/Concentrate Feed	56/44	45/55
NFC/CP	2.54	2.36
ME/CP	0.19	0.11
Ration ID	1	2

*MY: milk yield, kg/day.
**ME (Mcal/kg DM): (3227+62.86%CF-31.79%CA-32.50%ADF)/1000.
***NFC (non-fiber carbohydrate):%DM-(CP+CF+NDF+CA).

3. Results and Discussion

Changes in the biochemical composition of milk can be used as a reflector of the physiological state of the cow (Hamann & Krömker, 1997). Especially in large-scale commercial herds, applying a feeding program appropriate to the lactation periods throughout lactation is very important in terms of monitoring the health status of the animals and milk yield and quality (Stoop et al., 2009). The optimum milk fat/milk protein ratio is between 1.2-1.4, indicating that cows are in positive energy balance, while values higher than 1.4 (or values where the milk protein/milk fat ratio is equal to or lower than 0.75) are a signal of energy deficiency and indicate a high risk of subclinical ketosis (Geishauser et al., 1998; Heuer et al., 1999). A milk fat/milk protein ratio lower than 1.2 is most probably an indicator of subclinical acidosis, and it has been reported that low values may negatively affect the reproductive performance of cows and increase the possibility of disorders in mineral metabolism (Čejna & Chladek, 2005). Insufficient dietary fiber levels in herds can cause health problems, such as a decrease in milk fat level, acidosis and laminitis (Heuer et al., 1999). It has been reported that a milk fat/milk protein ratio below 1.2 indicates ration cellulose deficiency and energy excess, values between 1.2 and 1.4 are the optimum value, and above 1.4 is considered an indicator of ration energy deficiency and fiber excess (Alphonsus et al., 2013; Čejna & Chiladek 2005).

In the study, the percentage of animals at risk of acidosis in the 1st, 2nd and 3rd lactation was 39.5, 32.4 and 38.9%, respectively, and 33.9% for the whole herd. While the percentage of animals in positive energy balance was highest in the 1st lactation (30.3%), it was 29.7% in the 2nd lactation and 22.2% in the 3rd lactation, and was found to be 29.6% in the herd overall (Table 2). The percentage of animals at risk of ketosis in the 1st, 2nd and 3rd lactation was 30.2, 37.9 and 38.9%, respectively, and 36.5% for the herd overall. According to the results of the research, the problems seen in positive energy balance were found to be higher in young animals in the first lactation than in adult animals, especially since young animals in the first lactation, which constitute the majority of the herd, are more sensitive to pregnancy, hormonal changes and diseases.

It is so important to take into account the indicators used to estimate metabolic energy balances, to detect possible errors in feeding early enough and to manage the herd as well as to take the necessary precautions, especially for animals in the first lactation. If the negative effects observed in energy balance cannot be prevented in the 1st lactation, it is highly likely that more significant health and productivity losses will be experienced in the 2nd and 3rd lactation periods.

Table 2. The estimation of energy balance based on the milk fat/milk protein ratio for lactation number (x̄±Sx).

Limit values for milk fat/milk protein ratio		**1st Lactation (1.30±0.30)**		**2nd Lactation (1.37±0.3)**		**3rd Lactation (1.29±0.21)**		**TOTAL**	
		N	%	N	%	N	%	N	%
<1.2	Acidosis	68	39.5	227	32.4	7	38.9	302	33.9
>1.4	Ketosis	52	30.2	266	37.9	7	38.9	325	36.5
=1.2-1.4	Positive energy balance	57	30.3	208	29.7	4	22.2	264	29.6
TOTAL		172	100	701	100	18	100	891	100

The milk fat-to-protein ratio variable reached its highest value in the second lactation and its lowest value in the first lactation ($P<0.05$). Milk protein variable reached its highest value in the first lactation (3.21±0.39) and its lowest value in the second lactation (2.94±0.31). Milk fat variable reached its highest value in the first lactation (3.99±0.68) and its lowest value in the second lactation (3.34±0.42).

According to the research results, the percentage of ketosis was lowest (28%) in the mid-lactation period, and the highest percentage value (37%) was detected in the early lactation period (Table 3). Hanuš et al. (2013) reported that ketosis is a lack of energy and means an insufficient level of glucose in the blood. In ketosis, milk fat content increases due to the breakdown of body fats, while on the contrary, protein content decreases. Negative energy balance, which occurs due to inadequate feeding of cows despite high milk yield in the early lactation period, may be the possible cause of reproductive disorders such as abomasum displacement, mastitis and retention of end, which reduce milk yield and threaten milk quality in the postpartum period (Hanuš et al., 2013). It has been reported that ketosis cases, which are mostly seen in the first 50 days of lactation, can be diagnosed accurately within the first 10 days of lactation and that the use of milk analysis records in the early lactation period is common in practice, but the use of daily milk records for the first 10 days of lactation, which is a risky period for ketosis, will give more accurate results (Manzenreiter et al., 2013).

Table 3. The estimation of energy balance based on the milk fat/milk protein ratio for lactation stages (x̄±Sx).

Limit values for milk fat/milk protein ratio		**Early lactation (1.31±0.34)**		**Mid lactation (1.42±0.38)**		**Late lactation (1.35±0.36)**		**TOTAL**	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
<1.2	Acidosis	54	31	44	30	228	35	326	34
>1.4	Ketosis	65	37	41	28	226	35	332	34
=1.2-1.4	Positive energy balance	56	32	63	42	191	30	310	32
TOTAL		175	100	148	100	645	100	968	100

The milk fat-to-protein ratio variable reached its highest value in the early lactation and its lowest value in the late lactation ($P<0.05$). Milk protein variable reached its highest value in the late lactation (3.04±0.37) and its lowest value in the early lactation (2.84±0.30). Milk fat variable reached its highest value in the late lactation (4.06±0.97) and its lowest value in the early lactation (3.71±0.92).

In a study conducted by Čejna and Chladek (2005), the milk fat/milk protein ratio of individual milk samples taken from Holstein cows on days 25, 45, 73, 101, 133, 166, 199, 224, 253 and 280 of lactation was found to be 1.91, 1.45, 1.38, 1.28, 1.22, 1.14, 1.26, 1.21, 1.09 and 1.18 respectively. The high milk fat/milk protein ratio seen in the first phase of lactation has been attributed to energy deficiency. The sediment quality of milk obtained from these animals was also found to be low. Researchers have reported that the milk fat/milk protein ratio changes throughout the lactation period, and that a high ratio at the beginning of lactation indicates that the cows are undernourished in terms of energy and have a negative energy balance.

In the current study, the percentage of animals at risk of acidosis showed the highest value (35%) in the late lactation period, while the lowest value (30%) was observed in the mid-lactation period (Table 3). The rate of animals at risk of acidosis in the herd overall is 34%, and the rate of animals at risk of ketosis is 34%. While the percentage of animals in positive energy balance was highest in the mid-lactation period (42%), it was lower in the early and late lactation periods (32% and 30%, respectively).

4. Conclusion

The variations observed in the components of milk may reflect metabolic disorders and changes in energy balance. Preliminary symptoms of low milk fat percentage problems, as well as important yield and health problems in the 2nd and 3rd lactate periods, which are commonly encountered on large farms in Türkiye, can be obtained by using individual milk analyses. Thus, they will be able to prevent economic losses by protecting herd health by means of making changes in their feeding programs. In the research, the suitability and effectiveness of the feeding program applied in the evaluation of metabolic disorders were evaluated by using individual milk analyses on the farm.

Compliance with Ethical Standards

The milk samples used in the study originated from the milked cows in a commercial dairy cattle farm. Milks were obtained as a result of the routine milking processes at the farm. Cows were not subjected to any extraordinary applications nor given any agents. Moreover, according to the "Regulation on Working Procedures and Principles of Animal Trials Ethical Committees" (published in Turkish Republic Official Gazette, Number: 28914), milking procedure is not subjected to ethical approval.

Conflict of Interest

The author has no conflict of interest to declare.

References

Akyıldız, R. (1984). *Yemler bilgisi laboratuvar kılavuzu*. Ankara Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Alphonsus, C., Akpa, G. N., Nwagu, B. I., Barje, P. P., Orunmuyi, M., Yashim, S. M., Zanna, M., Ayigun A. E., & Opoola, E. (2013). Evaluation of nutritional status of Friesian x Bunaji dairy herd based on milk composition analysis. *Journal of Animal Science Advances*, *3*(5), 219-225. https://doi.org/10.5455/jasa.20130517033450

Arnould, V. M.-R., Reding, R., Bormann, J., Gengler, N., Soyeurt, H. (2013). Review: milk composition as management tool of sustainability. *Biotechnology, Agronomy, Society and Environment*, *17*(4), 613-621.

Beever, D. E. (1993). *Ruminant animal production from forages: Present position and future opportunities*. Proceedings of the XVIII International Grassland Congress. New Zealand.

Beever, D. E. (2006). The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. *Animal Reproduction Science*, *96*(3-4), 212-226. https://doi.org/10.1016/j.anireprosci.2006.08.002

Čejna, V., & Chládek, G. (2005). The importance of monitoring changes in milk fat to milk protein ratio in Holstein cows during lactation. *Journal of Central European Agriculture*, *6*(4), 539-546.

Duffield, T. F., Kelton, D. F., Leslie, K. E., Lissemore, K. D., & Lumsden, J. H. (1997). Use of test day milk fat and protein to predict subclinical ketosis in Ontario dairy herds. *Canadian Veterinary Journal*, *38*, 713-718.

Geishauser, T., Leslie, K., Duffield, T., & Edge, V. (1998). An evaluation of protein/fat ratio in first DHI test milk for prediction of subsequent displaced abomasum in dairy cows. *Canadian Journal of Veterinary Research*, *62*(2), 144-147.

Hamann, J., & Krömker, V. (1997). Potential of specific milk composition variables for cow health management. *Livestock Production Science*, *48*(3), 201-208. https://doi.org/10.1016/S0301-6226(97)00027-4

Hanuš, O., Klımešová, V. M., Gustav, C., Petr, R., & Seydlová, R. (2013). Metaanalysis of ketosis milk indicators in terms of their threshold estimation. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *61*(6), 1681-1692. https://doi.org/10.11118/actaun201361061681

Heuer, C., Schukken, Y. H., & Dobbelaar, P. (1999). Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. *Journal of Dairy Science*, *82*(2), 295-304. https://doi.org/10.3168/jds.s0022-0302(99)75236-7

Lean, I. J., & Golder, H. M. (2024). Milk as an indicator of dietary imbalance. *Australian Veterinary Journal*, *102*(1-2), 19-25. https://doi.org/10.1111/avj.13294

Mackle, T. R., Dwyer, D. A., Ingvartsen, K. L., Chouinard, P. Y., Ross, D. A., & Bauman, D. E. (2000). Effects of insulin and postruminal supply of protein on use of amino acids by the mammary gland for milk protein synthesis. *Journal of Dairy Science*, *83*(1), 93-105. https://doi.org/10.3168/jds.s0022-0302(00)74860-0

Mäntysaari, P., & Mäntysaari, E. A. (2010). Predicting early lactation energy balance in primiparous Red Dairy Cattle using milk and body traits. *Acta Agriculturae Scandinavica, Section A — Animal Science*, *60*(2), 79-87. https://doi.org/10.1080/09064702.2010.496002

Manzenreiter, H., Fürst–Waltl, B., Egger– Danner, C., & Zollitsch, W. (2013). Zur Eignung des Gehalts an Milchinhaltsstoff en als Ketoseindikator. *40. Viehwirtschaftliche Fachtagung*, 9-19. (In German)

Nelson, A. J., & Redlus, H. W. (1989). Dairy practice management: The key role of records in a production medicine practice. *Veterinary Clinics of North America: Food Animal Practice*, *5*(3), 517-552. https://doi.org/10.1016/S0749-0720(15)30947-6

Pavlata, L., Pechova, A., & Dvorak, R. (2008). Differential diagnosis of cows lying down syndrome. *Veterinarstvi*, *58*, 43-51.

Rathwell, A. C. (1990). *Dairy production medicine: Effective nutrition, dry cow management and body condition on reproduction*. Proceedings Society Ontario Veterinary Conference Hamilton, Ontario.

Stoop, W. M., Bovenhuis, H., Heck, J. M. L., & Van Arendonk, J. A. M. (2009). Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *Journal of Dairy Science*, *92*(4), 1469-1478. https://doi.org/10.3168/jds.2008-1468

Van Soest, P. J. (1994). *Nutritional ecology of the ruminant*. Cornell University Press.

Yang, W. Z., & Beauchemin, K. A. (2007). Altering physically effective fiber intake through forage proportion and particle length: Chewing and ruminal pH. *Journal of Dairy Science*, *90*(6), 2826-2838. https://doi.org/10.3168/jds.2007-0032

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RESEARCH ARTICLE

Genome-Wide Analysis of Vacuolar Iron Transporter (*VIT*) Gene Family in *Phaseolus vulgaris* L.: Functional Roles in Heavy Metal Stress

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ABSTRACT

Vacuolar Iron Transporter (*VIT*) genes have been characterized and indicated to play critical roles in iron homeostasis in various plants. Heavy metals pose a significant challenge to bean cultivation, necessitating the development of heavy metal-resistant cultivars as a key strategy to mitigate their impacts. Vacuolar detoxification is a crucial strategy for plants to survive and adapt to the adverse environment caused by heavy metal stress. The current study used various bioinformatic tools to characterize the *VIT* gene in the bean, a significant member of the legume family and an important agricultural product, for the first time. The study identified and characterized 11 *VIT* genes (PhvulVIT-1–PhvulVIT-11) in the bean's genome. These genes displayed molecular weights (MW) ranging from 16.48 to 28.92 kDa and comprised 155–269 amino acid residues. The distribution of the 11 *PhvulVIT* genes on the four chromosomes was not homogeneous, and eight genes were observed to be located on chromosome 2. Gene duplication events suggested purifying selection as the primary evolutionary force, ensuring functional stability of duplicated genes. Phylogenetic analysis classified *PhvulVIT* genes into three clades, reflecting evolutionary relationships with orthologs in *Arabidopsis thaliana* and *Glycine max*. Cis-regulatory element analysis of promoter regions revealed key stress-responsive motifs like MYB, MYC, and ABRE, which are essential for plant responses to environmental stresses and phytohormone signaling. Additionally, the expression patterns of PhvulVIT under heavy metal conditions were examined using RNAseq. This study enhances our understanding of the functional roles of *VIT* genes in nutrient homeostasis and environmental stress adaptation, offering valuable insights for crop improvement strategies, including biofortification and the development of stress-tolerant cultivars.

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1. Introduction

The common bean (*Phaseolus vulgaris* L.), a vital legume from the Fabaceae family, is widely grown worldwide, particularly in developing nations (Hammami et al., 2022). It is a rich source of protein, dietary fiber, starch, potassium, thiamine, B vitamins, and folic acid (Silva-Gigante et al., 2023; Wainaina et al., 2021). Legumes' nutritional and functional characteristics make them important for many demographic groups, not only in low-income nations but also in rich ones (Lisciani et al., 2024). With the world population expected to exceed 9.7 billion by 2050, achieving food security necessitates crops that are both nutrient-dense and environmentally sustainable (Gu et al., 2021; Hall et al., 2017). One of the most significant developmental issues in the coming years will be the ability of global food markets to fulfill the demands of a fast-rising population (Daszkiewicz, 2022). Furthermore, this substantial increase in population is accompanied by an increase in the pace of numerous anthropogenic activities, which degrade plant development and productivity by

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introducing abiotic stressors such as heavy metals (Aizaz et al., 2023).

Heavy metals have a dual role in plant metabolism: they function as essential micronutrients such as cobalt (Co), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn) and nickel (Ni) by acting as co-factors for critical metabolic enzymes, but they become toxic when their levels exceed permissible limits (Khalil et al., 2021; Salam et al., 2023). Non-essential heavy metals, including cadmium (Cd) and lead (Pb), exhibit phytotoxicity at minimal doses, negatively impacting plant growth and development (J. Li et al., 2020). When metal levels exceed an organelle's needs, it can upset the organelle's internal homeostasis, ultimately causing significant physiological and metabolic issues in plants (X. Chen et al., 2023). Excessive heavy metal exposure causes reduced biomass, leaf chlorosis, delayed root growth, and plant morphological anomalies, eventually culminating in plant death (Kumar et al., 2023). Bano et al. (2019) studied the impact of arsenic (As) on the growth of common bean plants, revealing reductions in shoot height, root length, and leaf surface area. Shahid et al. (2019) revealed that elevated Cd levels in beans lead to accumulation in roots and shoots, increased hydrogen peroxide (H_2O_2) production, and a significant reduction in chlorophyll content. Plants have developed mechanisms to avoid or tolerate heavy metal stress, such as antioxidant enzyme enhancement, ion homeostasis regulation, gene activation, and stress protein production (Kosakivska et al., 2021; Noor et al., 2022).

Vacuoles are exclusively found in eukaryotic species, vary in size, and can comprise up to 90% of the cell's volume (Cui et al., 2020). The vacuole has several functions, such as plant growth and development, turgor formation, storage of nutrients and metabolites, breakdown of proteins, and plant defense, the most important being maintaining ion and toxin balance (Mansour, 2023; Sharma et al., 2016). Metal transporters can remove metal ions from the cell or sequester them in vacuoles or other intracellular compartments to reduce HM-induced cytotoxicity (X. Chen et al., 2023). Tonoplast-localized vacuolar iron transporters (VIT) and VIT-likes (VTL) regulate plant Fe homeostasis and transport cytosolic ferrous ions into the vacuole via proton motive force (Kaur et al., 2021; Khoudi, 2021). This mechanism supports nutrient storage and redistribution while offering buffering capacity against heavy metal toxicity, which is crucial for plants in metal-contaminated soils. Previously, VITs were found to significantly function in keeping Fe within the appropriate physiological range and preventing cellular toxicity (Connorton et al., 2017). These *VIT* genes exhibit strong homology with a small family of nodulin-like proteins having a Ccc1 (Ca^{2+}-Sensitive Cross Complementer) like domain, with yeast Ccc1p1 (Gollhofer et al., 2011; Ram et al., 2021). Arabidopsis VIT1 was the first VIT protein discovered in plants, with 62% amino acid identity to Ccc1 and five projected transmembrane domains, which are compatible with the Ccc1 model. The AtVIT1 protein can transport iron into vacuoles, counteracting toxicity and promoting seedling development under high iron circumstances (Kim et al., 2006). In addition to their involvement in iron transport, VIT members have shown little selectivity for divalent metal ions (Zhu et al., 2016).

Using bioinformatics tools, previous studies have identified several VIT proteins across various plant species; however, a genome-wide investigation of this gene family in common bean has not yet been conducted. As an essential crop worldwide, characterizing vacuolar transporters in beans is crucial for preventing stress-induced crop losses and addressing global biofortification challenges. While biofortification remains a long-term objective, the primary goal of this study is to understand the molecular mechanisms underlying heavy metal tolerance in beans, particularly by enhancing metal homeostasis and stress adaptation through vacuolar sequestration. This study presents a genome-wide characterization of Phaseolus vulgaris VIT genes and includes in-silico expression analyses under different heavy metal treatments to explore the regulation of *VIT* genes under heavy metal stress.

2. Materials and Methods

2.1. Identification and Analysis of *VIT* Gene Family

The genome, CDS (CoDing Sequence) and amino acid sequences corresponding to the *VIT* gene family in *Phaseolus vulgaris* (v2.1), *Arabidopsis thaliana* (TAIR10) and *Glycine max* (Wm82.a4.v1) were obtained from the Phytozome v. 13 database (https://phytozome-next.jgi.doe.gov/) using the PFAM ID: PF01988 (Sharma et al., 2020). To identify VIT proteins in the genomes of the three selected plants, the blast tool from the Phytozome database and the Hidden Markov Model (HMM) (http://www.ebi.ac.uk) were employed. The HMMER (http://hmmer.org/) database was utilized to assess the presence of the VIT domain in the retrieved sequences. The ProtParam (http://au.expasy.org/tools) tool was used to predict the protein's theoretical molecular weight (MW), amino acid numbers (aa), putative isoelectric point (pI), and instability index. The subcellular localization of *PhvulVIT* genes was predicted using the WoLF PSORT (https://wolfpsort.hgc.jp/) using peptide sequences (Horton et al., 2007).

2.2. Identification of Chromosomal Position, Structure and Conserved Motif Analysis of the *PhvulVIT* Genes

The sizes and locations of *PhvulVIT* genes on the bean chromosome were determined using the Phytozome database and this information was then used to create a genetic map using MapChart software (Voorrips, 2002). After determining the location of *PhvulVIT* genes on chromosomes, a synteny map was generated using TBtools (C. Chen et al., 2023). The *PhvulVIT* family gene structures were shown using Gene Structure Display Server (GSDS) v. 2.0 (Hu et al., 2015). CDS and genome sequences were used to obtain exon-intron information of PhvulVIT proteins.

The Multiple Em for Motif Elicitation (MEME) v. 5.5.7 tool was utilized to analyze conserved motifs of the 11 PhvulVIT sequences (Bailey et al., 2009). The parameters are as follows: the motif regions were revised between 2 and 300; the maximum motif number was set at 10; the site distribution was set at any number of repetitions (anr); the minimum width was set at 6; and the maximum width was set at 50. Furthermore, for the analysis of conserved regions in bean sequences, sequence logo analysis of motifs of PhvulVIT proteins was obtained using the MEME suit online tool.

2.3. Gene Duplication and Ka/Ks Analysis of *PhvulVIT* Genes

TBtools program was utilized to determine gene duplication events. The paralogous gene pairs were used to calculate the synonymous (Ks), non-synonymous (Ka), and non-synonymous to synonymous (Ka/Ks) ratio using the TBtools basic Ka/Ks calculator algorithm (Isıyel et al., 2024). The formula $T = Ks/2\lambda$ ($\lambda = 6.56E\text{-}9$) was used to calculate the divergence time (T) million years ago (Mya) (Aygören et al., 2023).

2.4. Sequence and Phylogenetic Analysis of VIT Family Genes in *P. vulgaris, A. thaliana*, and *G. max*

For the PhvulVIT proteins phylogenetic analysis, all the VIT proteins from *P. vulgaris*, *A. thaliana*, and *G. max* were aligned using ClustalW. The phylogenetic tree structure was generated using the Neighbor-Joining (NJ) method and 1000 bootstrap replicates in MEGA v. 11 (Tamura et al., 2021). The Interactive Tree of Life (iTOL) v. 6 online tool was used to draw the phylogenetic tree (Letunic & Bork, 2024).

2.5. Syntenic and Homology Modeling of VIT Proteins

Orthologous protein sequence information of *P. vulgaris, A. thaliana*, and *G. max*, was retrieved using Phytozome v13. The TBtools' One Step MCScanX tool was used to compare genome and transcript sequences of *P. vulgaris* with *A. thaliana* and *G. max*, analyzing homology and collinearity of *VIT* genes. The obtained files screened the *VIT* genes present in these three plants, and the interspecific collinearity analysis was visualized with the Multiple Synteny Plot function in the TBtools (Qi et al., 2023). The Phyre2 v. 2.0 (https://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) program was used to generate model three-dimensional (3D) structures using VIT amino acid sequences and the best 3D image was selected after evaluating the reliability rates (Kelley et al., 2015).

2.6. Cis-Regulatory Element Analysis and Protein-Protein Interactions of PhvulVIT Family

Cis-regulatory elements of *PhvulVIT* genes were investigated using 2 kb genomic sequences (excluding 5'UTR sequences) upstream of the start codon (ATG) with the PlantCARE database (Rombauts et al., 1999). The results obtained were visualized with TBtools. Protein-protein interaction (PPIs) was analyzed in the STRING database v. 12.0 (https://string-db.org/) using the amino acid sequences of each PhvulVIT family member (Szklarczyk et al., 2023).

2.7. In-silico Gene Expression Analysis

Illumina RNA-seq data were acquired from NCBI's Sequence Read Archive (SRA) database to analyze the *PhvulVIT* genes. The accession numbers for control [SRR21012358, SRR21012359, and SRR21012360 (root HM control with Na) and HM stress [SRR21012367, SRR21012369, and SRR21012370 (Zn stress treated root), SRR21012397, SRR21012398, and SRR21012399 (Mn stress treated root), SRR21012417, SRR21012418, and SRR21012419 (Cu stress treated root), and SRR21012427, SRR21012428, and SRR21012429 (Co stress treated root)] were used to find relevant RNA-seq data (Fang et al., 2022). Log2-transformed RNA-seq RPKM values were used for expression profiling (C. Chen et al., 2023). Heat maps were generated with CIMminer to illustrate high-dimensional data sets such as gene expression profiles (https://discover.nci.nih.gov/cimminer/oneMatrix.do).

3. Results and Discussion

3.1. Identification and Chromosomal Distributions of *VIT* Genes in Bean

The PFAM accession number (PF01988) was used to identify *VIT* gene family members in the *Phaseolus vulgaris* v2.1 genome, accessible in the Phytozome database VIT family genes in *P. vulgaris* were higher than in *A. thaliana* but lower than in *G. max*, with 6, 11, and 19 Arabidopsis, *P. vulgaris*, and *G. max* candidate *VIT* genes identified, respectively. Every *VIT* gene was given a specific name, i.e., from PhvulVIT-1 to PhvulVIT-11. Cao (2019) identified 114 *VIT* genes in 14 different plant species, including *A. thaliana, G. max*, and *Zea mays.* PhvulVIT-2 was the smallest protein (155 amino acids), while PhvulVIT-1 was the largest (269 amino acids). The molecular weights (MW) of *PhvulVIT* genes ranged from 16.48 (PhvulVIT-2) to 28.92 kDa (PhvulVIT-1), while their predicted isoelectric points (pI) varied from 4.69 (PhvulVIT-2) to 9.36 (PhvulVIT-6). Except *PhvulVIT-6* and *PhvulVIT-7, PhvulVIT* genes are generally characterized as acidic, with pI < 7. The instability index of a protein defines its structure and stability; values above 40 indicate instability and values below 40 indicate stability (Singh & Mukhopadhyay, 2021). The instability index of PhvulVIT proteins ranged between 29.41 to

43.54. All proteins are stable except PhvulVIT-3, 5, 7, and 11 (Table 1).

According to the subcellular localization prediction results by WoLF PSORT, all PhvulVIT proteins are localized in the plasma membrane, and all proteins except PhvulVIT-6 are localized in the vacuole. PhvulVIT proteins were also localized in organelles such as the golgi, endoplasmic reticulum, and cytoplasm (Table 1). Sharma et al. (2020) reported that TaVIT proteins in wheat are predominantly localized in the plasma membrane and chloroplast thylakoid membrane, while TaVTL proteins are found in the vacuolar membrane. Zhang et al. (2012) demonstrated that the transient expression of OsVIT1:EGFP and OsVIT2:EGFP protein fusions revealed the localization of OsVIT1 and OsVIT2 to the vacuolar membrane, suggesting they may also act as vacuolar membrane transporters. The chromosomal positions of VITs were determined using the TBtool program by uploading a mapchart file containing chr data.

Table 1. Detailed information of all *VIT* genes identified in the bean genome.

Gene Name	**Phytozome Gene ID**	**Chromosomal Location**				**aa**	**MW (kDa)**	**pI**	**Instability Index**	**Aliphatic Index**	**Subcellular Localization**
		Chr Name	**Start**	**End**	**Strand**						
PhvulVIT-1	Phvul.008G070000	Chr08	6339673	6342165	R	269	28.92	5.44	37.43	0.171	vacu: 5, golg: 4, plas: 3, extr: 1, E.R.: 1
PhvulVIT-2	Phvul.002G323700	Chr02	48857306	48858670	R	155	16.48	4,69	33.80	98.32	cyto: 5, plas: 3, E.R.: 3, mito: 1, vacu: 1, golg: 1
PhvulVIT-3	Phvul.002G113500	Chr02	24370428	24371205	F	232	24.64	6.59	40.48	109.35	vacu: 8, plas: 6
PhvulVIT-4	Phvul.002G322800	Chr02	48774555	48776307	F	231	24.34	5.13	32.60	0.371	chlo: 6, plas: 3, mito: 2, vacu: 2, E.R.: 1
PhvulVIT-5	Phvul.007G079100	Chr07	7671484	7672393	F	223	23.7	5.44	41.69	101.93	vacu: 7, golg: 4, plas: 2, extr: 1
PhvulVIT-6	Phvul.002G205100	Chr02	37176245	37177230	R	221	22.94	9.36	29.73	99.37	plas: 8, E.R.: 5, chlo: 1
PhvulVIT-7	Phvul.004G096500	Chr04	15848520	15849195	R	224	23.71	6.15	43.23	99.38	vacu: 8, plas: 2, extr: 2, E.R.: 1, golg: 1
PhvulVIT-8	Phvul.002G205000	Chr02	37162537	37163665	R	229	24.24	8.63	29.41	104.37	vacu: 10, plas: 2, extr: 1, golg: 1
PhvulVIT-9	Phvul.002G322900	Chr02	48779469	48782786	R	247	26.17	5.48	31.68	105.22	plas: 10, vacu: 4
PhvulVIT-10	Phvul.002G205200	Chr02	37188536	37189462	R	221	23.25	6.74	30.14	105.57	plas: 4.5, vacu: 4, cyto_plas: 3, E.R.: 2, golg: 2, extr: 1
PhvulVIT-11	Phvul.002G205300	Chr02	37195862	37196667	F	209	22.7	4.71	43.54	108.76	vacu: 9, plas: 5

Chr: Chromosome, R: Reverse, F: Forward, aa: Amino acid, MW: Molecular weight, pI: Theoretical isoelectric point, vacu: Vacuole, golg: Golgi apparatus, plas: Plasma, extr: Extracellular, E.R: Endoplasmic reticulum, mito: Mitochondria, chlo: Chloroplast, cyto: Cytosol.

The unequal distribution of VITs on chromosomes is depicted in Figure 1. Chr2 (chromosomal 2) included the largest number of PhvulVIT (8), while Chr4, Chr7, and Chr8 each contained only one. Connorton et al. (2017) determined that the *TaVIT1* and *TaVIT2* genes of wheat are located on chromosomes 2 and 5, respectively. Thirty-one distinct VIT families of wheat have been identified in hexaploid wheat, with the majority of genes firmly positioned on chr2 (Sharma et al., 2020).

3.2. VIT Structure Analysis and Conserved Motif Composition

PhvulVIT genes exons and introns were discovered using GSDS structural analysis. The variety of exons and introns in gene families involves three processes: the gain or loss of exons and introns; exonization, which converts intronic or intergenic sequences into exonic states; and pseudoexonization, which reverses this transformation (Isıyel et al., 2024). The exon numbers of *PhvulVIT* genes range from 1 to 4, while the intron counts range from 2 to 3 (Figure 2). PhvulVIT-3, 5, 6, 7, 8, 10, and 11 are intronless genes with a single exon (Figure 2). PhvulVIT-2, PhvulVIT-4, and PhvulVIT-9 had the highest exon numbers, and of the 11 *PhvulVIT* genes, 2 had no UTRs (Figure 2). The *VIT* genes in wheat possess three to four intronic and exonic sections, whereas the *VTL* genes consist of a single exon apiece without introns. This structural distinction further categorises the VIT family into two sub-families (Sharma et al., 2020).

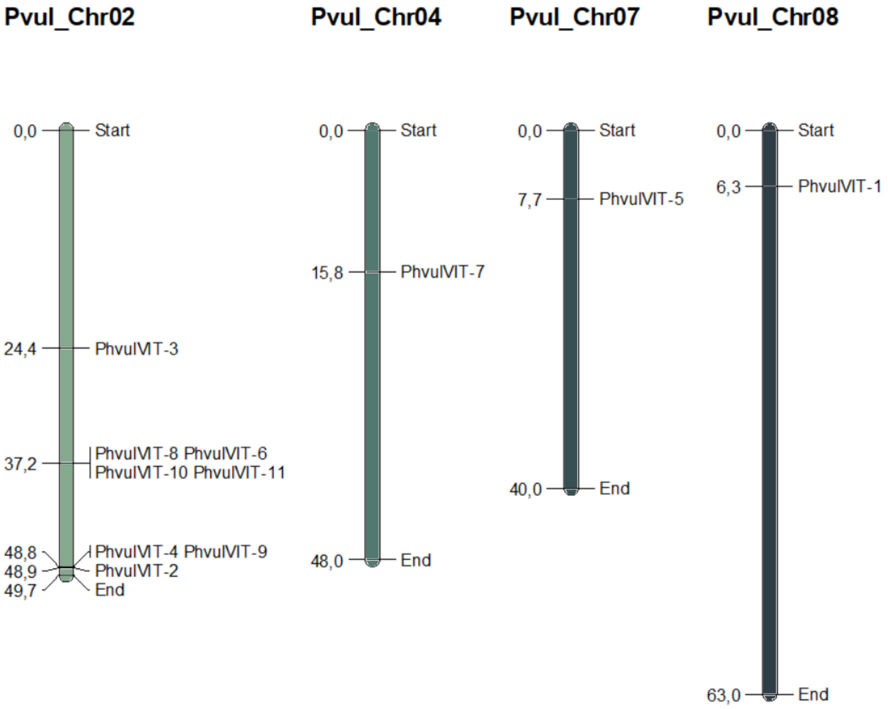


Figure 1. *PhvulVIT* genes distribution in bean chromosomes.

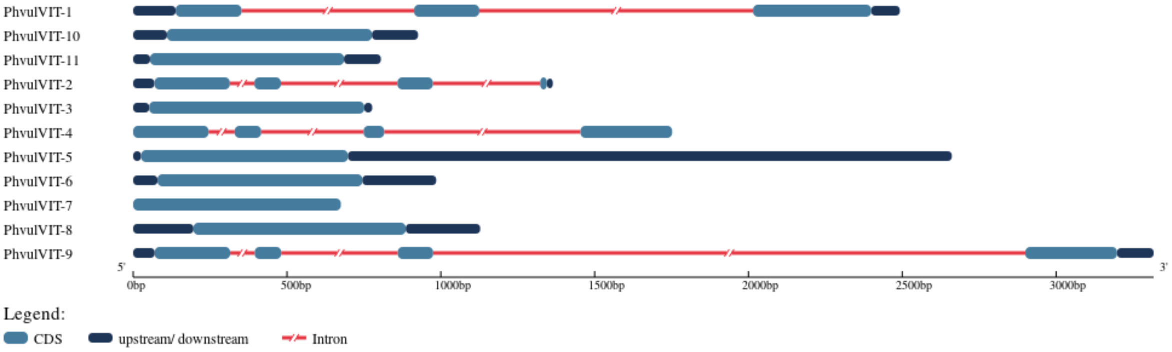
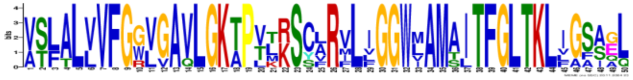


Figure 2. Gene structure of *VIT* genes in bean constructed by the GSDS 2.0. Exons, introns, and untranslated (UTR) regions are marked by blue boxes, red lines, and navy-blue boxes, respectively. The scale bar at the bottom shows the lengths of the exons, introns, and UTR sections.

Ten conserved motifs were identified in the conserved motif analysis conducted on PhvulVIT proteins using the MEME suite v. 5.5.7 and named Motif 1–10. Furthermore, the MEME suite database was used to find conserved domain sequences and motif logos of *P. vulgaris VIT* gene families (Table 2). The number of amino acids in conserved motifs ranges from 6 to 50 (Table 2). Table 2 displays the sequences that correlate to the motifs that are identified as being the best possible matches. It was found that Motifs 1 was present in all the common bean *PhvulVIT* genes that belong to the same gene family. This suggests that these motifs could act as identifiers for distinguishing the *PhvulVIT* gene.

Table 2. Sequence details of possible motifs and motif logo in PhvulVIT proteins.

MOTIF ID	WIDTH	LOGO	POSSIBLE BEST MATCH	DOMAIN
1	41		LMMGVGAVKQDIKAMLTAGFAGLVAGACSMAIGEFVSVYTQ	Ccc1
2	50		VSLALVVFGWVGAVLGKTPVTKSCLRVLIGGWMAMAITFGLTKLIGSAQL	Ccc1
3	41		EPEKEKLPNPFQAALASALAFSIGALVPLLPYAFIRSYKIR	Ccc1
4	29		HHNIDYSQRAQWLRDAVLGANDGLVSPFS	N/A
5	21		YDIYMRQMKREQERNNGGPRD	N/A
6	26		GHRTNEYSINQHEYPPHYNGLEPNPN	N/A
7	50		VVVTMFALLVFGYAKGHFTGNKPFRSALETALIGAIASAAAFGMAKAFHP	Ccc1
8	15		EHGISLDQEKTNLPN	N/A
9	6		WLDFMM	N/A
10	7		QDNKHWK	N/A

Motif 1, Motif 2, Motif 3, and Motif 8 contain the Ccc1 domain. The Ccc1 family comprises a group of putative vacuolar ion transporters. This protein family includes yeast Ccc1, which is involved in Ca and Mn homeostasis (Lapinskas et al., 1996). Arabidopsis VIT1, the first plant VIT protein to function as a vacuolar Fe^{2+} uptake transporter, is 62% amino acid similar to Ccc1 and has five predicted transmembrane domains consistent with the Ccc1 mode (Kim et al., 2006). The proteins with the fewest motifs are PhvulVIT-1, PhvulVIT-8, and PhvulVIT-11 (5 motifs), while the gene with the most motifs is PhvulVIT-9 (8 motifs) (Figure 3). All *VIT* and *VTL* genes exhibited the characteristic Ccc1-like superfamily domains observed in yeast (Sharma et al., 2020).

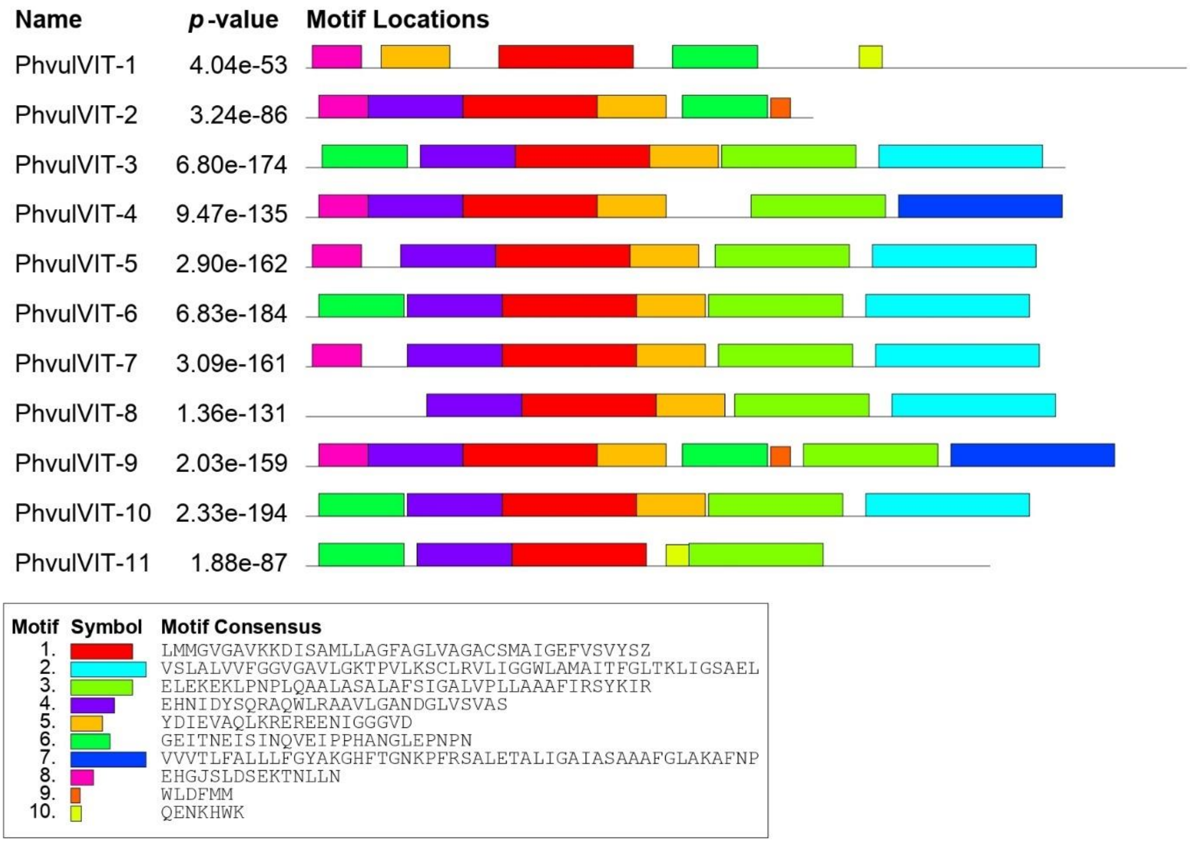


Figure 3. Motif composition of VIT proteins in bean. Boxes of various colors represent numerous motifs. Motif's location in each sequence is marked.

3.3. Gene Duplication Events and Ka/Ks Analysis of *PhvulVIT* Genes

In this research, it was determined four tandem duplicated genes (*PhvulVIT-4*/*PhvulVIT-9*, *PhvulVIT-6*/*PhvulVIT-10*, *PhvulVIT-8*/*PhvulVIT-6*, and *PhvulVIT-10*/*PhvulVIT-11*) pair among 11 *PhvulVIT* genes. The Ka/Ks ratio is a useful tool for describing the degree of selection; values above 1 suggest positive selection, values below 1 indicate purifying selection, and a value of 1 indicates neutral selection (Inal et al., 2024). The Ka/Ks ratio of genes exhibiting tandem duplication was smaller than 1. This hypothesis shows that the duplicated *PhvulVIT* genes underwent purifying selection pressure. Table 3 shows that the *PhvulVIT-8* and *PhvulVIT-6* genes were determined to be the first to diverge approximately 216 billion years ago. Cao (2019) determined that tandem/segmental duplication and transposition events contributed to the evolution of the *VIT* gene family.

Table 3. Divergence time of the VIT paralogues in bean.

Gene 1	Gene 2	Ka	Ks	Ka/Ks	Selection Pressure	Duplication Types	Divergence Time (Mya)
PhvulVIT-4	*PhvulVIT-9*	0,076324	0,266241	0,286673	Purifying Selection	Tandem Duplication	43,65
PhvulVIT-6	*PhvulVIT-10*	0,056855	0,202275	0,281078	Purifying Selection	Tandem Duplication	33,16
PhvulVIT-8	*PhvulVIT-6*	0,316047	1,32E+15	0,239951	Purifying Selection	Tandem Duplication	2,16E+17
PhvulVIT-10	*PhvulVIT-11*	0,348542	0,810983	0,429778	Purifying Selection	Tandem Duplication	132,95

3.4. Phylogenetic Analysis of VITs

The analysis of multiple sequence alignments for PhvulVIT protein sequences demonstrates the presence of highly conserved regions characterized by specific amino acid sequences. A phylogenetic tree was constructed using the VIT protein sequences from *A. thaliana* and *G. max*, elucidating the relationships among PhvulVIT proteins. Phylogenetic tree analysis of 36 VIT proteins from these three plant species was performed utilizing the NJ approach in MEGA v11 software, with a bootstrap value of 1000 repetitions. The 36 *VIT* genes were classified into three clades based on their homology percentage. Clade A consisted of 3 PhvulVIT, 1 Arabidopsis, and 5 soybean members; Clade B had 3 PhvulVIT, 2 Arabidopsis, and 6 soybean members; and Clade C had 5 PhvulVIT, 3 Arabidopsis, and 8 soybean members (Figure 4). Combining the gene structure with the phylogenetic tree revealed that the exon numbers of *PhvulVIT* genes in various subgroups were related to their classification. For example, *PhvulVIT-6* and *PhvulVIT-10* genes in Clade B and *PhvulVIT-2* and *PhvulVIT-9* genes in Clade C were found to have 1 and 4 exons, respectively, and were subdivided in parallel with their exon numbers. The phylogenetic analysis divided 114 *VIT* genes from 14 different plant species into seven groups based on their intron-exon structure and the presence of different motifs (Cao, 2019).

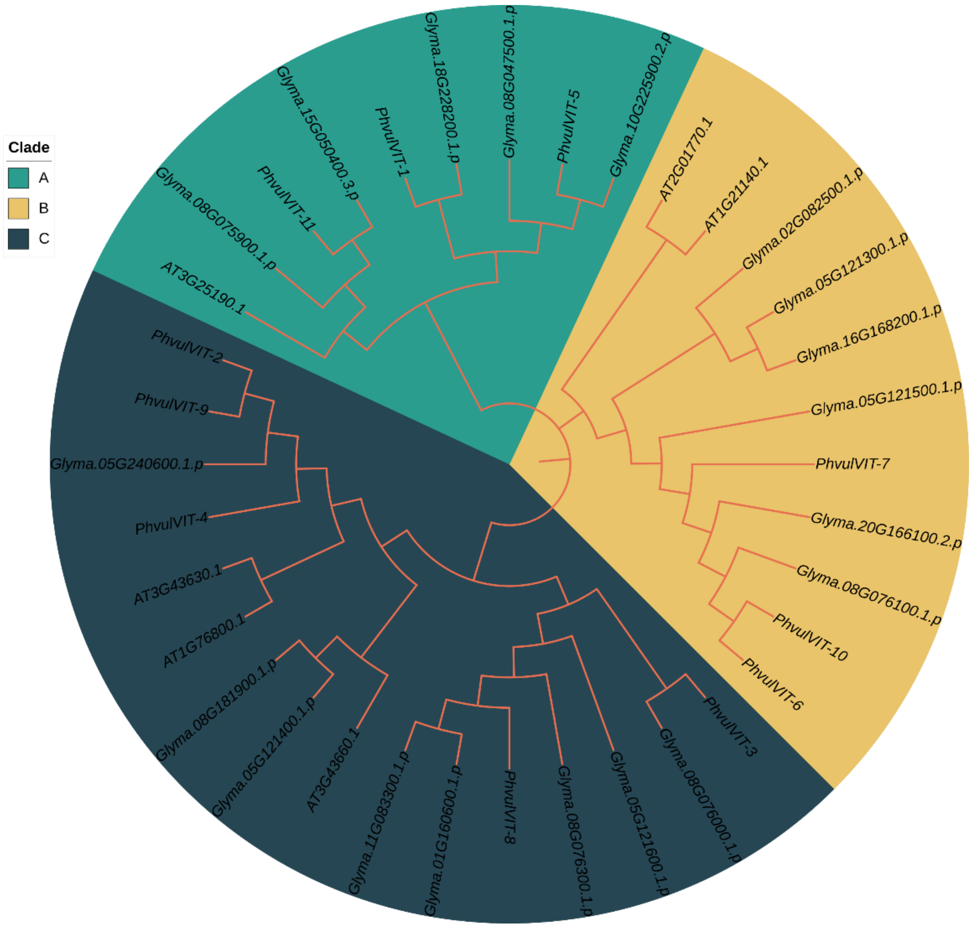


Figure 4. Phylogenetic relationships among 36 VIT proteins in bean, *Arabidopsis*, and soybean. The full-length sequences of bean VIT proteins were used to build the phylogenetic tree using the MEGA 11 software.

3.5. Synteny Analysis of VIT Protein in *P.vulgaris*, *A.thaliana* and *G. max*

The study demonstrated orthologous relationships of *VIT* genes among *P. vulgaris, A. thaliana* and *G. max* genomes. One syntenic relationship was determined among *P. vulgaris* and *A. thaliana VIT* genes, while fifteen syntenic relationships were determined among *P. vulgaris* and *G. max VIT* genes (Figure 5). Orthology was found between *PhvulVIT-2* and *PAC:19642412* genes in bean and Arabidopsis. Moreover, orthology was identified between the *PhvulVIT- 1 /Glyma.18G228200.1; PhvulVIT-2* and *Glyma.05G240600.1-Glyma.08G047500.1; PhvulVIT-3* and *Glyma.01G160600.1-Glyma.11G083300.1; PhvulVIT-5* and *Glyma.02G082500.1-Glyma.10G225900.2-Glyma.16G168200.1-Glyma.20G166100.2; PhvulVIT-6* and *Glyma.05G121300.1; PhvulVIT-7* and *Glyma.02G082500.1-Glyma.16G168200.1*, *PhvulVIT-8* and *Glyma.05G121500.1-Glyma.08G076000.1*, and *PhvulVIT-11* and *Glyma.08G075900.1* genes. There was no collinearity between the *PhvulVIT-4*, *PhvulVIT-9*, and *PhvulVIT-10* genes and *A. thaliana* or *G. max* which suggests that the homologous gene pairs formed after the species split up (Dey et al., 2023).

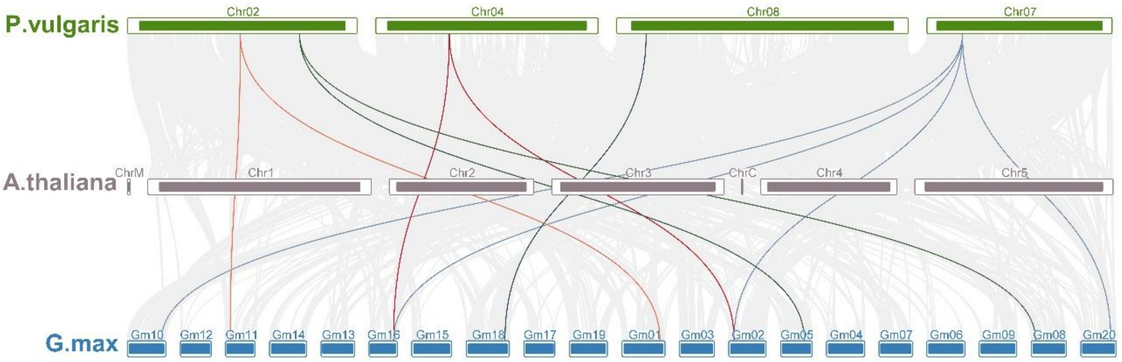


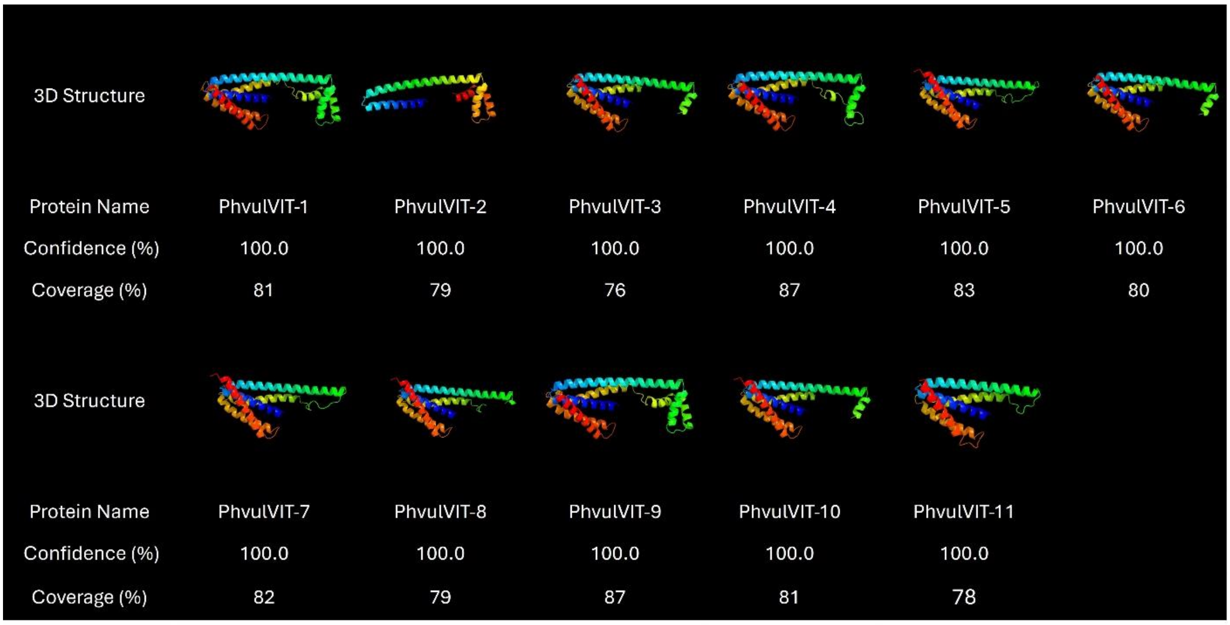
Figure 5. Synteny analysis of the *VIT* genes among the *P. vulgaris, A. thaliana.* and *G. max.* Different color lines delineate the syntenic *VIT* gene pairs.

3.6. Homology Modeling of Identified PhvulVITs

The proteins' structure and function were predicted using the Phyre 2 database. With a 100% modeling confidence level, the 3D structures of the 11 PhvulVIT proteins were predicted using homology modeling. Coverage values also ranged between 76-87%. The results showed that most of the secondary structures of PhvulVIT proteins consisted of disordered structures (Table 4). Specifically, a distinctive alpha (α) helix and TM-helix structure were observed in PhvulVIT proteins (Table 4). Specifically, 7 out of the 11 VIT proteins in *P. vulgaris* displayed similar proportions of alpha and TM-helix structures (Figure 6). The alpha helix is critical in signal transduction and dynamic motions in some proteins. For instance, G-proteins and receptor proteins use this structure in signal transmission (Palczewski et al., 2000). TM-helix, hydrophobic amino acids, facilitate protein passage through biological membranes, ensuring correct localization of membrane proteins by interacting with the hydrophobic region of the lipid bilayer (White & Wimley, 1999).

Table 4. The 3D structure of VIT proteins in bean.

Protein Name	Disordered Structures (%)	Alpha Helix (%)	TM-Helix (%)
PhvulVIT-1	25	73	39
PhvulVIT-2	26	68	23
PhvulVIT-3	34	69	43
PhvulVIT-4	28	73	43
PhvulVIT-5	35	68	44
PhvulVIT-6	35	69	45
PhvulVIT-7	35	67	44
PhvulVIT-8	38	65	44
PhvulVIT-9	22	70	44
PhvulVIT-10	34	68	45
PhvulVIT-11	35	67	46



3D Structure						
Protein Name	PhvulVIT-1	PhvulVIT-2	PhvulVIT-3	PhvulVIT-4	PhvulVIT-5	PhvulVIT-6
Confidence (%)	100.0	100.0	100.0	100.0	100.0	100.0
Coverage (%)	81	79	76	87	83	80

3D Structure					
Protein Name	PhvulVIT-7	PhvulVIT-8	PhvulVIT-9	PhvulVIT-10	PhvulVIT-11
Confidence (%)	100.0	100.0	100.0	100.0	100.0
Coverage (%)	82	79	87	81	78

Figure 6. Homology modeling of PhvulVIT proteins.

3.7. Cis-Elements Analysis in the Promoter Regions of *PhvulVIT* Genes

Cis-acting elements are distinct motifs in each gene's promoter region that regulates gene transcription (Buttanri et al., 2024). The 2000 bp upstream region of the *PhvulVIT* gene promoter was examined to identify key cis-acting elements in the 11 *PhvulVIT* gene. The analyses conducted in the PlantCARE database indicated the cis-regularity elements in the *PhvulVIT* gene sequences using the TBtools (Figure 7). The promoter sequences of bean *VIT* genes show a wealth of potential cis-acting elements that play a critical role in mediating stress responses, phytohormone synthesis, plant growth and development, and adaptations to anaerobic conditions (Figure 7). This demonstrates the significant regulatory pathways involved in providing plant resilience and adaptation. All PhvulVIT includes the promoter elements MYB, MYC, TCT-motif, and Box 4 (Figure 7).

The 172 elements shown to be linked with environmental stress included MYC, MBS, and MYB (drought-related regulatory), DRE core (dehydration-responsive element), W-box and box S (pathogen response elements), LTR (low-temperature responsiveness), WUN-motif (wound-responsive element), WRE3 (high-temperature elements), TC-rich repeats, and STRE (defense and stress-responsive element) (Figure 7). J. Li et al. (2020) identified ten cis-elements associated with abiotic and biotic stresses, including DRE, MYB, MBS, MYC, LTR, STRE, TC-rich repeats, W box, WUN motif, and WRE3.

The 72 cis-acting elements related to phytohormones primarily include the ABRE (abscisic acid-responsive elements), ERE (estrogen-responsive element), AuxRR-core and TGA- element (auxin-responsive element), and TCA-element (salicylic acid responsiveness) (Figure 7). Rasool et al. (2023) found cis elements connected to hormones, including the ABRE, TCA element, GARE motif, P and TATC boxes, TGA element, and AuxRR core. The ARE (regulatory anaerobic induction) cis element was present in 4 *PhvulVIT* genes and was revealed to be responsible for anaerobic induction. In PhvulVIT, light sensitivity was associated with 127 cis-elements, e.g., the TCT motif, Box 4, MRE, G-Box, AE-Box, ACE, LS7, and L-Box (Figure 7). These findings suggest that PhvulVIT proteins are crucial in signaling pathways during plant growth and development and in responding to different abiotic stresses.

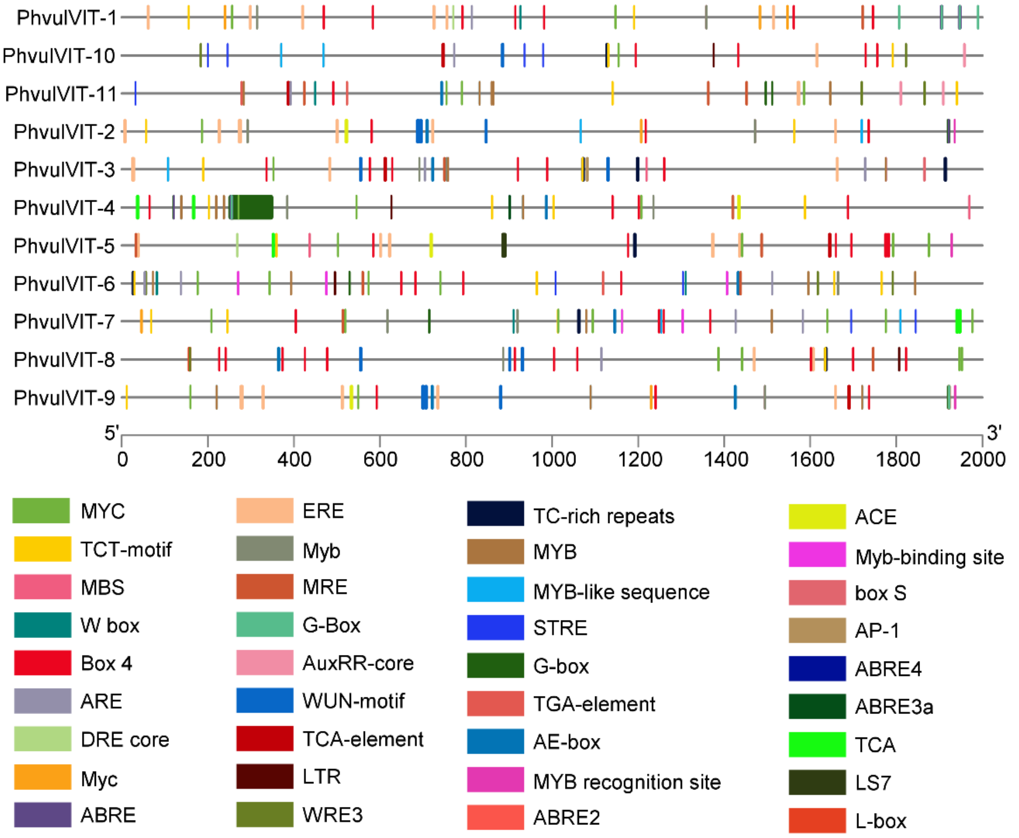


Figure 7. Analysis of cis-acting elements in promoter regions of bean *VIT* genes.

3.8. PhvulVIT Protein–Protein Interactions

Protein-protein interactions (PPIs) provide information about the role of proteins in biological processes and molecular functions (Peng et al., 2017). Figure 8 presents a graphical representation of protein-protein interactions among PhvulVIT proteins using the STRING database (Szklarczyk et al., 2023). PhvulVIT-2 and PhvulVIT-11 interact with other proteins more than any other molecules. PhvulVIT-2 interacts with PhvulVIT-3, PhvulVIT-5, PhvulVIT-6, PhvulVIT-7, and PhvulVIT-8 proteins, while PhvulVIT-11 interacts with PhvulVIT-2, PhvulVIT-4, and PhvulVIT-9 proteins (Figure 8). This suggests that they may play a part in building complex networks and interacting with many different molecules inside cells. V7ASI1_PHAVU, V7CXI9_PHAVU, and V7CX76_PHAVU proteins contain glutamine amidotransferase type-2. The V7B1N1_PHAVU and V7AT93_PHAVU proteins are uncharacterized.

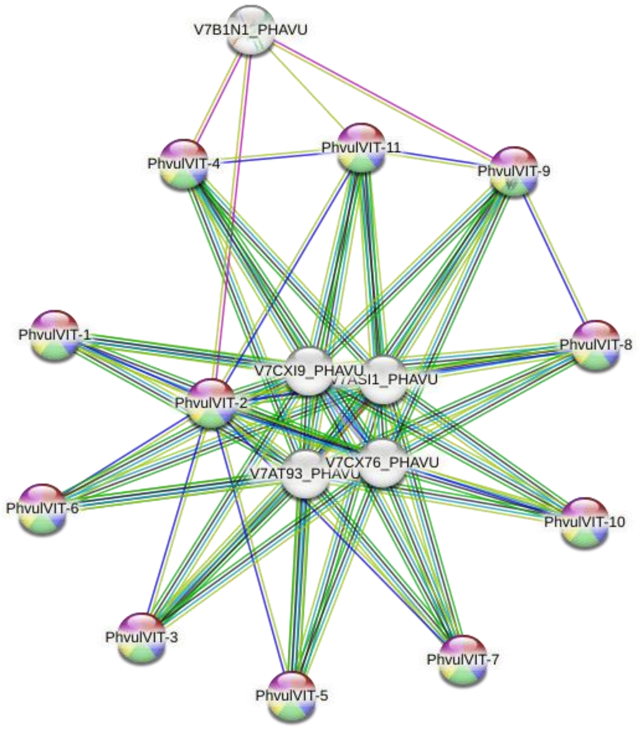


Figure 8. Protein–protein interactions schematic demonstration found in *PhvulVIT* genes. The different colors in the circle show biological functions (red: cellular manganese ion homeostasis, purple: intracellular sequestering of iron ion, green: manganese ion transmembrane transport) and molecular functions (yellow: iron ion transmembrane transporter activity, pink: manganese ion transmembrane transporter activity).

According to the results of PPI analysis, all PhvulVIT proteins are involved in biological processes such as cellular manganese ion homeostasis, intracellular sequestration of iron ion, and manganese ion transmembrane transport (Figure 9A). Moreover, PhvulVIT proteins have molecular functions such as iron and manganese ion transmembrane transporter activity (Figure 9B). Iron-excess-dependent transporters may facilitate iron detoxification or sequestration for homeostasis in rice and wheat (Krishna et al., 2023).

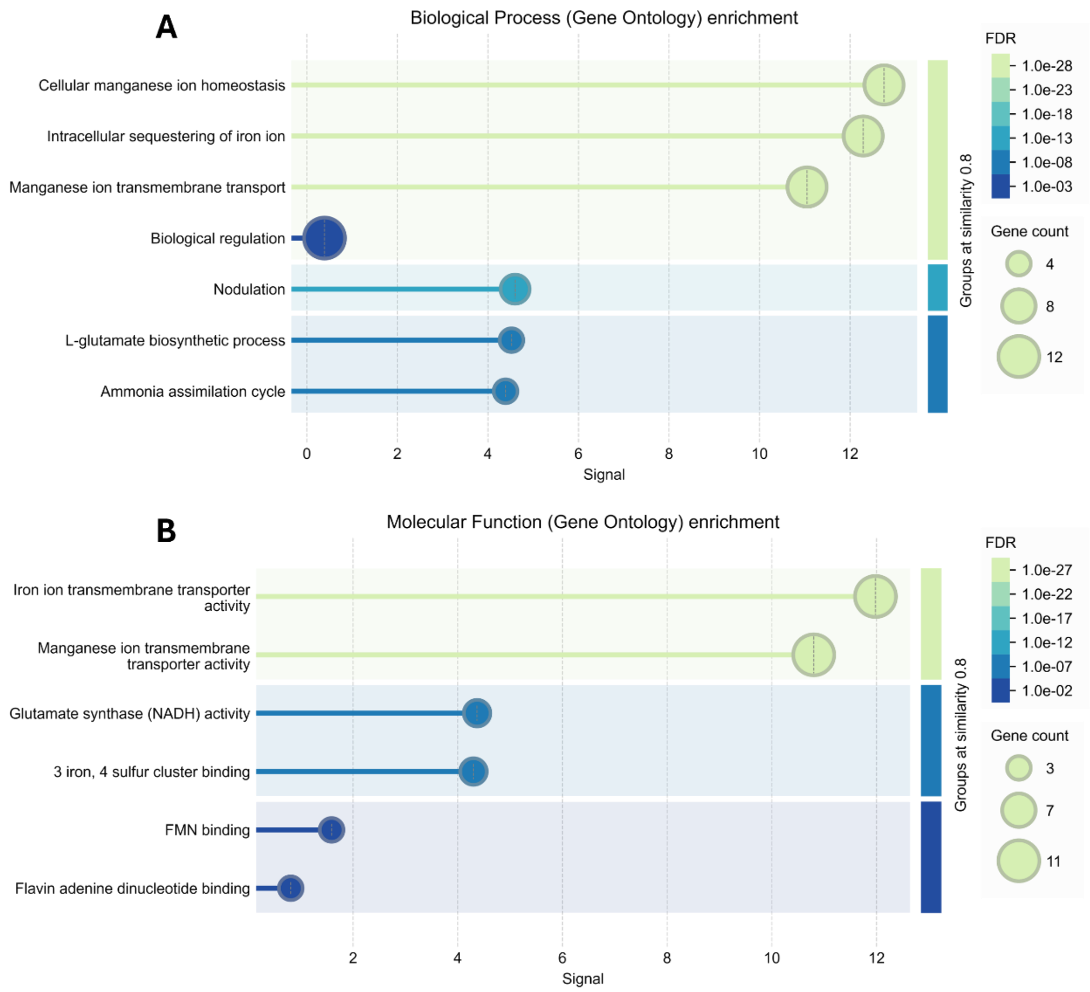


Figure 9. Functional enrichment visualization of PhvulVIT proteins covering biological processes (A) and molecular functions (B) visualized employing the STRING database.

3.9. In Silico Expression Profiles *PhvulVIT* Genes Under Heavy Metal Stress

VIT genes are crucial in plant metal homeostasis and stress responses. They primarily sequester iron into vacuoles, thereby controlling its availability within the cell (Shekhawat et al., 2023). Recent investigations have expanded our understanding of *VIT* genes, revealing their involvement in transporting and detoxifying various heavy metals beyond Fe (T. Li et al., 2023; Sharma et al., 2020).

The expression profiles of *PhvulVIT* genes in the root tissues of bean were examined utilizing SRA data under heavy metal stress conditions (Control, Cu, Co, Zn, and Mn). It is observed that the expression levels of *PhvulVIT* genes in root tissue vary according to different heavy metals (Figure 10). When Cu treatment was compared with the control, it was determined that the expression of *PhvulVIT-1* and *PhvulVIT-3* genes increased significantly, the expression of *PhvulVIT-4* and *PhvulVIT-7* genes did not change much, and the expression levels of *PhvulVIT-2*, *PhvulVIT-6*, *PhvulVIT-9*, and *PhvulVIT-10* genes decreased significantly. In roots exposed to Co metal, the *PhvulVIT-7* gene was significantly up-regulated compared to the control, and the *PhvulVIT-2*, *PhvulVIT-6*, and *PhvulVIT-9* genes were significantly down-regulated. In response to Zn stress, the expression levels of the *PhvulVIT-7*, *PhvulVIT-10*, and *PhvulVIT-11* genes were remarkably increased compared to the control. Conversely, the expression levels of the *PhvulVIT-2* and *PhvulVIT-9* genes showed a significant decline. On the other hand, when Mn treatment was evaluated, *PhvulVIT-6*, *PhvulVIT-7*, *PhvulVIT-10*, and *PhvulVIT-11* genes were found to have higher expression compared to the control, while *PhvulVIT-2* and *PhvulVIT-9* genes had significantly decreased expression. When all treatments were evaluated, a significant decrease was observed in the expression profiles of *PhvulVIT-2* and *PhvulVIT-9* genes compared to the control. The *PhvulVIT-10* gene demonstrates a notable increase, particularly when exposed to Zn and Mn stress, outperforming other treatment conditions. This highlights its potential significance in stress response mechanisms (Figure 10).

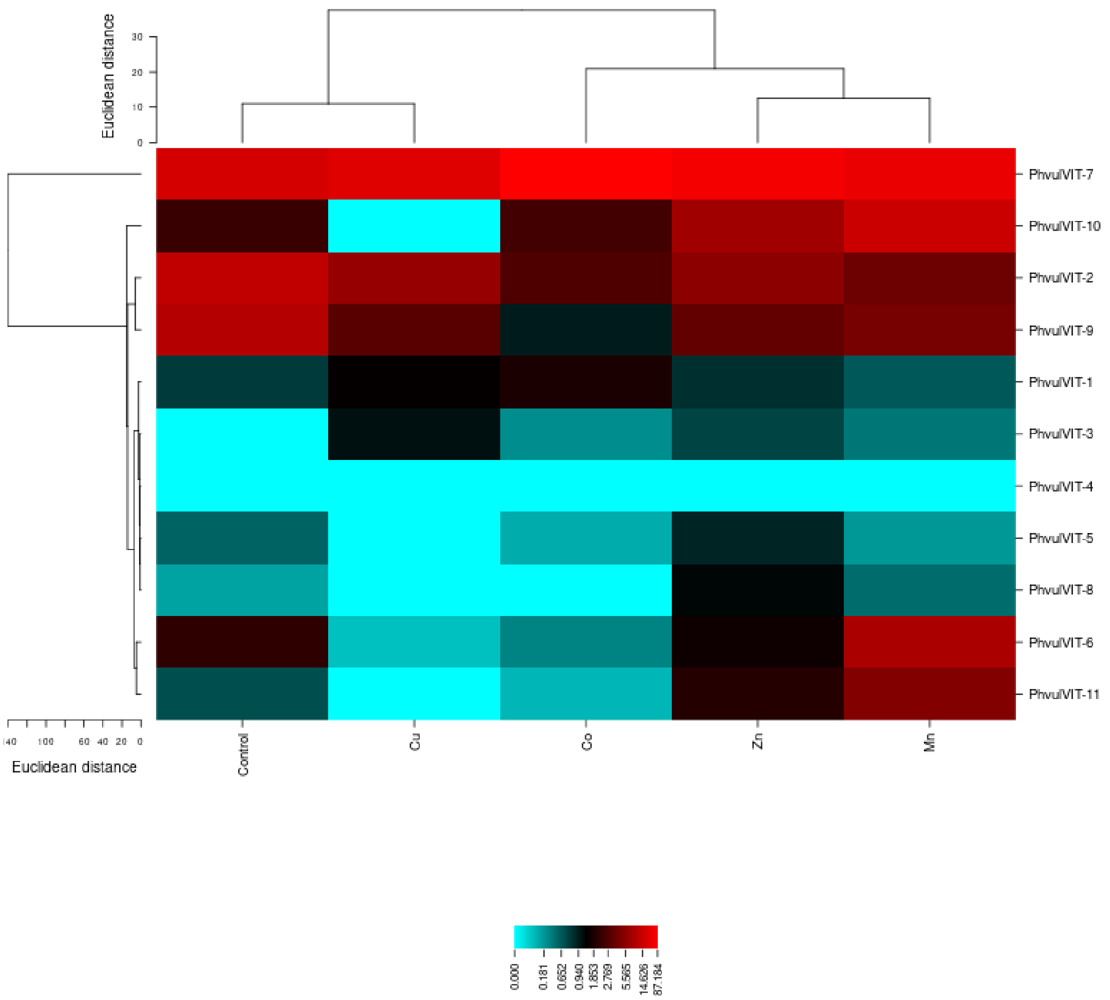


Figure 10. Figure 10. Expression profiles of *PhvulVIT* genes in *P. vulgaris* roots under heavy metal stress conditions. Each row corresponds to a specific *PhvulVIT* gene, while the columns represent different treatment conditions (Control, Cu, Co, Zn, and Mn). The color gradient indicates the expression levels of the *PhvulVIT* genes, ranging from blue (indicating low expression) to red (indicating high expression).

Sharma et al. (2020) expression study results indicate that *VTL* genes may play a role in giving tolerance to high Fe and Zn levels in soil. Furthermore, under Ni, Cd and Co stress, down-regulation of the majority of *TaVTL* genes was shown in roots, and up-regulation of the majority of *TaVTL* genes occurred in Co stress. A transgenic study characterizes *OsVIT1* and *OsVIT2* in rice, orthologs of *Arabidopsis VIT1*, as vacuolar transporters for Fe, Zn, and Mn. Disrupting *OsVIT1* and *OsVIT2* increased Fe/Zn accumulation in rice seeds while reducing levels in flag leaves, enhancing nutrient translocation (Zhang et al., 2012). Consequently, these results emphasize the crucial role of VIT/VTL proteins in maintaining Fe, Mn, and Zn homeostasis, as their altered expression profiles under stress conditions reveal differential gene responses, highlighting their potential in heavy metal stress adaptation and suggesting that VIT gene modulation could be a promising strategy for biofortifying staple crops.

4. Conclusion

This study provides a comprehensive genome-wide analysis of the vacuolar iron transporter (*VIT*) gene family in *Phaseolus vulgaris*, highlighting their critical roles in heavy metal stress tolerance. A total of 11 *PhvulVIT* genes were determined and systematically characterized, revealing significant insights into their structural features, regulatory mechanisms, and evolutionary relationships. It was also found that *PhvulVIT* genes have conserved Ccc1 domains, which are necessary for them to function as metal ion transporters. Protein-protein interaction networks revealed the involvement of PhvulVIT proteins in complicated pathways regulating homeostasis and stress responses. Homology modeling confirmed conserved 3D structures, with alpha-helices and transmembrane regions enabling efficient ion transport across vacuolar membranes. Differential expression profiles of *PhvulVIT* genes under various metal stress conditions in bean root tissues suggest their different roles in metal stress adaptation. *PhvulVIT-10* appears as a key player in responding to Zn and Mn stress, while *PhvulVIT-2* and *PhvulVIT-9* consistently exhibit decreased expression across all treatments. The study highlights the *PhvulVIT* gene family's role in enhancing stress resilience in *Phaseolus vulgaris*, paving the way for future research in biofortification, environmental stress management, and sustainable agriculture.

Conflict of Interest

The author has no financial or non-financial interests that could impact this study's research.

References

Aizaz, M., Khan, I., Lubna, Asaf, S., Bilal, S., Jan, R., Khan, A. L., Kim, K. M., & AL-Harrasi, A. (2023). Enhanced physiological and biochemical performance of mung bean and maize under saline and heavy metal stress through application of endophytic fungal strain SL3 and exogenous IAA. *Cells*, *12*(15), 1960. https://doi.org/10.3390/cells12151960

Aygören, A. S., Güneş, E., Muslu, S., Kasapoğlu, A. G., Yiğider, E., Aydın, M., Büyük I., & İlhan, E. (2023). Genome-wide analysis and characterization of *SABATH* gene family in *Phaseolus vulgaris* genotypes subject to melatonin under drought and salinity stresses. *Plant Molecular Biology Reporter*, *41*, 242-259. https://doi.org/10.1007/s11105-022-01363-5

Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., Ren, J., Li, W. W., & Noble, W. S. (2009). MEME SUITE: Tools for motif discovery and searching. *Nucleic Acids Research*, *37*(suppl_2), W202-W208. https://doi.org/10.1093/nar/gkp335

Bano, Y., Khan, R., Sharma, J., & Shrivastav, A. (2019). Changes in activities of nitrogen metabolism enzymes in arsenic stressed *Phaseolus vulgaris*. *International Journal of Scientific Research and Review*, *8*(4), 336-341.

Buttanri, A., Kasapoğlu, A. G., Öner, B. M., Aygören, A. S., Muslu, S., İlhan, E., Yildirim, E., & Aydin, M. (2024). Predicting the role of *β-GAL* genes in bean under abiotic stress and genome-wide characterization of *β-GAL* gene family members. *Protoplasma*, *262*, 365-383. https://doi.org/10.1007/s00709-024-01998-z

Cao, J. (2019). Molecular evolution of the *Vacuolar Iron Transporter (VIT)* family genes in 14 plant species. *Genes*, *10*(2), 144. https://doi.org/10.3390/genes10020144

Chen, C., Wu, Y., Li, J., Wang, X., Zeng, Z., Xu, J., Liu, Y., Feng, J., Chen, H., & He, Y., (2023). TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. *Molecular Plant*, *16*(11), 1733-1742. https://doi.org/10.1016/j.molp.2023.09.010

Chen, X., Zhao, Y., Zhong, Y., Chen, J., & Qi, X. (2023). Deciphering the functional roles of transporter proteins in subcellular metal transportation of plants. *Planta*, *258*, 17. https://doi.org/10.1007/s00425-023-04170-8

Connorton, J. M., Jones, E. R., Rodríguez-Ramiro, I., Fairweather-Tait, S., Uauy, C., & Balk, J. (2017). Wheat vacuolar iron transporter TaVIT2 transports Fe and Mn and is effective for biofortification. *Plant Physiology*, *174*(4), 2434-2444. https://doi.org/10.1104/pp.17.00672

Cui, Y., Zhao, Q., Hu, S., & Jiang, L. (2020). Vacuole biogenesis in plants: How many vacuoles, how many models? *Trends in Plant Science*, *25*(6), 538-548. https://doi.org/10.1016/j.tplants.2020.01.008

Daszkiewicz, T. (2022). Food production in the context of global developmental challenges. *Agriculture*, *12*(6), 832. https://doi.org/10.3390/agriculture12060832

Dey, S., Malviya, R., Pandey, A., Banavath, H. N., Muthamilarasan, M., & Gayen, D. (2023). Identification and expression analysis of the FtsH protein family in chickpea in response to drought stress. *ResearchSquare*, 1-34. https://doi.org/10.21203/rs.3.rs-3505392/v1

Fang, P., Hu, Y., Xia, W., Wu, X., Sun, T., Pandey, A. K., Ning, K., Zhu, C., & Xu, P. (2022). Transcriptome dynamics of common bean roots exposed to various heavy metals reveal valuable target genes and promoters for genetic engineering. *Journal of Agricultural and Food Chemistry*, *71*(1), 223-233. https://doi.org/10.1021/acs.jafc.2c06301

Gollhofer, J., Schläwicke, C., Jungnick, N., Schmidt, W., & Buckhout, T. J. (2011). Members of a small family of nodulin-like genes are regulated under iron deficiency in roots of *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, *49*(5), 557-564. https://doi.org/10.1016/j.plaphy.2011.02.011

Gu, D., Andreev, K., & Dupre, M. E. (2021). Major trends in population growth around the world. *China CDC Weekly*, *3*(28), 604-613. https://doi.org/10.46234/ccdcw2021.160

Hall, C., Dawson, T. P., Macdiarmid, J. I., Matthews, R. B., & Smith, P. (2017). The impact of population growth and climate change on food security in Africa: Looking ahead to 2050. *International Journal of Agricultural Sustainability*, *15*(2), 124-135. https://doi.org/10.1080/14735903.2017.1293929

Hammami, H., Parsa, M., Bayat, H., & Aminifard, M. H. (2022). The behavior of heavy metals in relation to their influence on the common bean (*Phaseolus vulgaris*) symbiosis. *Environmental and Experimental Botany*, *193*, 104670. https://doi.org/10.1016/j.envexpbot.2021.104670

Horton, P., Park, K. J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. J., & Nakai, K. (2007). WoLF PSORT: Protein localization predictor. *Nucleic Acids*

Research, *35*(suppl_2), W585-W587. https://doi.org/10.1093/nar/gkm259

Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J., & Gao, G. (2015). GSDS 2.0: An upgraded gene feature visualization server. *Bioinformatics*, *31*(8), 1296-1297. https://doi.org/10.1093/bioinformatics/btu817

Inal, B., Muslu, S., Yigider, E., Kasapoglu, A., Ilhan, E., Ciltas, A., Yildirim, E., & Aydin, M., (2024). In silico analysis of *Phaseolus vulgaris* L. metalloprotease *FtsH* gene: Characterization and expression in drought and salt stress. *Genetic Resources and Crop Evolution*, *72*, 1065-1088. https://doi.org/10.1007/s10722-024-02031-1

Isıyel, M., İlhan, E., Kasapoğlu, A. G., Muslu, S., Öner, B. M., Aygören, A. S., Yigider, E., Aydin, M., & Yıldırım, E. (2024). Identification and characterization of *Phaseolus vulgaris CHS* genes in response to salt and drought stress. *Genetic Resources and Crop Evolution*, *72*, 271-293. https://doi.org/10.1007/s10722-024-01980-x

Kaur, R., Das, S., Bansal, S., Singh, G., Sardar, S., Dhar, H., & Ram, H. (2021). Heavy metal stress in rice: Uptake, transport, signaling, and tolerance mechanisms. *Physiologia Plantarum*, *173*(1), 430-448. https://doi.org/10.1111/ppl.13491

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, *10*, 845-858. https://doi.org/10.1038/nprot.2015.053

Khalil, R., Haroun, S., Bassyoini, F., Nagah, A., & Yusuf, M. (2021). Salicylic acid in combination with kinetin or calcium ameliorates heavy metal stress in *Phaseolus vulgaris* plant. *Journal of Agriculture and Food Research*, *5*, 100182. https://doi.org/10.1016/j.jafr.2021.100182

Khoudi, H. (2021). Significance of vacuolar proton pumps and metal/H+ antiporters in plant heavy metal tolerance. *Physiologia Plantarum*, *173*(1), 384-393. https://doi.org/10.1111/ppl.13447

Kim, S. A., Punshon, T., Lanzirotti, A., Li, L., Alonso, J. M., Ecker, J. R., Kaplan, J., & Guerinot, M. L. (2006). Localization of iron in *Arabidopsis* seed requires the vacuolar membrane transporter VIT1. *Science*, *314*(5803), 1295-1298. https://doi.org/10.1126/science.1132563

Kosakivska, I. V., Babenko, L. M., Romanenko, K. O., Korotka, I. Y., & Potters, G. (2021). Molecular mechanisms of plant adaptive responses to heavy metals stress. *Cell Biology International*, *45*(2), 258-272. https://doi.org/10.1002/cbin.11503

Krishna, T. P. A., Ceasar, S. A., & Maharajan, T. (2023). Biofortification of crops to fight anemia: Role of vacuolar iron transporters. *Journal of Agricultural and Food Chemistry*, *71*(8), 3583-3598. https://doi.org/10.1021/acs.jafc.2c07727

Kumar, N., Sharma, V., Kaur, G., Lata, C., Dasila, H., Perveen, K., Khan, F., Gupta, V. K., & Khanam, M. N. (2023). Brassinosteroids as promoters of seedling growth and antioxidant activity under heavy metal zinc stress in mung bean (*Vigna radiata* L.). *Frontiers in Microbiology*, *14*, 1259103. https://doi.org/10.3389/fmicb.2023.1259103

Lapinskas, P. J., Lin, S. J., & Culotta, V. C. (1996). The role of the *Saccharomyces cerevisiae CCC1* gene in the homeostasis of manganese ions. *Molecular Microbiology*, *21*(3), 519-528. https://doi.org/10.1111/j.1365-2958.1996.tb02561.x

Letunic, I., & Bork, P. (2024). Interactive Tree of Life (iTOL) v6: Recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Research*, *52*(W1), W78-W82. https://doi.org/10.1093/nar/gkae268

Li, J., Zhang, M., Sun, J., Mao, X., Wang, J., Liu, H., Zheng, H., Li, X., & Zou, D. (2020). Heavy metal stress-associated proteins in rice and *Arabidopsis*: Genome-wide identification, phylogenetics, duplication, and expression profiles analysis. *Frontiers in Genetics*, *11*, 477. https://doi.org/10.3389/fgene.2020.00477

Li, T., Zhang, X. M., Gao, J. L., Wang, L., Si, L., Shu, Y. J., Guo, C. H., Lai, Y. C., Bi, Y. D., & Guo, D. L. (2023). Soybean *GmVIT1* gene confers plant tolerance to excess Fe/Mn stress. *Agronomy*, *13*(2), 384. https://doi.org/10.3390/agronomy13020384

Lisciani, S., Marconi, S., Le Donne, C., Camilli, E., Aguzzi, A., Gabrielli, P., Gambelli, L., Kunert, K., Marais, D., Vorster, B. J., Alvarado-Ramos, K., Reboul, E., Cominelli, E., Preite, C., Sparvoli, S., Losa, A., Sala, T., Botha, A. M., & Ferrari, M. (2024). Legumes and common beans in sustainable diets: Nutritional quality, environmental benefits, spread and use in food preparations. *Frontiers in Nutrition*, *11*, 1385232. https://doi.org/10.3389/fnut.2024.1385232

Mansour, M. M. F. (2023). Role of vacuolar membrane transport systems in plant salinity tolerance. *Journal of Plant Growth Regulation*, *42*, 1364-1401. https://doi.org/10.1007/s00344-022-10655-9

Noor, I., Sohail, H., Sun, J., Nawaz, M. A., Li, G., Hasanuzzaman, M., & Liu, J. (2022). Heavy metal and metalloid toxicity in horticultural plants: Tolerance mechanism and remediation strategies. *Chemosphere*, *303*(Part 3), 135196. https://doi.org/10.1016/j.chemosphere.2022.135196

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Trong, I. L., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., & Miyano, M. (2000). Crystal structure of rhodopsin: A G protein-coupled receptor. *Science*, *289*(5480), 739-745. https://doi.org/10.1126/science.289.5480.739

Peng, X., Wang, J., Peng, W., Wu, F. X., & Pan, Y. (2017). Protein–protein interactions: Detection, reliability assessment and applications. *Briefings in*

Bioinformatics, *18*(5), 798-819. https://doi.org/10.1093/bib/bbw066

Qi, M., Wang, S., Li, N., Li, L., Zhang, Y., Xue, J., Wang, J., Wu, R., & Lian, N. (2023). Genome-wide analysis of *TPX2* gene family in *Populus trichocarpa* and its specific response genes under various abiotic stresses. *Frontier Plant Science*, *14*, 1159181. https://doi.org/10.3389/fpls.2023.1159181

Ram, H., Sardar, S., & Gandass, N. (2021). Vacuolar Iron Transporter (Like) proteins: Regulators of cellular iron accumulation in plants. *Physiologia Plantarum*, *171*(4), 823-832. https://doi.org/10.1111/ppl.13363

Rasool, A., Azeem, F., Ur-Rahman, M., Rizwan, M., Hussnain Siddique, M., Bay, D. H., Binothman, N., Al Kashgry, N. A.T., & Qari, S. H. (2023). Omics-assisted characterization of two-component system genes from *Gossypium raimondii* in response to salinity and molecular interaction with abscisic acid. *Frontiers in Plant Science*, *14*, 1138048. https://doi.org/10.3389/fpls.2023.1138048

Rombauts, S., Déhais, P., Van Montagu, M., & Rouzé, P. (1999). PlantCARE, a plant *cis*-acting regulatory element database. *Nucleic Acids Research*, *27*(1), 295-296. https://doi.org/10.1093/nar/27.1.295

Salam, A., Afridi, M. S., Khan, A. R., Azhar, W., Shuaiqi, Y., Ulhassan, Z., Qi J., Xuo N., Chunyan Y., Chen N., & Gan, Y. (2023). Cobalt induced toxicity and tolerance in plants: Insights from omics approaches. In M. A. Hossain, AKM. Z. Hossain, S. Bourgerie, M. Fujita, O. P. Dhankher & P. Haris (Eds.), *Heavy metal toxicity and tolerance in plants: A biological, omics, and genetic engineering approach* (pp. 207-229). John Wiley & Sons Ltd. https://doi.org/10.1002/9781119906506.ch10

Shahid, M., Shamshad, S., Farooq, A. B. U., Rafiq, M., Khalid, S., Dumat, C., Zhang, Y., Hussain, I., & Niazi, N. K. (2019). Comparative effect of organic amendments on physio-biochemical traits of young and old bean leaves grown under cadmium stress: a multivariate analysis. *Environmental Science and Pollution Research*, *26*, 11579-11590. https://doi.org/10.1007/s11356-018-2689-4

Sharma, S. S., Dietz, K. J., & Mimura, T. (2016). Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. *Plant, Cell & Environment*, *39*(5), 1112-1126. https://doi.org/10.1111/pce.12706

Sharma, S., Kaur, G., Kumar, A., Meena, V., Ram, H., Kaur, J., & Pandey, A. K. (2020). Gene expression pattern of vacuolar-iron transporter-like (VTL) genes in hexaploid wheat during metal stress. *Plants*, *9*(2), 229. https://doi.org/10.1101/863084

Shekhawat, P. K., Sardar, S., Yadav, B., Salvi, P., Soni, P., & Ram, H. (2023). Meta-analysis of transcriptomics studies identifies novel attributes and set of genes involved in iron homeostasis in rice. *Functional & Integrative Genomics*, *23*, 336. https://doi.org/10.1007/s10142-023-01265-z

Silva-Gigante, M., Hinojosa-Reyes, L., Rosas-Castor, J. M., Quero-Jiménez, P. C., Pino-Sandoval, D. A., & Guzmán-Mar, J. L. (2023). Heavy metals and metalloids accumulation in common beans (*Phaseolus vulgaris* L.): A review. *Chemosphere*, *335*, 139010. https://doi.org/10.1016/j.chemosphere.2023.139010

Singh, P., & Mukhopadhyay, K. (2021). Comprehensive molecular dissection of TIFY Transcription factors reveal their dynamic responses to biotic and abiotic stress in wheat (*Triticum aestivum* L.). *Scientific Reports*, *11*, 9739. https://doi.org/10.1038/s41598-021-87722-w

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., & Pyysalo, S., (2023). The STRING database in 2023: Protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, *51*(D1), 638-646. https://doi.org/10.1093/nar/gkac1000

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022-3027. https://doi.org/10.1093/molbev/msab120

Voorrips, R. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, *93*(1), 77-78. https://doi.org/10.1093/jhered/93.1.77

Wainaina, I., Wafula, E., Sila, D., Kyomugasho, C., Grauwet, T., Van Loey, A., & Hendrickx, M. (2021). Thermal treatment of common beans (*Phaseolus vulgaris* L.): Factors determining cooking time and its consequences for sensory and nutritional quality. *Comprehensive Reviews in Food Science and Food Safety*, *20*(4), 3690-3718. https://doi.org/10.1111/1541-4337.12770

White, S. H., & Wimley, W. C. (1999). Membrane protein folding and stability: physical principles. *Annual Review of Biophysics and Biomolecular Structure*, *28*, 319-365. https://doi.org/10.1146/annurev.biophys.28.1.319

Zhang, Y., Xu, Y. H., Yi, H. Y., & Gong, J. M. (2012). Vacuolar membrane transporters OsVIT1 and OsVIT2 modulate iron translocation between flag leaves and seeds in rice. *The Plant Journal*, *72*(3), 400-410. https://doi.org/10.1111/j.1365-313x.2012.05088.x

Zhu, W., Zuo, R., Zhou, R., Huang, J., Tang, M., Cheng, X., Liu, Y., Tong, C., Xiang, Y., Dong, C., & Liu, S. (2016). Vacuolar iron transporter *BnMEB2* is involved in enhancing iron tolerance of *Brassica napus*. *Frontiers in Plant Science*, *7*, 1353. https://doi.org/10.3389/fpls.2016.01353

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RESEARCH ARTICLE

Enhanced Lipid Yield from Olive-Mill Wastewater by *Yarrowia lipolytica* NRRL YB-423

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ABSTRACT

Lipid production from olive-mill wastewater (OMW) by *Yarrowia lipolytica* NRRL YB-423 was optimized (biomass concentration and lipid yield based on dry cell weight) using multi-response criteria based on the Taguchi orthogonal array. Sixteen experimental runs were performed using the L16 orthogonal array. Dilution rates of OMW (15, 30, 45, and 60%), Tween 80 (0, 0.2, 0.4, and 0.6%), sodium chloride (NaCl; 0, 1, 2, and 3%), and sterility were selected as factors. The significance of the parameters was determined using analysis of variance (ANOVA). The effects of all factors on the lipid yield were statistically significant ($p<0.05$). The results showed that sterility had a maximum contribution of 48.12% to lipid yield. The highest lipid yield (40.88 %) was achieved in sterile medium supplemented with 15% diluted OMW, 0.6% Tween 80, and 3% NaCl.

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1. Introduction

The global annual olive oil production is approximately 3.32 million, predominantly concentrated in Europe, accounting for 72% of the total. The primary producers are Spain (42%), Italy (17%), and Greece (11%), whereas other Mediterranean countries such as Türkiye (6%), Syria (6%), Tunisia (6%), Morocco (4%), Jordan (3%), and Lebanon (1.5%) contribute to the remaining production share (Khdair & Abu-Rumman, 2020). Olive oil is obtained from olives using physical methods such as crushing, malaxation, and oil phase separation. These steps are entegrated to three methods: traditional discontinuous pressing, continuous three-phase horizontal centrifuge systems, and continuous two-phase horizontal centrifuge systems. The first two processes necessitate the use of hot water, resulting in the generation of substantial quantities of liquid waste, commonly known as olive mill wastewater (OMW). The two-phase system separates olive pulp into oil and wet solid residue (Abrunhosa et al., 2013). Approximately 20% of the final product is in the oily phase, with the remainder comprising of solid waste (approximately 30%) and OMW (up to 50%) (Alique et al., 2020).

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OMW elevated organic content necessitates considerable oxygen consumption, resulting in surface waters eutrophication. Additionally, OMW diminishes soil quality by inducing water repellence, which inhibits seed germination and plant growth (Ayadi et al., 2022). Direct disposal of OMW into sewage systems or its use as an organic fertilizer in agriculture is not advisable because of its potential to disrupt the efficiency of sewage treatment plants and adversely affect microbial soil properties. On the other hand, the phytotoxic and antibacterial effects of OMW are primarily due to its polyphenolic content. Therefore, biological degradation of OMW phenolic compounds is regarded as a safer, more effective, and less costly approach for pollutant reduction than other methods (Dahmen-Ben Moussa et al., 2021). The phenolic content of OMW varies widely based on the olive variety, maturity, and oil extraction technology (Diamantis et al., 2022).

Biodiesel has attracted increasing attention as an alternative to petroleum-based fuel, driven by concerns over the energy crisis and the environmental consequences of fossil fuel combustion. Biodiesel, which is derived from renewable sources such as vegetable oils, animal fats, and waste oils, is a biodegradable and non-toxic biofuel. Moreover, microorganisms are now recognized as novel sources of lipids, known as “second-generation biodiesel” (Arous et al., 2016). Microbial oils are lipids synthesized by oleaginous microorganisms. Conventionally, bacteria, yeasts, molds, and microalgae capable of accumulating lipids exceeding 20% of their dry weight are classified as oleaginous microorganisms (Huang et al., 2013). Microbial lipids exhibit unique compositions and structures that are useful in the highly interesting to food and pharmaceutical industries (Sarris et al., 2019). Considering the depletion of crude oil, the “food-or-fuel” debate regarding plant oils for biodiesel production, overfishing, and the need to reduce greenhouse gas emissions, microbial lipids offer promising alternatives to crude oil, plant oil, and fish oil. Microbial lipid production is independent of season, climate, and location (Ochsenreither, 2016).

An estimated 10-30 m^3 million OMW are produced globally annually (Dias et al., 2021). Bacteria typically struggle to break down the complex polyphenols responsible for OMW’s dark coloration of OMWs. Filamentous fungi are constrained by the challenges of obtaining a homogeneous culture and long fermentation cycles. In contrast, yeasts are promising because of their adaptability and resistance to high phenolic concentrations and low pH. OMW contains essential carbohydrates, proteins, and minerals, which provide the necessary components for fermentation (Benhoula et al., 2023). *Yarrowia lipolytica*, an oleaginous yeast, has been extensively studied owing to its fully sequenced genome and well-known metabolism (Fabiszewska et al., 2019). *Y. lipolytica* is commonly employed in OMW bioremediation systems because of its high capacity to catabolize polyphenols and produce value-added products (Hamimed et al., 2021). This study focused on optimizing lipid production from OMW using *Y. lipolytica* NRRL YB-423. To achieve this objective, the Taguchi method was employed to systematically investigate the effects of the OMW dilution ratio, amount of Tween 80 and sodium chloride (NaCl), and sterility on the biomass concentration and lipid yield.

2. Materials and Methods

2.1. OMW, Microorganism and Culture Conditions

OMW was supplied by a local olive oil production plant located in İzmir, Türkiye, which has a three-phase olive oil extraction process. The samples were stored at -18°C until analysis. Before analysis, the samples were defrozen, and the solid parts were removed by centrifugation at 5000 rpm for 10 min (Thermo Fisher MR23I, Germany). *Yarrowia lipolytica* NRRL YB-423 (ATCC 18942) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Lipid production experiments were conducted using a modified culture medium with the following composition (g/L): glucose 35, KH_2PO_4 7, Na_2HPO_4 2.5, $CaCl_2.2H_2O$ 0.15, $MnSO_4.H_2O$ 0.06, $ZnSO_4.7H_2O$ 0.02, $FeCl_3.6H_2O$ 0.15, and $MgSO_4.7H_2O$ 1.5 (Sarris et al., 2014). The culture medium was sterilized in an autoclave at 121°C for 15 min. Shake-flask experiments were carried out in 250 mL Erlenmeyer flasks, each containing 50 mL of culture medium. The medium was inoculated with 1 mL of pre-culture containing 10^6 cells. After inoculation, the cultures were incubated on a rotary shaker (JSSI-100; JS Research, Gongju, Korea) at 28°C and 180 rpm. The initial pH value of fermentation medium was 6.5, and the fermentation lasted for 6 days.

2.2. OMW Key Indicators: pH Level, Phenolic Content, Color, and Salt

The pH level of OMW was measured using a pH meter (Mettler Toledo, Switzerland). The phenol concentration in OMW was determined based on the method described by Qwele et al. (2013). Briefly, 0.1 mL of the sample was mixed with 0.2 mL of Folin-Ciocalteu reagent and 3 mL of 5% Na_2CO_3 solution. The reaction mixture was then vortexed and kept at 23°C for 1 h. Finally, the absorbance of the phenolic compounds in 10-fold diluted sample was measured at 765 nm using a UV-visible spectrophotometer (Aquamate 9423 AQA 2000E, Thermo Scientific, England). Phenolic concentrations were quantified using a standard curve prepared with gallic acid and expressed as gallic acid equivalents per liter of OMW. The color of OMW was measured at 395 nm using a UV-visible spectrophotometer. As the absorbance of the undiluted sample exceeded the measurable limit, measurements were conducted using 25-fold diluted samples (Flouri et al., 1996). The NaCl content of the OMW samples was analyzed using the Volhard method (ISO 1841-1, 1996).

2.3. Determination of Biomass Concentration

Dry cell weight was determined to calculate biomass production. For this purpose, the culture broths were centrifuged (Thermo Fisher MR23I, Germany) at 5000 rpm for 10 min. The cell pellets were washed twice with distilled water, dried to a constant weight at 80°C, and then weighed (Chatzifragkou et al., 2011).

2.4. Total Lipid Production

In the modified method combined with mechanical disruption, the biomass was treated with 8 mL of 4 M HCl solution and 0.7 mm diameter glass beads, followed by waited in an ultrasonic bath at 60°C for 2 h (Kuttiraja et al., 2016; Yu et al., 2015). The acid-hydrolyzed biomass was stirred in 16 mL of a chloroform/methanol mixture (1:1) for 2-3 h at room temperature. Finally, centrifugation was applied at 5000 rpm for 5 min to separate the aqueous upper phase from the organic lower phase. The lipid-containing sub-phase was then removed, and the solvents were evaporated at 40°C in a vacuum rotary evaporator (Buchi Heating Bath B-490, Switzerland) (Enshaeieh et al., 2014). Lipid content was determined as the weight (g) of lipid produced per liter of medium and lipid yields were calculated based on the dry weight of the cells (El-Fadaly et al., 2009).

2.5. Statistical Analysis

The primary reason for choosing the Taguchi method is its ability to simultaneously examine multiple parameters under a limited number of experimental conditions. The Signal-to-Noise (S/N) ratio is a statistical measure used to determine the optimal levels of the control factors by minimizing variability and maximizing performance. This reflects the robustness of a process by evaluating the relationship between the desired signal (mean performance) and undesired noise (variability). The factors and levels were selected to optimize the biomass concentration and lipid yield, as shown in Table 1. The experimental design was created by using a mixed-level design option in the Taguchi model [L16($4^3 \times 2^1$)]. Experiments were conducted in duplicate under the conditions outlined in the experimental design. Analysis of variance (ANOVA) was used to determine the statistical significance of the experimental parameters. The experiments were designed using the Taguchi method in Minitab (version 19; State College, PA, USA). The experimental results were presented as the mean ± standard deviation.

Table 1. Factors and levels for the process optimization.

Factors	Level 1	Level 2	Level 3	Level 4
Dilution rate (%)	15	30	45	60
Tween 80 (%)	0	0.2	0.4	0.6
NaCl (%)	0	1	2	3
Sterility	0	1		

3. Results and Discussion

3.1. Physicochemical Analysis of OMW

Physicochemical analyses conducted for the characterization of OMW, along with their corresponding results, are shown in Table 2. The chemical composition of OMW varies significantly based on factors such as the olive variety, growing method, harvest timing, and oil extraction method (Roig et al., 2006). Ochando-Pulido et al. (2017) reported that pH values in three-phase and two-phase extraction processes range between 3.5-5.5 and 3.5-6.0, respectively. These results clearly indicated that the OMWs exhibited acidic pH levels, primarily owing to the presence of organic acids. The pH levels are also influenced by olive ripeness and storage conditions after harvest. In contrast, the standard methods of olive processing do not affect pH levels (Barbera et al., 2013). The phenolic compound concentration in OMW varies from 0.5 to 24 g/L (Paraskeva & Diamadopoulos, 2006). Previous studies have reported significantly higher phenol contents (Buchmann et al., 2015; Dourou et al., 2016; Sarris et al., 2013) and lower pH values (Aggoun et al., 2016; Paredes et al., 1999; Sarris et al., 2023a,b). The mineral salt content of OMW typically ranges from 0.4% to 2.5%, as reported by Fattoum et al. (2023). However, in our sample, the mineral salt content was observed to be lower than this range.

Table 2. Characterization of OMW.

Parameter	Results
pH	6.50±0.05
Salt (%)	0.27±0.01
Color	0.22±0.00
Phenol content (mg/L gallic acid)	557.20±7.06

Data are mean values ± standard deviation.

3.2. Optimization of Lipid Production

Yeasts are capable of degrading phenols, exhibiting resistance to phages, and demonstrating greater tolerance to high osmolarity, pH, and solvents (Singh et al., 2022). OMW is characterized by a high content of phenolic compounds, which have antioxidant and antibacterial activities (Roila et al., 2024). For this reason, one of the simplest methods to reduce the initial phenolic content is to dilute OMW to decrease its antimicrobial effect in the fermentation medium. In this study, different OMW dilution rates were evaluated in terms of biomass and lipid production by *Y. lipolytica* NRRL YB-423. On the other hand, Tween 80 is a widely recognized non-ionic polyoxyethylene detergent and surfactant (Sipiczki et al., 2024). Tween 80 is used as a vehicle for the addition of water-insoluble compounds. It can alter cell membrane permeability, thereby facilitating the uptake of nutrients from the environment into the cells (Taoka et al., 2011). In this study, we investigated the effects of different Tween 80 amounts on biomass and lipid production. Moreover, NaCl was added to the

OMW-based medium to evaluate the formation of biomass and lipids under osmotic stress. Finally, considering the advantages of non-sterile production, particularly on an industrial scale, the effects of this factor on production under various conditions were investigated.

Table 2 shows biomass and lipid concentrations, lipid yields, and S/N ratios. When the biomass level exceeded 1 g/L, it was determined that this occurred under non-sterile conditions, likely due to contamination, which did not cause an increase in lipid yield. The highest lipid yield (40.88 %) was observed in a sterile medium containing 15% diluted OMW, 0.6% Tween 80, and 3% NaCl. The second and third highest lipid yield were 38.40% (with 0.2% Tween 80) and 32.34% (with 1% NaCl), respectively, under sterile conditions, but with 30% diluted OMW. The S/N ratio should be at the maximum level for optimum conditions to minimize the effect of noise. As shown in Table 2, the S/N ratio was the highest under the conditions where the highest biomass concentration and lipid yield were achieved.

Table 2. Experimental design and results for biomass and lipid concentrations, lipid yields, and S/N ratios.

Run	Dilution rate (%)	Tween 80 (%)	NaCl (%)	Sterility	Biomass (g/L)	Lipid (g/L)	Lipid content (w/w dry cell %) ($Y_{L/B}$)	S/N ratio for biomass	S/N ratio for lipid production
1	15	0	0	0	1.23±0.14	0.25±0.02	19.97±0.85	1.79810	26.0076
2	15	0.2	1	0	1.31±0.03	0.32±0.01	24.42±0.39	2.34543	27.7549
3	15	0.4	2	1	0.46±0.00	0.11±0.02	24.65±3.98	-6.74484	27.8363
4	15	0.6	3	1	0.49±0.00	0.20±0.01	40.88±1.02	-6.19608	32.2302
5	30	0	1	1	0.38±0.01	0.12±0.01	32.34±2.66	-8.40433	30.1948
6	30	0.2	0	1	0.53±0.00	0.20±0.02	38.40±3.02	-5.51448	31.6866
7	30	0.4	3	0	1.19±0.11	0.21±0.02	17.73±0.01	1.51094	24.9742
8	30	0.6	2	0	1.27±0.03	0.19±0.01	14.98±0.78	2.07607	23.5102
9	45	0	2	0	0.90±0.02	0.09±0.01	9.97±0.68	-0.91515	19.9739
10	45	0.2	3	0	1.21±0.09	0.20±0.01	16.58±0.54	1.65571	24.3917
11	45	0.4	0	1	0.49±0.03	0.13±0.02	25.77±5.08	-6.19608	28.2223
12	45	0.6	1	1	0.42±0.03	0.14±0.01	31.06±1.31	-7.53501	29.8440
13	60	0	3	1	0.37±0.02	0.10±0.01	26.37±2.88	-8.63597	28.4222
14	60	0.2	2	1	0.44±0.01	0.11±0.00	23.97±0.50	-7.13095	27.5934
15	60	0.4	1	0	0.78±0.02	0.20±0.03	25.42±2,46	-2.15811	28.1035
16	60	0.6	0	0	0.83±0.00	0.21±0.02	24.70±2.37	-1.61844	27.8539

The high cost of microbial lipid production is attributed to the low productivity of oleaginous microorganisms and significant energy requirements for medium sterilization. One solution that can decrease the cost of microbial lipid production is to use a non-sterile culture technique, as it can reduce energy consumption, save time, and reduce the required workload (Polburee & Limtong, 2020). Lipid production is typically performed under sterile conditions to prevent microbial contamination. However, non-sterile lipid production can be successfully achieved through careful optimization of various factors. These include adjusting inoculum size and pH, employing carbon source-only or nutrient-starvation media, supplementing with antimicrobial agents, and utilizing metabolic engineering in oleaginous species (He et al., 2025). In a related study, raw glycerol was converted into microbial lipids by *Zygorhynchus moelleri* under non-aseptic conditions, using nitrogen-limited media containing essential oils and/or antibiotics. The findings revealed that lipid accumulation was not affected by the presence of bacteria in the growth medium compared with aseptic conditions (Moustogianni et al., 2014). As highlighted in the aforementioned study, the successful implementation of a lipid-producing bioprocess under non-aseptic conditions requires the presence of inhibitory factors such as high concentrations of NaCl, essential oils, antibiotics, or phenolic compounds (Filippousi et al., 2022). Although OMW was utilized in this study, maintaining sterility proved to be important. This was likely due to the low phenolic and salt content of the OMW and its further dilution, which diminished its ability to inhibit contamination effectively.

Sarris et al. (2011) found that the presence of OMWs in the medium promoted the accumulation of storage lipids in *Y. lipolytica* W29 strain. In another study, mixtures of OMW and crude glycerol were used for lipid production of *Y. lipolytica* LMBF Y-46 and *Y. lipolytica* ACA-YC 5033 and it was reported that $Y_{L/B}$ did not exceed 16.6% (Sarris et al., 2023b). Dourou et al. (2016) reported that *Lipomyces starkeyi* NRRL Y-11557 and *Y. lipolytica* strains accumulated lipids in OMW-based media ($Y_{L/B}$= 15-25%, w/w). Sarris et al. (2019) explored the potential of *Y. lipolytica* ACA-DC 5029 to grow and produce metabolites in crude glycerol and OMW blends with nitrogen-limited submerged shake-flask cultures. The pH of the culture medium was maintained between at 5.0 and 6.0. They found that the accumulation of microbial oil increased with the addition of OMW (L_{max} ~2.0 g/L, $Y_{L/X}$ ~20% w/w). The $Y_{L/X}$ values remained below 20% despite nitrogen-limited conditions in the growth medium. This result can be attributed to the fact that citric acid production increases at neutral pH levels, whereas lipid production is enhanced under acidic conditions (Zhang et al., 2019). Tzirita et al. (2019) reported that the highest lipid yield (35.1%) was achieved with *Y. lipolytica* ACA-YC 5031 in medium containing glycerol blended with OMW and 5% NaCl, after 308 h. In addition, other studies have reported lipid yields of 60% and above but did not use *Y. lipolytica* (Bellou et al., 2014; Herrero et al., 2018).

The ANOVA results for the biomass concentrations are presented in Table 3. The table shows that among the selected factors, sterility had a stronger influence (87.95%) on the biomass concentration, whereas the amount of NaCl showed the least influence (1.09%). The effects of the dilution rate and sterility were statistically significant ($p<0.05$). Papanikolaou et al. (2008) enriched OMW-based media with commercial glucose for citric acid and lipid production by *Y. lipolytica* ACA-DC 50109. In contrast, the presence of OMWs in the growth medium had almost no effect on the maximum biomass produced, maximum specific growth rate, and biomass yield on consumed glucose.

Table 3. ANOVA for biomass concentrations.

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F	P
Dilution rate (%)	3	16.892	6.38%	16.892	5.631	5.85	0.043
Tween 80 (%)	3	7.333	2.77%	7.333	2.444	2.54	0.170
NaCl (%)	3	2.892	1.09%	2.892	0.964	1.00	0.464
Sterility	1	232.961	87.95%	232.961	232.961	242.11	0.000
Error	5	4.811	1.82%	4.811	0.962		
Total	15	264.890	100%				

DF: Degrees of freedom, Seq SS: Sequential sums of squares, Adj SS: Adjusted sum of square, Adj MS: Adjusted mean square (R^2:98.18%, R^2adj:94.55%).

The ANOVA results (Table 4) showed that among the selected factors, sterility had a stronger influence (48.12%) on lipid yield, whereas the amount of Tween 80 showed the least influence (7.44%). As an important finding, the effects of all selected factors on the process were statistically significant ($p<0.05$). In contrast to the results obtained in our study, Sarris et al. (2017) found that the addition of OMWs to the medium resulted in the accumulation of lipid reserves of *Y. lipolytica* ACA-YC 5033 and no significant differences were observed between aseptic and pasteurized cultures in terms of biomass and total cellular lipid production.

Table 4. ANOVA for lipid yields.

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F	P
Dilution rate (%)	3	18.868	12.98%	18.868	6.2895	10.85	0.013
Tween 80 (%)	3	10.823	7.44%	10.823	3.6075	6.22	0.038
NaCl (%)	3	42.852	29.47%	42.852	14.2839	24.64	0.002
Sterility	1	69.973	48.12%	69.973	69.9729	120.70	0.000
Error	5	2.899	1.99%	2.899	0.5797		
Total	15	145.414	100%				

DF: Degrees of freedom, Seq SS: Sequential sums of squares, Adj SS: Adjusted sum of square, Adj MS: Adjusted mean square (R^2:98.01%, R^2adj:94.02%).

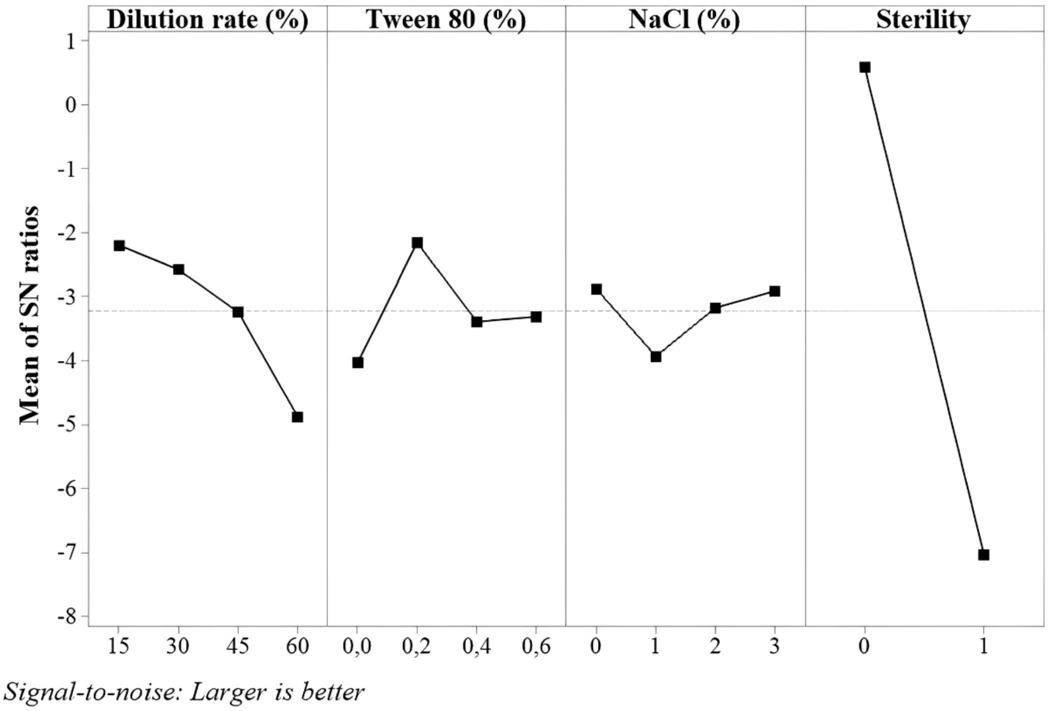

Figure 1. Main effect plot for S/N ratios for biomass concentration.

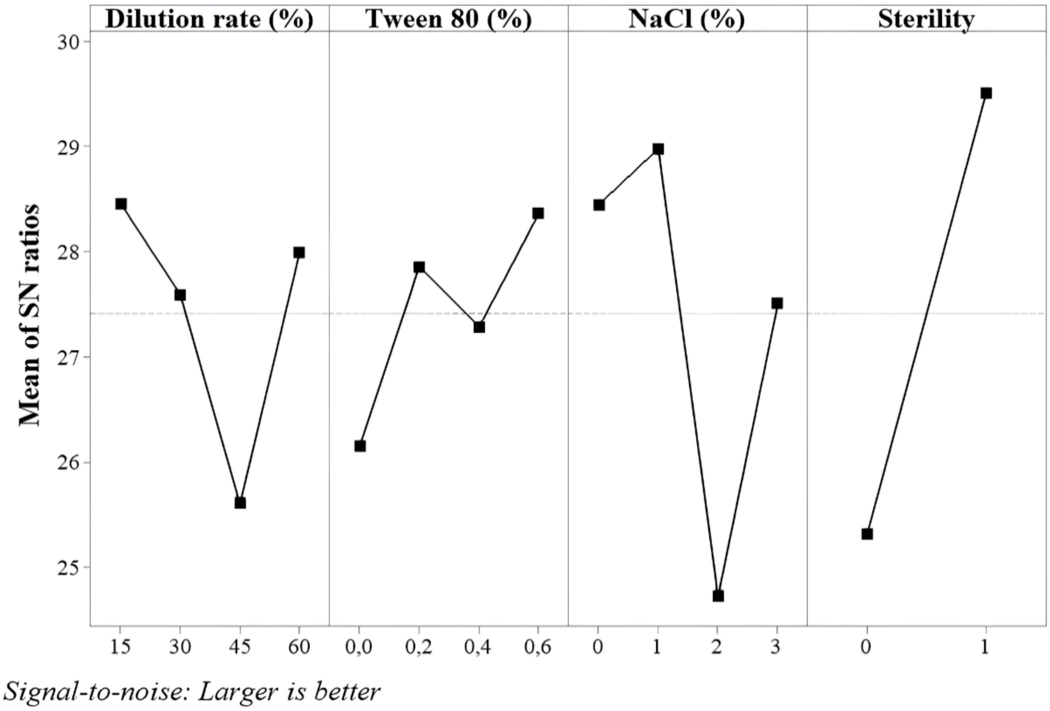

Figure 2. Main effect plot for S/N ratios for lipid yield.

The main effect plots for the S/N ratios of the biomass concentrations and lipid yields are presented in Figure 1 and 2, respectively. The goal of optimizing the process parameters was to improve the S/N ratio for better results. The peak S/N ratio for lipid yield was observed at a 15% dilution rate, 0.6% Tween 80, 1% NaCl, and under sterile process conditions. For biomass concentration, the optimal conditions were 15% dilution rate, 0.2% Tween 80, no NaCl addition, and non-sterile process conditions.

4. Conclusion

The treatment of olive-mill wastewater (OMW), a highly toxic by-product of olive oil production, remains a key environmental challenge, particularly in the Mediterranean countries. In light of this, the conversion of OMW into value-added bioproducts presents a promising solution for mitigating the environmental impact of its disposal. This study focused on the optimization of biomass and lipid production from OMW using *Yarrowia lipolytica* NRRL YB-423. Through the application of the Taguchi method, the experimental conditions were optimized to maximize the lipid yield, demonstrating the potential of *Y. lipolytica* as an efficient microorganism for biotransformation processes. These results underscore the significance of process optimization in enhancing lipid production from OMW, providing a sustainable approach for both waste management and the generation of valuable bioproducts. Future research could focus scaling up the process and further refining the medium composition to increase efficiency.

Conflict of Interest

The authors have no conflict of interest to declare.

References

Abrunhosa, L., Oliveira, F., Dantas, D., Gonçalves, C., & Belo, I. (2013). Lipase production by *Aspergillus ibericus* using olive mill wastewater. *Bioprocess and Biosystems Engineering*, *36*, 285-291. https://doi.org/10.1007/s00449-012-0783-4

Aggoun, M., Arhab, R., Cornu, A., Portelli, J., Barkat, M., & Graulet, B. (2016). Olive mill wastewater microconstituents composition according to olive variety and extraction process. *Food Chemistry*, *209*, 72-80. https://doi.org/10.1016/j.foodchem.2016.04.034

Alique, D., Bruni, G., Sanz, R., Calles, J. A., & Tosti, S. (2020). Ultra-pure hydrogen via co-valorization of olive mill wastewater and bioethanol in Pd-membrane reactors. *Processes*, *8*(2), 219. https://doi.org/10.3390/pr8020219

Arous, F., Frikha, F., Triantaphyllidou, I. E., Aggelis, G., Nasri, M., & Mechichi, T. (2016). Potential utilization of agro-industrial wastewaters for lipid production by the oleaginous yeast *Debaryomyces etchellsii*. *Journal of Cleaner Production*, *133*, 899-909. https://doi.org/10.1016/j.jclepro.2016.06.040

Ayadi, K., Meziane, M., Rouam, D., Bouziane, M. N., & El-Miloudi, K. (2022). Olive mill wastewater for bioethanol production using immobilised cells. *Kemija u Industriji: Časopis Kemičara i Kemijskih Inženjera Hrvatske*, *71*(1-2), 21-28. https://doi.org/10.15255/KUI.2021.015

Barbera, A. C., Maucieri, C., Cavallaro, V., Ioppolo, A., & Spagna, G. (2013). Effects of spreading olive mill wastewater on soil properties and crops, a review. *Agricultural Water Management*, *119*, 43-53. https://doi.org/10.1016/j.agwat.2012.12.009

Bellou, S., Makri, A., Sarris, D., Michos, K., Rentoumi, P., Celik, A., Papanikolaou, S., & Aggelis, G. (2014). The olive mill wastewater as substrate for single cell oil production by *Zygomycetes*. *Journal of Biotechnology*, *170*, 50-59. https://doi.org/10.1016/j.jbiotec.2013.11.015

Benhoula, M., Azzouz, Z., Bettache, A., Le Roes-Hill, M., Djoudi, W., Maibeche, R., Hamma, S., Bensaad, M. S., Amghar, Z., Boudjelal, A., Benallaoua, S., & Boucherba, N. (2023). Olive mill wastewater biodegradation for bacterial lipase production using a response surface methodology. *Biomass Conversion and Biorefinery*, *14*, 1187-1200. https://doi.org/10.1007/s13399-023-04051-w

Buchmann, C., Felten, A., Peikert, B., Muñoz, K., Bandow, N., Dag, A., & Schaumann, G. E. (2015). Development of phytotoxicity and composition of a soil treated with olive mill wastewater (OMW): An incubation study. *Plant and Soil*, *386*, 99-112. https://doi.org/10.1007/s11104-014-2241-3

Chatzifragkou, A., Petrou, I., Gardeli, C., Komaitis, M., & Papanikolaou, S. (2011). Effect of *Origanum vulgare* L. essential oil on growth and lipid profile of *Yarrowia lipolytica* cultivated on glycerol-based media. *Journal of the American Oil Chemists' Society, 88*, 1955-1964. https://doi.org/10.1007/s11746-011-1870-4

Dahmen-Ben Moussa, I., Maalej, A., Masmoudi, M. A., Feki, F., Choura, S., Baccar, N., Jelail, L., Karray, F., Chamkha, M., & Sayadi, S. (2021). Effect of olive mill wastewaters on *Scenedesmus* sp. growth, metabolism and polyphenols removal. *Journal of the Science of Food and Agriculture*, *101*(13), 5508-5519. https://doi.org/10.1002/jsfa.11200

Diamantis, I., Melanouri, E. M., Dedousi, M., Panagopoulou, I., Papanikolaou, S., Stoforos, N. G., & Diamantopoulou, P. (2022). Sustainable and eco-friendly conversions of olive mill wastewater-based media by *Pleurotus pulmonarius* cultures. *Fermentation*, *8*(3), 129. https://doi.org/10.3390/fermentation8030129

Dias, B., Lopes, M., Ramôa, R., Pereira, A. S., & Belo, I. (2021). *Candida tropicalis* as a promising oleaginous yeast for olive mill wastewater bioconversion. *Energies*, *14*(3), 640. https://doi.org/10.3390/en14030640

Dourou, M., Kancelista, A., Juszczyk, P., Sarris, D., Bellou, S., Triantaphyllidou, I. E., Rywińska, A., Papanikolaou, S., & Aggelis, G. (2016). Bioconversion of olive mill wastewater into high-added value products. *Journal of Cleaner Production*, *139*, 957-969. https://doi.org/10.1016/j.jclepro.2016.08.133

El-Fadaly, H. A., El-Naggar, N. E., & Marwan, E. M. (2009). Single cell oil production by an oleaginous yeast strain in a low-cost cultivation medium. *Research Journal of*

Microbiology, 4(8), 301-313. https://doi.org/10.3923/jm.2009.301.313

Enshaeieh, M., Nahvi, I., & Madani, M. (2014). Improving microbial oil production with standard and native oleaginous yeasts by using Taguchi design. *International Journal of Environmental Science and Technology*, *11*, 1691-1698. https://doi.org/10.1007/s13762-013-0373-2

Fabiszewska, A., Misiukiewicz-Stępień, P., Paplińska-Goryca, M., Zieniuk, B., & Białecka-Florjańczyk, E. (2019). An insight into storage lipid synthesis by *Yarrowia lipolytica* yeast relating to lipid and sugar substrates metabolism. *Biomolecules*, *9*(11), 685. https://doi.org/10.3390/biom9110685

Fattoum, H., Cherif, A. O., Trabelsi, S., & Messaouda, M. B. (2023). Identification of phenolic compounds extracted from OMW Using LC-MS. *Journal of Oleo Science*, *72*(12), 1113-1123. https://doi.org/10.5650/jos.ess23109

Filippousi, R., Diamantopoulou, P., Stavropoulou, M., Makris, D. P., & Papanikolaou, S. (2022). Lipid production by *Rhodosporidium toruloides* from biodiesel-derived glycerol in shake flasks and bioreactor: Impact of initial C/N molar ratio and added onion-peel extract. *Process Biochemistry*, *123*, 52-62. https://doi.org/10.1016/j.procbio.2022.10.008

Flouri, F., Sotirchos, D., Ioannidou, S., & Balis, C. (1996). Decolorization of olive oil mill liquid wastes by chemical and biological means. *International Biodeterioration & Biodegradation*, *38*(3-4), 189-192. https://doi.org/10.1016/S0964-8305(96)00050-9

Hamimed, S., Landoulsi, A., & Chatti, A. (2021). The bright side of olive mill wastewater: Valuables bioproducts after bioremediation. *International Journal of Environmental Science and Technology*, *18*, 4053-4074. https://doi.org/10.1007/s13762-021-03145-0

He, M., Li, L., Zhou, W., Huang, H., Ma, Q., & Gong, Z. (2025). Non-sterile fermentation for enhanced lipid production by *Cutaneotrichosporon oleaginosum* using bifunctional benzamide as selective antibacterial agent and unique nitrogen source. *Bioresource Technology*, *418*, 131926. https://doi.org/10.1016/j.biortech.2024.131926

Herrero, O. M., Villalba, M. S., Lanfranconi, M. P., & Alvarez, H. M. (2018). *Rhodococcus* bacteria as a promising source of oils from olive mill wastes. *World Journal of Microbiology and Biotechnology*, *34*, 114. https://doi.org/10.1007/s11274-018-2499-3

Huang, C., Chen, X. F., Xiong, L., Ma, L. L., & Chen, Y. (2013). Single cell oil production from low-cost substrates: The possibility and potential of its industrialization. *Biotechnology Advances*, *31*(2), 129-139. https://doi.org/10.1016/j.biotechadv.2012.08.010

ISO 1841-1. (1996). *Meat and meat products-determination of chloride content-part 1: Volhard method*. https://standards.iteh.ai/catalog/standards/iso/f7a5c841-dbb3-46c1-a30d-bfab93312a26/iso-1841-1-1996

Khdair, A., & Abu-Rumman, G. (2020). Sustainable environmental management and valorization options for olive mill byproducts in the Middle East and North Africa (MENA) region. *Processes*, *8*(6), 671. https://doi.org/10.3390/pr8060671

Kuttiraja, M., Douha, A., Valéro, J. R., & Tyagi, R. D. (2016). Elucidating the effect of glycerol concentration and C/N ratio on lipid production using *Yarrowia lipolytica* SKY7. *Applied Biochemistry and Biotechnology*, *180*, 1586-1600. https://doi.org/10.1007/s12010-016-2189-2

Moustogianni, A., Bellou, S., Triantaphyllidou, I. E., & Aggelis, G. (2014). Feasibility of raw glycerol conversion into single cell oil by Zygomycetes under non-aseptic conditions. *Biotechnology and Bioengineering*, *112*(4), 827-831. https://doi.org/10.1002/bit.25482

Ochando-Pulido, J. M., Pimentel-Moral, S., Verardo, V., & Martinez-Ferez, A. (2017). A focus on advanced physico-chemical processes for olive mill wastewater treatment. *Separation and Purification Technology*, *179*, 161-174. http://doi.org/10.1016/j.seppur.2017.02.004

Ochsenreither, K., Glück, C., Stressler, T., Fischer, L., & Syldatk, C. (2016). Production strategies and applications of microbial single cell oils. *Frontiers in Microbiology*, *7*, 1539. https://doi.org/10.3389/fmicb.2016.01539

Papanikolaou, S., Galiotou-Panayotou, M., Fakas, S., Komaitis, M., & Aggelis, G. (2008). Citric acid production by *Yarrowia lipolytica* cultivated on olive-mill wastewater-based media. *Bioresource Technology*, *99*(7), 2419-2428. https://doi.org/10.1016/j.biortech.2007.05.005

Paraskeva, P., & Diamadopoulos, E. (2006). Technologies for olive mill wastewater (OMW) treatment: A review. *Journal of Chemical Technology & Biotechnology*, *81*(9), 1475-1485. https://doi.org/10.1002/jctb.1553

Paredes, C., Cegarra, J., Roig, A., Sánchez-Monedero, M. A., & Bernal, M. P. (1999). Characterization of olive mill wastewater (alpechin) and its sludge for agricultural purposes. *Bioresource Technology*, *67*(2), 111-115. https://doi.org/10.1016/S0960-8524(98)00106-0

Polburee, P., & Limtong, S. (2020). Economical lipid production from crude glycerol using *Rhodosporidiobolus fluvialis* DMKU-RK253 in a two-stage cultivation under non-sterile conditions. *Biomass and Bioenergy*, *138*, 105597. https://doi.org/10.1016/j.biombioe.2020.105597

Qwele, K., Hugo, A., Oyedemi, S. O., Moyo, B., Masika, P. J., & Muchenje, V. (2013). Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. *Meat Science*, *93*(3), 455-462. http://doi.org/10.1016/j.meatsci.2012.11.009

Roig, A., Cayuela, M. L., & Sánchez-Monedero, M. A. (2006). An overview on olive mill wastes and their valorisation methods. *Waste Management*, *26*(9), 960-969. https://doi.org/10.1016/j.wasman.2005.07.024

Roila, R., Primavilla, S., Ranucci, D., Galarini, R., Paoletti, F., Altissimi, C., Valiani, A., & Branciari, R. (2024). The effects of encapsulation on the in vitro anti-clostridial activity of olive mill wastewater polyphenolic extracts: A promising strategy to limit microbial growth in food systems. *Molecules*, *29*(7), 1441. https://doi.org/10.3390/molecules29071441

Sarris, D., Galiotou-Panayotou, M., Koutinas, A. A., Komaitis, M., & Papanikolaou, S. (2011). Citric acid, biomass and cellular lipid production by *Yarrowia lipolytica* strains cultivated on olive mill wastewater-based media. *Journal of Chemical Technology and Biotechnology*, *86*(11), 1439-1448. https://doi.org/10.1002/jctb.2658

Sarris, D., Giannakis, M., Philippoussis, A., Komaitis, M., Koutinas, A. A., & Papanikolaou, S. (2013). Conversions of olive mill wastewater-based media by *Saccharomyces cerevisiae* through sterile and non-sterile bioprocesses. *Journal of Chemical Technology and Biotechnology*, *88*(5), 958-969. https://doi.org/10.1002/jctb.3931

Sarris, D., Matsakas, L., Aggelis, G., Koutinas, A. A., & Papanikolaou, S. (2014). Aerated vs non-aerated conversions of molasses and olive mill wastewaters blends into bioethanol by Saccharomyces cerevisiae under non-aseptic conditions. *Industrial Crops and Products*, *56*, 83-93. https://doi.org/10.1016/j.indcrop.2014.02.040

Sarris, D., Stoforos, N. G., Mallouchos, A., Kookos, I. K., Koutinas, A. A., Aggelis, G., & Papanikolaou, S. (2017). Production of added-value metabolites by *Yarrowia lipolytica* growing in olive mill wastewater-based media under aseptic and non-aseptic conditions. *Engineering in Life Sciences*, *17*, 695-709. https://doi.org/10.1002/elsc.201600225

Sarris, D., Rapti, A., Papafotis, N., Koutinas, A. A., & Papanikolaou, S. (2019). Production of added-value chemical compounds through bioconversions of olive-mill wastewaters blended with crude glycerol by a *Yarrowia lipolytica* strain. *Molecules*, *24*(2), 222. https://doi.org/10.3390/molecules24020222

Sarris, D., Tsouko, E., Photiades, A., Tchakouteu, S. S., Diamantopoulou, P., & Papanikolaou, S. (2023a). Growth response of non-conventional yeasts on sugar-rich media: Part 2: Citric acid production and circular-oriented valorization of glucose-enriched olive mill wastewaters using novel *Yarrowia lipolytica* strains. *Microorganisms*, *11*(9), 2243. https://doi.org/10.3390/microorganisms11092243

Sarris, D., Tsouko, E., Kothri, M., Anagnostou, M., Karageorgiou, E., & Papanikolaou, S. (2023b). Upgrading major waste streams derived from the biodiesel industry and olive mills via microbial bioprocessing with non-conventional *Yarrowia lipolytica* strains. *Fermentation*, *9*(3), 251. https://doi.org/10.3390/fermentation9030251

Singh, S., Bharadwaj, T., Verma, D., & Dutta, K. (2022). Valorization of phenol contaminated wastewater for lipid production by *Rhodosporidium toruloides* 9564T. *Chemosphere*, *308*(Part 2), 136269. https://doi.org/10.1016/j.chemosphere.2022.136269

Sipiczki, G., Micevic, S. S., Kohari-Farkas, C., Nagy, E. S., Nguyen, Q. D., Gere, A., & Bujna, E. (2024). Effects of olive oil and Tween 80 on production of lipase by *Yarrowia* yeast strains. *Processes*, *12*(6), 1206. https://doi.org/10.3390/pr12061206

Taoka, Y., Nagano, N., Okita, Y., Izumida, H., Sugimoto, S., & Hayashi, M. (2011). Effect of Tween 80 on the growth, lipid accumulation and fatty acid composition of *Thraustochytrium aureum* ATCC 34304. *Journal of Bioscience and Bioengineering*, *111*(4), 420-424. https://doi.org/10.1016/j.jbiosc.2010.12.010

Tzirita, M., Kremmyda, M., Sarris, D., Koutinas, A. A., & Papanikolaou, S. (2019). Effect of salt addition upon the production of metabolic compounds by *Yarrowia lipolytica* cultivated on biodiesel-derived glycerol diluted with olive-mill wastewaters. *Energies*, *12*(19), 3649. https://doi.org/10.3390/en12193649

Yu, X., Dong, T., Zheng, Y., Miao, C., & Chen, S. (2015). Investigations on cell disruption of oleaginous microorganisms: Hydrochloric acid digestion is an effective method for lipid extraction. *European Journal of Lipid Science and Technology*, *117*(5), 730-737. https://doi.org/10.1002/ejlt.201400195

Zhang, S., Jagtap, S. S., Deewan, A., & Rao, C. V. (2019). pH selectively regulates citric acid and lipid production in *Yarrowia lipolytica* W29 during nitrogen-limited growth on glucose. *Journal of Biotechnology*, *290*, 10-15. https://doi.org/10.1016/j.jbiotec.2018.10.012

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RESEARCH ARTICLE

The Effects of Water Quality Parameters on Summer Dynamics of Phytoplankton and Zooplankton in the Tributaries of Murat River (Hınıs, Erzurum)

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ABSTRACT

This study aimed to investigate the spatial variations in phytoplankton and zooplankton composition in relation to selected water quality parameters across five tributaries of the Murat River. The research encompassed physical and chemical parameters, chlorophyll-*a* levels, zooplankton and phytoplankton composition and biodiversity indices within the tributaries. A total of seven zooplankton species were identified, comprising 40% Copepoda, 37.14% Cladocera and 22.86% Rotifera, with *Cyclops vicinis* emerging as the dominant species. Additionally, 34 species from the Bacillariophyta, Chlorophyta and Cyanobacteria groups were recorded. The Shannon-Wiener and Margalef Biodiversity Indices revealed that zooplankton diversity peaked at the 5th station, while phytoplankton diversity was highest at the 3rd station. The average concentrations of total phosphorus (TP) and ammonia nitrogen (NH_3-N) were measured as 5.93 mg L^{-1} and 3.02 mg L^{-1}, respectively. Chlorophyll-*a* values ranged from 0.001 mg L^{-1} at the 1st station to 0.011 mg L^{-1} at the 5th station. According to the Trophic Diatom Index (TDI), the water quality was categorized as poor, while other indices indicated medium water quality. In conclusion, the findings highlight that the tributaries of the Murat River are under significant threat from anthropogenic pollution. Urgent mitigation measures are recommended to safeguard these water resources.

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1. Introduction

Streams are dynamic aquatic ecosystems containing suspended organic and inorganic matter, dissolved nutrients and gases. These systems are highly vulnerable to anthropogenic pressures such as climate change, population growth and pollution. Therefore, understanding the current ecological status of stream systems is essential for their protection and sustainable management.

Environmental pollution, with its multifaceted impacts, has become a global issue. The European Water Framework Directive, implemented by the European Union in 2000, marked a significant step in the management of inland waters. This directive categorizes surface waters into four main types:

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lakes, rivers, transitional waters and coastal waters. It also identifies biological quality indicators—phytoplankton, phytobenthos, macrophytes, macroinvertebrates and fish—to assess the ecological status of these water bodies (Anonymous, 2003a; T.C. Başbakanlık Devlet Planlama Teşkilatı, 1998). In Türkiye, research on monitoring and evaluating water bodies in accordance with the necessities of Water Framework Directive has gained importance.

Diatom indices have been developed and widely applied to determine the trophic status of streams in various countries (Kıvrak et al., 2012). Examples of these indices include the Descy and Coste Diatom Index (Descy & Coste, 1991), Generic Diatom Index (Coste & Ayphassorho, 1991), Leclercq and Maquet Index (Leclercq & Maquet, 1987), Steinberg and Schiefele Index (Steinberg & Schiefele, 1988) and the Trophic Diatom Index (Kelly & Whitton, 1995; Kelly, 1998).

Plankton, which migrate passively within aquatic environments, play a critical role in sustaining life. While they are abundant in marine systems, they also thrive in inland waters. The dynamics of these organisms in streams differ from those in lakes or reservoirs, as they are influenced by constantly changing physical conditions, such as temperature, pH, dissolved oxygen and flow rate. Nutrient inputs from agricultural, industrial and urban activities further impact these dynamics (Tanyolaç, 2009). Assessing the distribution of planktonic organisms in streams is therefore crucial for ecological evaluations. Furthermore, phytoplankton, macrophytes, benthic invertebrates and fish have been recognized as key biological indicators (Bakır, 2015).

This study was conducted in the Başköy and Kocasu streams, tributaries of the Murat River located in the Hınıs district of Erzurum. It aimed to analyze zooplankton and phytoplankton populations, water quality parameters, chlorophyll-*a* (chl-*a*) and biodiversity indices in the region. The objective was to determine the index values of plankton dynamics and explore the relationship between primary producers and nutrient levels. Given the lack of prior studies on these streams, this research fills a critical knowledge gap and provides a foundation for future investigations. The findings are expected to contribute significantly to understanding the ecological dynamics of tributaries with substantial water potential, such as the Murat River.

2. Materials and Methods

2.1. Study Area

The Murat River, with a length of 722 km, is one of the longest rivers in the region. Originating in the Muratbaşı Mountains near Lake Van, it flows southward, irrigating the Ağrı region and collecting waters from tributaries such as the Hınıs streams (Kocasu and Başköy). It eventually merges with the Karasu River to form the Euphrates River (Kirici et al., 2016; Koyun, 2011).

The study was conducted in the Hınıs region, which contains over 20 large and small streams. These streams converge and flow into the Murat River approximately 30 km downstream. Sampling was performed in five selected streams at altitudes ranging from 1560 to 1650 meters. Phytoplankton, zooplankton and water samples were collected from these locations in June 2024. Details of the sampling stations, including coordinates and hydro-morphological characteristics, are provided in Table 1, and the map of the stations is shown in Figure 1.

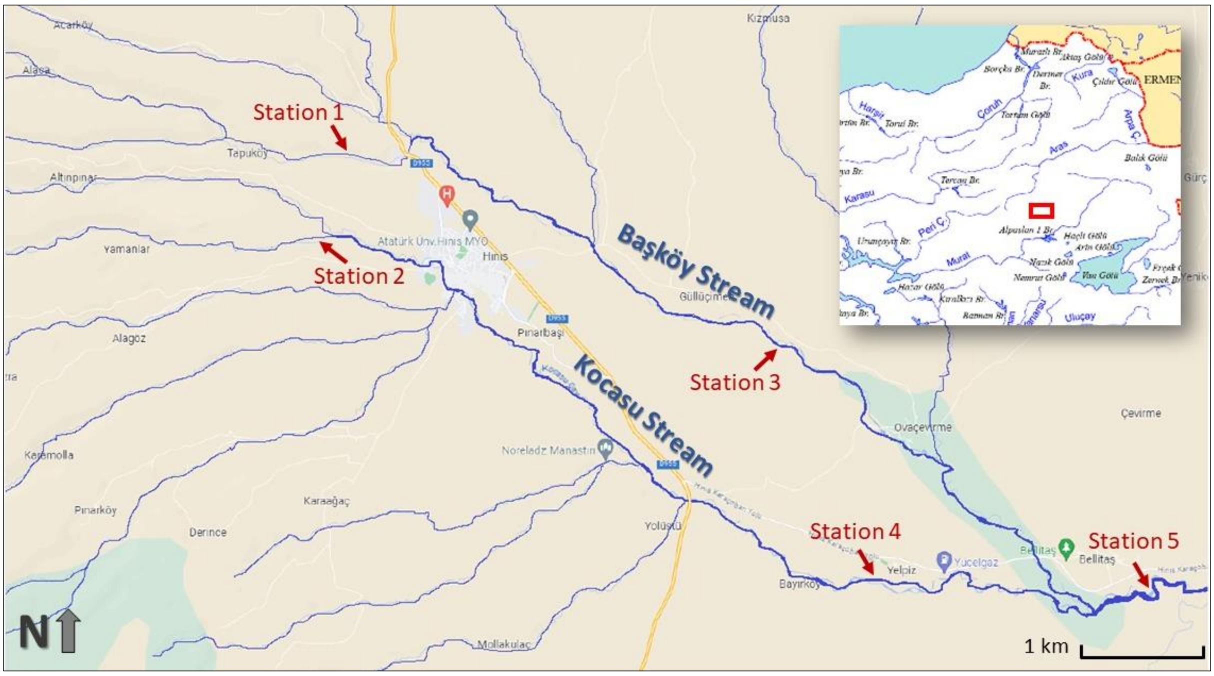

Figure 1. Map of study area.

Table 1. Hydro-morphological characteristics of the tributaries of the Murat River.

Stations	Name of Streams	Altitude	Coordinate	Flow rate (m/s)	Flow (m^3/s)	Depth (m)
1	Alaca Creek	1650m	39°22'34"N, 41°41'16"E	0.9	0.1	0.78
2	Şeyhnadir Creek	1750m	39º21'39"N, 41º39'20"E	1.33	0.09	0.38
3	Başköy Creek	1615m	39°21'40"N 41°43'44"E	0.83	0.28	0.38
4	Kocasu Creek	1595m	39°19'07"N 41°45'03"E	1.5	0.27	0.4
5	The location after intersection of Başköy and Kocasu Creeks	1560m	39°18'21"N 41°51'13"E	1.21	1.06	0.5

2.2. Sampling and Analysis

2.2.1. Physicochemical parameters

Water temperature, pH, dissolved oxygen and conductivity were measured *in situ* using a YSI multiparameter probe. Turbidity measurements were recorded with an Extech turbidimeter. Water samples for nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_3-N), orthophosphate (PO_4^{3-}) and total phosphorus (TP) were transported to the laboratory and analyzed spectrophotometrically following APHA protocols (APHA, 2001). Chl-*a* was measured using spectrophotometry after filtering samples through 45 µm Whatman filter papers on the day of sampling (Strickland & Parsons, 1972). Total hardness was determined titrimetrically as per APHA (1995) guidelines.

2.2.2. Zooplankton sampling and identification

Zooplankton samples were collected by filtering water through standard plankton nets with a mesh size of 60 µm. The samples were preserved in 4% formalin for further analysis. Zooplankton species were identified and quantified under a light microscope using taxonomic keys, including Edmondson (1959), Scourfield and Harding (1969), Kolisko (1974) Stemberger (1979) and Segers (1995) for Rotifera; Kiefer and Fryer (1978), Koste (1978a,b) and Negrea (1983) for Cladocera; and Borutskii (1964), Flössner (1972), Einsle (1996) and Dussart and Defaye (2001) for Copepoda.

2.2.3. Phytoplankton sampling and identification

Phytoplankton samples were collected using plankton nets with a 10 µm mesh size. Species composition was identified using a Zeiss binocular microscope (magnifications: 100x, 200x and 400x) following taxonomic literature (Cox, 1991, 1996; John et al., 2002). Final taxonomic names were verified using the AlgaeBase database (https://www.algaebase.org). Quantitative samples were collected with a Ruttner water sampler, and phytoplankton counts were conducted in Hydrobios plankton counting chambers after preserving samples with Lugol's solution (Anonymous, 2003b; Utermöhl, 1958).

2.3. Biodiversity Indices

2.3.1. Shannon-Wiener Diversity Index (H')

The Shannon-Wiener Index, derived by Shannon in 1948, is calculated using the formula:

$$\mathrm{H}' = -\sum_{i=1}^{s} pi \log_e pi \tag{1}$$

Where, s: Total number of species; pi: Proportion of individuals belonging to species (ni) to total number of individuals (n).

2.3.2. Simpson Diversity Index (D)

The Simpson Diversity Index is calculated as (Hill, 1973; James & Aderaje, 2010; Krebs, 1998; Kwak & Peterson, 2007):

$$1 - D = \frac{\sum ni(ni-1)}{N(N-1)} \tag{2}$$

Where, ni: Number of individuals belonging to species; N: Total number of individuals.

2.3.3. Margalef Diversity Index (Dmg)

The Margalef Index is given by (James & Aderaje, 2010):

$$D = \frac{S-1}{\log N} \tag{3}$$

Where, S: Number of species; N: Total number of individuals.

2.4. Data Evaluation

Diatom indices were calculated using OMNIDIA 5.2 software (Lecointe et al., 1993). The results were interpreted according to the scoring criteria by Lenoir and Coste (1996). Statistical differences among environmental parameters at different sites were assessed using one-way ANOVA in IBM SPSS 20.0, followed by Duncan's test to determine significance levels. Cluster observation analysis was performed using MINITAB software. Canonical correspondence analysis (CCA) was conducted using PAST 4.03 to analyze the relationship between environmental factors and phytoplankton biomass.

3. Results

3.1. Water Quality Parameters

Significant variations in water temperature, dissolved oxygen, pH, electrical conductivity, turbidity, hardness, NH_3-N, NO_3-N, NO_2-N, TP, PO_4-P and chl-*a* were observed across the sampling stations ($p < 0.05$). The highest water temperature (21.7 °C) was recorded at 5th station, the lowest-altitude site, while 2nd station, the highest-altitude site, had the lowest temperature (18.0 °C). Dissolved oxygen values ranged from 9.51 mg L^{-1} at 1st station to 5.78 mg L^{-1} at 4th station. The highest pH was recorded at 1st station (8.04) and the lowest at 4th station (7.91). Electrical conductivity peaked at 5th station (0.35 mS/cm) and was lowest at 2nd station (0.10 mS/cm). Turbidity values varied significantly, with 1st station exhibiting the highest value (38.51 NTU) and 5th station the lowest (10.45 NTU).

Orthophosphate phosphorus (PO_4-P) values ranged from 0.01 mg L^{-1} to 0.02 mg L^{-1} across stations. Ammonia nitrogen (NH_3-N) had an average concentration of 2.96 mg L^{-1} across all stations, with the highest concentration at 5th station (7.73 mg L^{-1}). Nitrate nitrogen (NO_3-N) was lowest at 1st, 2nd and 3rd stations (0.04 mg L^{-1}) and slightly higher at 4th and 5th stations (0.05 mg L^{-1}). Nitrite nitrogen (NO_2-N) concentrations varied from 0.47 µg L^{-1} at 2nd station to 2.33 µg L^{-1} at 4th station (Table 2).

The results obtained in this study were evaluated in comparison with the limit values specified in the Surface Water Quality Regulation (SWQR) (YSKY, 2015). Based on these evaluations, the quality classes of the stations were determined according to specific parameters. All stations were classified as Class I waters based on water temperature, electrical conductivity, PO_4-P and NO_3-N concentrations according to SWQR. Dissolved oxygen levels indicated that the 1st and 5th stations were Class I waters, while the 2nd, 3rd and 4th stations were classified as Class II waters (Table 3).

According to the SWQR, the 1st station (1.10 mg L^{-1}) and 2nd station (1.24 mg L^{-1}) were classified as Class II, the 3rd station (1.61 mg L^{-1}) as Class III, and the 4th station (3.13 mg L^{-1}) and 5th station (7.72 mg L^{-1}) as Class IV waters based on ammonia nitrogen concentrations. These classifications were made following the Quality Criteria of Surface Water Quality Management Regulation for Intra-Continental Surface Water Resources (YSKY, 2015) (Table 3).

Table 2. Changes in water quality parameters and chl-*a* values across stations.

Parameter	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**Station 5**
Water temperature (°C)	20.04±0.0^{b*}	19.13±0.33^{c}	18.00±0.0^{d}	19.00±0.06^{c}	21.7±0.21^{a}
Dissolved Oxygen (mgL $^{-1}$)	9.51±1.89^{a}	7.07±0.77^{a}	6.94±0.40^{a}	5.78±0.33^{a}	9.38±0.18^{a}
pH	8.14±0.01^{a}	8.04±0.03^{a}	8.03±0.26^{a}	7.90±0.02^{a}	8.11±0.01^{a}
EC (mS/cm)	0.11±0.00^{c}	0.24±0.03^{b}	0.10±0.00^{c}	0.22±0.00^{b}	0.35±0.02^{a}
Turbidity (ntu)	38.51±4.85^{a}	34.76±2.67^{a}	17.38±0.20^{b}	17.51±0.97^{b}	11.13±1.07^{b}
NH_3-N (mg L $^{-1}$)	1.10±0.18^{a}	1.61±0.51^{a}	1.24±0.05^{a}	3.13±0.69^{a}	7.73±4.31^{a}
NO_3-N (mg L $^{-1}$)	0.04±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}	0.05±0.01^{a}	0.05±0.01^{a}
NO_2-N (µg L $^{-1}$)	0.94±0.04^{b}	1.73±0.33^{a}	0.47±0.09^{b}	2.33±0.07^{a}	2.23±0.01^{a}
TP (mg L $^{-1}$)	1.58±0.08^{a}	3.20±0.34^{a}	1.84±1.07^{a}	4.12±2.72^{a}	3.34±0.03^{a}
PO_4-P (mg L $^{-1}$)	0.02±0.00^{a}	0.02±0.00^{ab}	0.01±0.00^{c}	0.02±0.00^{a}	0.01±0.00^{bc}
Total Hardness (mg L $^{-1}$)	31.35±13.65^{a}	75.05±1.20^{a}	17.95±2.47^{a}	81.65±3.46^{a}	291±168^{a}
Chl-*a* (mg L $^{-1}$)	0.001±0.00^{d}	0.002±0.00^{c}	0.002±0.00^{d}	0.009±0.00^{b}	0.011±0.00^{a}

***** a, b, c, d indicate the difference in water quality parameters between stations. The difference between groups shown with different lowercase letters in the same line is statistically significant ($p<0.05$).

Table 3. Assessment of tributaries of Murat River based on selected water quality parameters (blue: Class I water, green: Class II water, yellow: Class III water and red: Class IV water).

Stations **Parameter**	**1**	**2**	**3**	**4**	**5**	**SWQR (YSKY, 2015)**
Water temperature (°C)	20.04	19.13	18.00	19.00	21.7	19.57
Dissolved oxygen (mg L^{-1})	9.51	7.07	6.94	5.78	9.38	7.74
pH	8.14	8.04	8.03	7.90	8.11	8.04
EC (mS/cm)	0.11	0.24	0.10	0.22	0.35	0.20
PO_4-P (mg L^{-1})	0.02	0.02	0.01	0.02	0.01	0.02
NH_3-N (mg L^{-1})	1.10	1.61	1.24	3.13	7.73	2.96
NO_3-N (mg L^{-1})	0.04	0.04	0.04	0.05	0.05	0.04
NO_2-N (mg L^{-1})	0.94	1.73	0.47	2.33	2.23	1.54

Chl-*a* concentrations varied between 0.001 mg L^{-1} to 0.011 mg L^{-1} across stations, with the highest value at 5th station (Figure 2), and the differences between stations were found to be statically significant ($p<0.05$, Table 2).

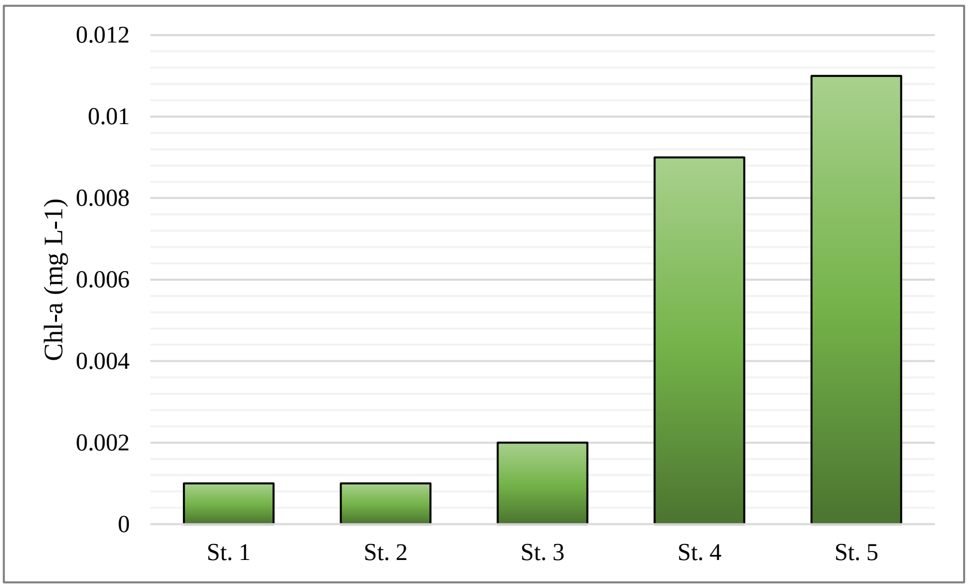

Figure 2. Changes in chl-*a* concentration across the stations (Mean±SD, n=4).

3.2. Phytoplankton and Zooplankton Composition

A total of 34 phytoplankton species from Bacillariophyta, Chlorophyta and Cyanobacteria groups and 7 zooplankton species from Cladocera, Copepoda and Rotifera groups were identified. *Encyonema latens*, *Navicula lanceolata* and *Cyclops vicinus* were present at all stations (Table 4).

Table 4. Phytoplankton and zooplankton species identified across stations.

Species	**Stations**				
	1	**2**	**3**	**4**	**5**
Phytoplankton					
Classis: Bacillariophyta					
***Caloneis* sp.**		+			
***Cocconeis placentula* Ehrenberg**	+	+	+		+
***Craticula accomoda* (Hustedt) D.G.Mann**					+
***Diatoma* vulgaris Bory**					+
***Encyonema latens* (Krasske) D.G.Mann**	+	+	+	+	+
***Epithemia* sp.**				+	
***Fragilaria capucina* Desmazières**	+	+			
***Fragilaria* sp.**				+	
***Fallacia insociabilis* (Krasske) D.G.Mann**	+		+	+	+
***Geissleria schoenfeldii* (Hustedt) Lange-Bertalot & Metzeltin**			+		
***Gomphonema* sp.**	+	+	+		+
***Gomphonema truncatum* Ehrenberg**					+
***Hannaea arcus* (Ehrenberg) R.M.Patrick**	+		+		
***Melosira varians* C.Agardh**				+	
***Navicula cryptotenella* Lange-Bertalot**	+		+		
***Navicula lanceolata* Ehrenberg**	+	+	+	+	+
***Navicula reinhardtii* (Grunow) Grunow**	+	+	+		
***Nitzschia capitellata* Hustedt**			+	+	+

Table 4. (continued).

Species	**Stations**				
	1	**2**	**3**	**4**	**5**
Phytoplankton					
Classis: Bacillariophyta					
***Nitzschia* sp.**	+	+	+	+	
***Pinnularia* sp.**				+	
***Placoneis* sp.**		+			
***Rhizosolenia* sp.**				+	+
***Rhopalodia* sp.**	+	+	+	+	
***Staurosira neoproducta* (Lange-Bertalot) Chudaev & Gololobova**			+		
***Stephanocyclus meneghinianus* (Kützing) Kulikovskiy, Genkal & Kociolek**	+	+	+	+	
***Surirella brebissonii* Krammer & Lange-Bertalot**			+		
***Surirella librile* (Ehrenberg) Ehrenberg**				+	
Classis: Chlorophyta					
***Comasiella arcuata* (Lemmermann) E.Hegewald, M.Wolf, Al.Keller, Friedl & Krienitz**		+			
***Scenedesmus* sp.**	+		+		
***Tetraedron minimum* (A.Braun) Hansgirg**		+			
***Tetradesmus bernardii* (G.M.Smith) M.J.Wynne**				+	
***Tetradesmus dimorphus* (Turpin) M.J.Wynne**			+	+	
***Tetradesmus obliquus* (Turpin) M.J.Wynne**			+		
Classis: Cyanobacteria					
***Oscillatoria* sp.**	+	+	+	+	
Zooplankton					
Group: Cladocera					
***Bosmina longirostris* (O.F. Müller, 1785)**	+		+		+
***Daphnia cucullata* (Sars, 1862)**		+		+	+
Group: Copepoda					
***Cyclops vicinus* (Sars, 1863)**	+	+	+	+	+
Group: Rotifera					
***Keratella quadrata* (O. F. Müller, 1785)**					+
***Polyarthra dolichoptera* (Idelson, 1925)**					+
***Philodina roseola* (Ehrenberg, 1832)**			+	+	+
***Brachionus angularis* (Gosse, 1851)**					+

3.2.1. Abundance and diversity

The average phytoplankton density was 130,493.7 cells/m^3, while the zooplankton count averaged 70 individuals/m^3. Phytoplankton abundance was highest at 3rd station, whereas zooplankton density peaked at 5th station (Figure 3).

Bacillariophyta was found to be the highest rate among phytoplankton groups, while the Copepoda was determinate to be approximately 50% within the zooplankton group (Figure 4).

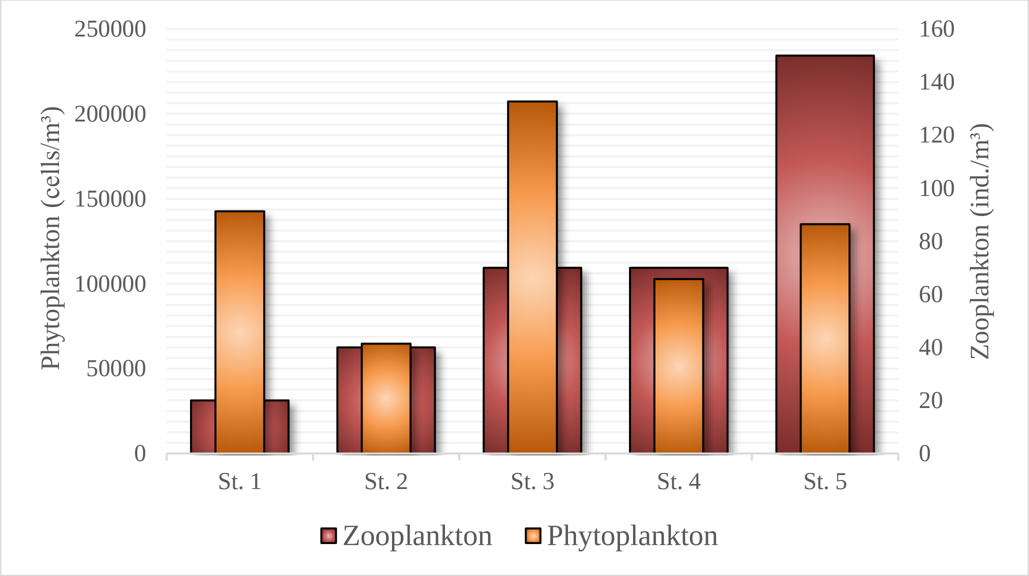

Figure 3. Phytoplankton and zooplankton abundance across stations.

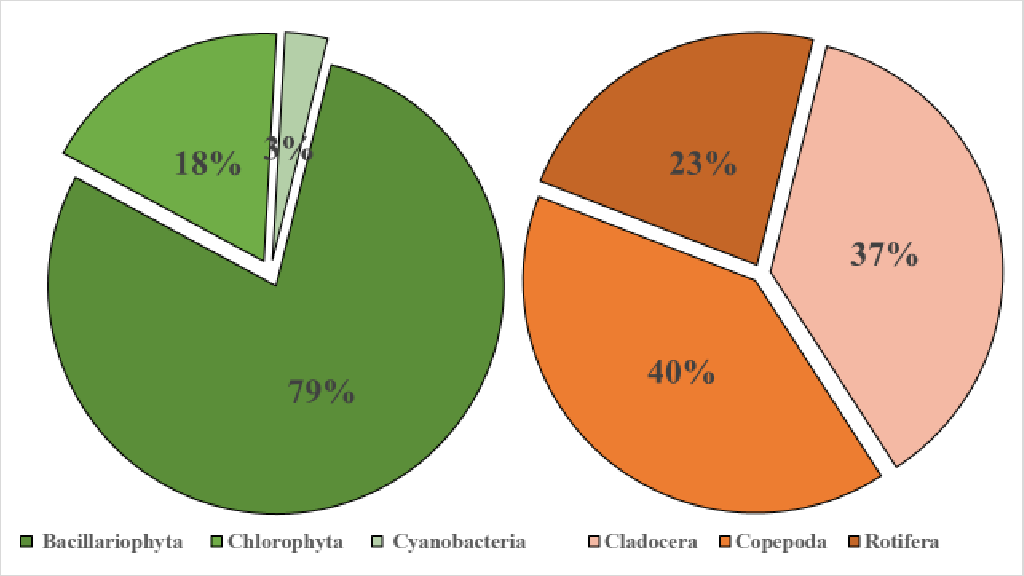

Figure 4. Distribution of phytoplankton and zooplankton groups in the study area.

3.2.2. Biodiversity indices

Moderate phytoplankton biodiversity was observed at all stations based on the Shannon-Wiener Index. However, Simpson and Margalef indices indicated low phytoplankton and zooplankton biodiversity, except at 5th station, which exhibited the highest zooplankton diversity (Figure 5).

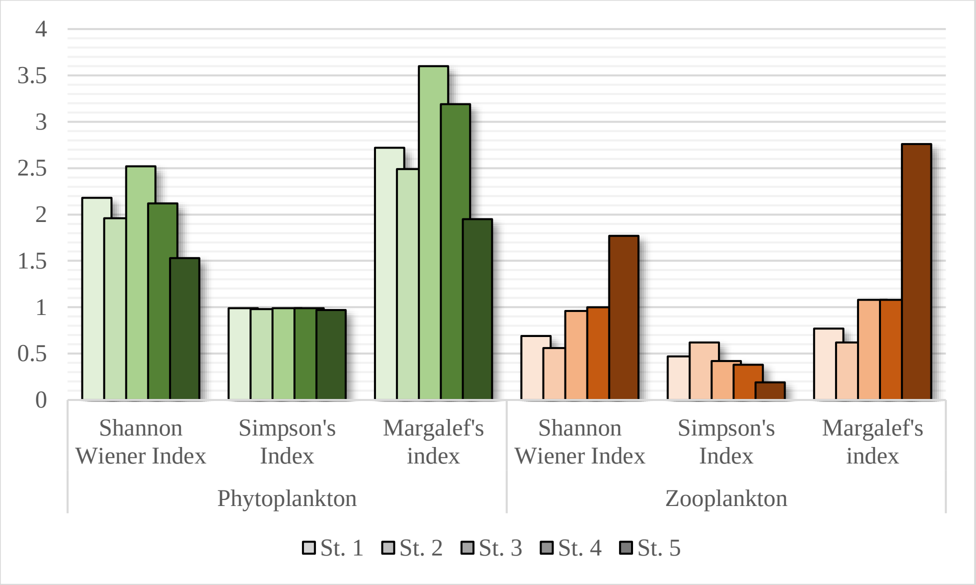

Figure 5. Biodiversity indices of phytoplankton and zooplankton groups in the study area (From light to dark colour for 1st to 5th station).

3.3. Canonical Correspondence Analysis (CCA)

CCA analysis revealed strong correlations between nutrient levels (TP, NH_3-N, NO_2-N, NO_3-N) and water temperature with diatom indices such as EPID, Rott SI and TDI. However, no significant relationships were observed for IBD, SHE and IPS indices (Figure 6).

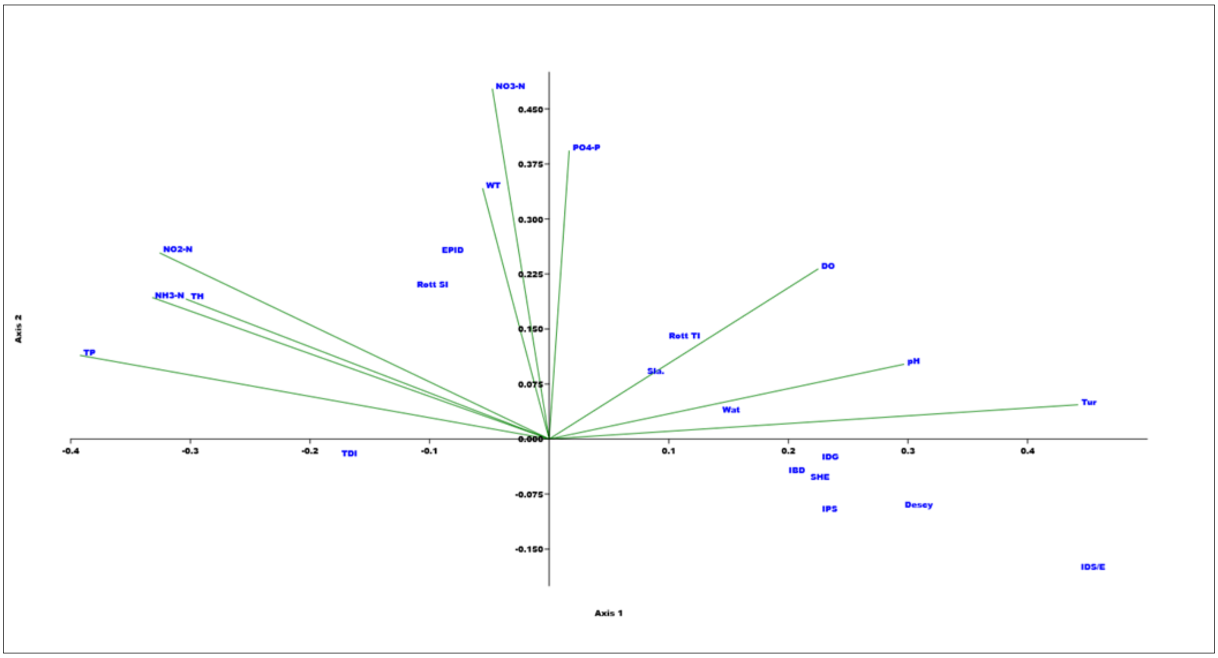

Figure 6. Canonical correspondence analysis of diatom indices and water quality parameters (WT: water temperature, DO: dissolved oxygen, NH_3-N: ammonia nitrogen, NO_2-N: nitrite-nitrogen, NO_3-N: nitrate-nitrogen, PO_4-P: orthophosphate phosphorus, TP: total phosphorus, TH: total hardness, Tur: turbidity).

4. Discussion

According to the SWQR, the streams and rivers examined in this study were categorized as clean river systems based on water quality parameters. However, ammonia nitrogen concentrations indicated that Başköy Stream, Kocasu Stream and the intersection of Başköy and Kocasu Streams were within the polluted water category. Koyun et al. (2020) was determinate that the Murat River within the Bingöl province, as "fourth class water quality" depend on pH and nitrogen derivative concentrations, and as "first class water quality" depend on other parameters according to the Surface Water Quality Management Regulation. These studies show that pollution related to nitrogen, because of anthropogenic impact, especially in the urban area like as in this result of research (Kerkmann et al., 2012; Koyun et al., 2020).

Physical and chemical parameters such as water temperature, dissolved oxygen and pH are important factors that affect lower food web relationships. These parameters, which have a significant impact on the life cycle of zooplankton and phytoplankton, were found to be at values suitable for the reproduction of these organisms for temperature (Mikschi, 1989), sufficient alkaline amount for pH (Bērziņš & Pejler, 1987) and tolerable levels for dissolved oxygen (Moss, 2007) in the study area.

The average results of pH, water temperature, dissolved oxygen, EC and total hardness was similar with a study conducted downstream of Murat River (Koyun et al., 2020). However, when the results are compared with studies conducted in the Euphrates River Basin, it is observed that dissolved oxygen concentrations decrease in relation to the hydro-morphological characteristics of the basin (Bulut & Saler, 2019).

Variations in conductivity can affect the ability of aquatic organisms to regulate their internal salt balance, potentially leading to stress or even death. Pulatsü et al. (2014) indicated that electrical conductivity values between 1 and 1000 µS/cm are acceptable for river systems. The findings of this study revealed that the electrical conductivity values for the tributaries of the Murat River fall within this range.

In river ecosystems, chl-*a* concentrations are associated with nutrient inputs. Elevated and sustained nutrient inputs, particularly beyond the eutrophication threshold of 0.2 mg L^{-1} for total nitrogen (TN) and 0.02 mg L^{-1} for total phosphorus (TP), can result in chl-*a* concentrations of up to 2.50 mg L^{-1} (Liao et al., 2021). In this context, the study revealed that nitrogen and phosphorus loads were lower at stations with higher altitudes and minimal pollutant influences. Conversely, stations in regions with intensive livestock activities exhibited higher nutrient loads.

In a study conducted on Murat River in Palu district, NH_4 values varied between 0.4 - 0.7 mg L^{-1} (Topal & Topal, 2016), which differs from this study. The high ammonia nitrogen concentrations in Kocasu and Başköy Streams can be attributed to the low regeneration ability due to the morphological characteristics of this stream.

Unlike in this study, a previous investigation on the Murat River found rotifers to be the dominant group (Bulut & Saler, 2014). The higher presence of copepods compared to rotifers in this study may suggest that the sampling area has cleaner waters than the downstream section of the Murat River. It is known that copepods are more abundant in oligotrophic waters, whereas rotifers thrive in eutrophic conditions (Herzig, 1987). The low concentration of water quality parameters (except ammonia nitrogen) observed in this study supports this finding. All zooplankton species identified during the research on Kocasu and Başköy Streams are included in the published checklists of zooplankton (Ustaoğlu, 2004) and rotifers (Ustaoğlu et al., 2012).

This study identified Bacillariophyta group algae, predominantly pennate diatoms such as *U. ulna* and *Gomphonema* species, which are considered moderately tolerant or pollution-tolerant taxa (Kelly et al., 2008; Salinas-Camarillo et al., 2021; Van Dam et al., 1994). Additionally, the presence of cyanobacterial species signified pollution within the streams and creeks. Although the Shannon-Wiener biodiversity index indicated high phytoplankton biodiversity, the Simpson Diversity Index and Margalef Diversity Index highlighted low biodiversity for both phytoplankton and zooplankton. The scarcity of identified plankton species and/or low counts, along with the absence of previous studies on the plankton community in this area, may contribute to the variable diversity observed. Therefore, it is recommended to expand the study and examine a wider area.

The study further evaluated the water quality of the tributaries using diatom indices. The results showed poor water quality based on the TDI and moderate quality according to the Steinberg and Schiefele Index (SHE), Sládeček Intermediate Index (SLA) and EPI-D index. Significant correlations were observed between EPI-D, TDI and ROTT indices with TP, NH_3-N and NO_2-N concentrations ($p < 0.01$). These findings align with Ongun Sevindik et al. (2023), who reported strong correlations of EPI-D, TDI, IDP and ROTT indices with NO_3-N and TN values, concluding that the TDI index provides a consistent measure of ecological status. Studies based on biological data have just begun in this region, and there is a need for this kind of studies to be examined in more detail.

5. Conclusion

This study investigated the ecological status of streams and creeks within the Murat River system, considering both water quality parameters and diatom index values. The findings indicate that these water sources are subjected to medium pollution levels. The presence of low biodiversity, elevated ammonia nitrogen concentrations, and pollution-tolerant taxa such as *Oscillatoria sp.* and pennate diatoms underscores the influence of anthropogenic pollutants on these aquatic systems. The results highlight an urgent need for measures to mitigate pollution and safeguard these critical water resources. These findings not only provide valuable insights into the chemical and biological status of the tributaries of the Murat River, but also establish a baseline for future research efforts. To build upon this work, it is recommended that subsequent studies expand to encompass the entire Murat River Basin and include comprehensive analyses of anthropogenic impacts and ecological trends over time. In summary, this study revealed that TP, NH_3-N, NO_2-N and NO_3-N concentrations are closely related to diatom indices, indicating that the streams in the study area face significant pollution threats. By addressing the pollution sources and implementing effective management strategies, it is possible to protect and improve the ecological health of the Murat River system for future studies.

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Conflict of Interest

There are no conflicts of interest regarding this research for any of the authors.

References

Anonymous. (2003a). *Su Çerçeve Direktifi'nin Türkiye'de uygulanması uygulama el kitabı*. Grantmij Advies and Techniek bv Vestiging Utrect, Houten.

Anonymous. (2003b). *Water quality-guidance standard for the routine analysis of phytoplankton abundance and composition using inverted microscopy (Utermöhl technique)*. CEN TC 230/WG 2/TG 3/N73.

APHA. (1995). *Standard methods for the examination of water and wastewater* (19th edition). American Public Health Association.

APHA. (2001). *Standard methods for the examination of water and wastewater* (20th edition). American Public Health Association.

Bakır, N. (2015). *Su çerçeve direktifine göre biyolojik kalite unsuru: Makrofit* (Speciality Thesis, Turkish Republic Ministry of Forestry and Water Affairs). (In Turkish)

Bērziņš, B., & Pejler, B. (1987). Rotifer occurrence in relation to pH. In L. May, R. Wallace & A. Herzig (Eds.), *Rotifer Symposium IV. Developments in Hydrobiology* (pp. 107-116). Springer. https://doi.org/10.1007/978-94-009-4059-8_16

Borutskii, E. V. (1964). *Fauna of U.S.S.R. Vol. 3, 4, crustacea: Freshwater Harpacticoida*. Jerusalem: Israel Program for Scientific Translations.

Bulut, H., & Saler, S. (2014). Murat Nehri'nin (Elazığ-Palu ilçe merkezi sınırları içindeki bölümün'de) zooplanktonu ve değişimi. *Türk Tarım – Gıda Bilim ve Teknoloji Dergisi*, *2*(1), 13-17. https://doi.org/10.24925/turjaf.v2i1.13-17.32 (In Turkish)

Bulut, H., & Saler, S. (2019). Effect of physicochemical parameters on zooplankton at a freshwater body of Euphrates Basin (Elazığ-Turkey). *Cellular and Molecular Biology*, *65*(1), 8-13. https://doi.org/10.14715/cmb/2019.65.1.2

Coste, M., & Ayphassorho, H. (1991). Étude de la qualité des eaux du Bassin Artois-Picardie à l'aide des communautés de diatomées benthiques : Application des indices diatomiques. *Rapport*, 227. (In French)

Cox, E. J. (1991). What is the basis for using diatoms as monitors of river quality? In B. A. Whitton, E. Rott & G. Fredrich (Eds.), *Use of algae for monitoring rivers* (pp. 33-40). Universitat Innsbruck Institut für Botanik.

Cox, E. J. (1996). *Identification of freshwater diatoms from live material*. Chapman and Hall.

Descy, J. P., & Coste, M. (1991). A test of methods for assessing water quality based on diatoms. *Verhandlungen des Internationalen Verein Limnologie*, *24*(4), 2112-2116. https://doi.org/10.1080/03680770.1989.11899905

Dussart, H. B., & Defaye, D. (2001). Introduction to the Copepoda. In H. J. F. Dumont (Ed.), *Guides to the identification of the microinvertebrates of the continental waters of the world* (pp. 1-344). SPB Academic Publishers.

Edmondson, W. T. (1959). *Fresh water biology*. University of Washington.

Einsle, U. (1996). Copepoda: Cyclopoida, genera cyclops, megacyclops, acanthocyclops. In H. J. F. Dumont (Ed.), *Guides to the identification of the microinvertebrates of the continental waters of the world* (pp. 1-82). SPB Academic Publishers.

Flössner, D. (1972). *Krebstiere, crustacea. Kiemen and Blattfüßer Brachiopoda fischlause, Branchiura, tierwelt-deutschlands*. 60. Tiel Veb. Gustav Fischer Verlag, Jena. (In German)

Herzig, A. (1987). The analysis of planktonic rotifer population: A plea for long-term investigations. *Hydrobiologia*, *147*, 163-180. https://doi.org/10.1007/BF00025739

Hill, M. O. (1973). Diversity and evenness: A unifying notation and its consequences. *Ecology*, *54*(2), 427-432.

James, B. K., & Adejare, L. I. (2010). Nutrients and phytoplankton production dynamics of a tropical harbor in relation to water quality indices. *Journal of American Science*, *6*(9), 261-275.

John, D. M., Whitton, B. A., & Brook, A. J. (2002). *The freshwater algal flora of the British Isles*. Cambridge University Press.

Kelly, M. G., & Whitton, B. A. (1995). The trophic diatom index: A new index for monitoring eutrophication in rivers. *Journal of Applied Phycology*, *7*, 433-444. https://doi.org/10.1007/BF00003802

Kelly, M. G. (1998). Use of the trophic diatom index to monitor eutrophication in rivers. *Water Research*, *32*(1), 236-242. https://doi.org/10.1016/S0043-1354(97)00157-7

Kelly, M., Juggins, S., Guthrie, R., Pritchard, S., Jamieson, J., Rippey, B., Hırst, H., & Yallop, M. (2008). Assessment of ecological status in UK rivers using diatoms. *Freshwater Biology*, *53*(2), 403-422. https://doi.org/10.1111/j.1365-2427.2007.01903.x

Kerkmann, G. R., Hatunoğlu, A., & Hatunoğlu, Y. (2012). Preliminary data regarding anthropogenic impact on the Murat River (Ağri region). *International Journal of Ecosystems & Ecology Sciences*, *2*(2), 55-60.

Kıvrak, E., Uygun, A., & Kalyoncu, H. (2012). Akarçay'ın (Afyonkarahisar, Türkiye) su kalitesini değerlendirmek için diyatome indekslerinin kullanılması. *Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, *12*(2), 27-38. (In Turkish)

Kiefer, F., & Fryer, G. (1978). *Das Zooplankton der Binnengewässer*. 2. Teil, E. Schweizerbart'sche Verlagsbuchhandlung. (In German)

Kirici, M., Kirici, M., Demir, Y., Beydemir, S., & Atamanalp, M. (2016). The effects of Al3+ and Hg2+ on glucose 6-phosphate dehydrogenase from Capoeta umbla kidney. *Applied Ecology and Environmental Research*, *14*(2), 253-264. https://doi.org/10.15666/aeer/1402_253264

Kolisko, W. R. (1974). *Planktonic rotifers biology and taxonomy biological station*. Lunz of The Austrian Academy of Science.

Koste, W. (1978a). *Die Rädertiere Mitteleuropas I*. Textband.

Koste, W. (1978b). *Die Rädertiere Mitteleuropas II*. Tofelband.

Koyun, M. (2011). Occurrence of monogeneans on some cyprinid fishes from Murat River in Turkey. *African Journal of Biotechnology*, *10*(79), 18285-18293. https://doi.org/10.5897/AJB11.2507

Koyun, M., Ulupınar, M., Özdemir, N. S., Kırıcı, M., & Caf, F. (2020). Seasonal changes of water quality of Murat River (Bingol, Turkey) in terms of physicochemical and biological parameters. *Acta Aquatica Turcica*, *16*(3), 305-312. https://doi.org/10.22392/actaquatr.626918

Krebs, C. J. (1998). *Ecological methodology*. Benjamin/Cummings.

Kwak, T. J., & Peterson, J. T. (2007). Community indices, parameters, and comparisons. In C. S. Guy & M. L. Brown (Eds.), *Analysis and interpretation of freshwater fisheries data* (pp. 677-763). American Fisheries Society.

Leclercq, L., & Maquet, B. (1987). *Deux nouveaux indices chimiques et diatomiques de qualité d'eau courante, application au Samson et ses affluents (bassin de la Meuse belge), comparaison avec d'autres indices chimiques biocénotiques et diatomiques*. Institut Royal des Sciences Naturelles de Belgique, Document de Travail. (In French)

Lecointe, C., Coste, M., & Prygiel, J. (1993). Omnidia: Software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia*, *269*(1), 509-513. https://doi.org/10.1007/BF00028048

Lenoir, A., & Coste, M. (1996). Development of a practical diatom index of overall water quality applicable to the French national water board network. In B. A. Whittonand & E. Rott (Eds.), *Use of algae for monitoring rivers II* (pp. 29-43). Universitat Innsbruck Institut für Botanik.

Liao, A., Han, D., Song, X., & Yang, S. (2021). Impacts of storm events on chlorophyll-a variations and controlling factors for algal bloom in a river receiving reclaimed water. *Journal of Environmental Management*, *297*, 113376. https://doi.org/10.1016/j.jenvman.2021.113376

Mikschi, E. (1989). Rotifer distributions in relation to temperature and oxygen content. *Hydrobiology*, *186*, 209-214. https://doi.org/10.1007/BF00048914

Moss, B. (2007). The art and science of lake restoration. *Hydrobiologia*, *581*, 15-24. https://doi.org/10.1007/s10750-006-0524-2

Negrea, S. T. (1983). *Fauna republici socialiste Romania, crustacea Cladocera*. Academia Republici Socialiste. (In Romanian)

Ongun Sevindik, T., Çetin, T., Tunca, H., Güzel, U., & Tekbaba, A. G. (2023). Ecological status estimation of minimally disturbed rivers of the Western Mediterranean Basin (Türkiye) using diatom indices. *Community Ecology*, *24*, 243-255. https://doi.org/10.1007/s42974-023-00147-5

Pulatsü, S., Topçu, A., & Atay, D. (2014). *Su kirlenmesi ve kontrolü*. Ankara Üniversitesi Basımevi. (In Turkish)

Salinas-Camarillo, V. H., Carmona-Jiménez, J., & Lobo, E. A. (2021). Development of the Diatom Ecological Quality Index (DEQI) for peri-urban mountain streams in the Basin of Mexico. *Environmental Science and Pollution Research*, *28*, 14555-14575. https://doi.org/10.1007/s11356-020-11604-3

Scourfield, D. J., & Harding, J. P. (1969). *Fresh-water biology*. As Sci Publications.

Segers, H. (1995). Rotifera 2. The Lecanidae (Monogononta). In H. J. Dumont & T. Nogrady (Eds.), *Guides to the identification of the continental waters of the world 6* (pp. 226). SPB Academic.

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, *27*(3), 379-423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

Steinberg, C., & Schiefele, S. (1988). Biological indication of trophy and pollution of running waters. *Z. WasserAbwasser-Forsch*, *21*, 227-234.

Stemberger, R. S. (1979). *A guide to rotifers of the Laurentian great lakes*. Environmental Monitoring and Support Laboratory Office of Research and Development.

Strickland, J. D. H., & Parsons, T. R. (1972). *A practical handbook of seawater analysis*. Bulletin 167.

T.C. Başbakanlık Devlet Planlama Teşkilatı. (1998). *Ulusal çevre eylem planı. Deniz kaynakları yönetimi ve kirlilik kontrolü*. TC. Başbakanlık Devlet Planlama Teşkilatı. (In Turkish)

Tanyolaç, J. (2009). *Limnoloji*. Hatipoğlu Yayınları. (In Turkish)

Topal, M., & Topal, E. I. A. (2016). *Murat Nehri (Elazığ)'nin bazı fizikokimyasal parametreler açısından su kalitesinin belirlenmesi*. International Symposium of Water and Wastewater Management. Malatya.

Ustaoğlu, M. R. (2004). A check-list for zooplankton of Turkish inland waters. *Ege University Journal of Fisheries & Aquatic Sciences*, *21*(3-4), 191-199.

Ustaoğlu, M. R., Altındağ, A., Kaya, M., Akbulut, N., Bozkurt, A., Özdemir Mis, D., Atasağun, S., Erdoğan, S., Bekleyen, A., Saler, S., & Okgerman, H. C. (2012). A check list of Turkish rotifers. *Turk Journal of Zoology*, *36*(5), 607-622. https://doi.org/10.3906/zoo-1110-1

Utermöhl, H. (1958). Zur Vervolkommnung deer quantitativen Phytoplankton-Methodik. Mitteilungen der Internationale Vereinigung der theoretretische und Angewandte. *Limnologie*, *9*, 39. (In German)

Van Dam, H., Mertens, A., & Sinkeldam, J. (1994). A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Netherlands Journal of Aquatic Ecology*, *28*, 117-133. https://doi.org/10.1007/BF02334251

YSKY. (2015). *Yüzeysel su kalitesi yönetimi yönetmeliği*. https://www.resmigazete.gov.tr/eskiler/2015/04/20150415-18.htm (In Turkish)

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RESEARCH ARTICLE

Balancing Trade and Sustainability: Türkiye's Squid and Cuttlefish Market Dynamics

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ABSTRACT

Türkiye's squid and cuttlefish production struggles to meet domestic demand due to limited local supply. This study examines production levels, trade balances, and economic impacts using data from the Turkish Statistical Institute (TÜİK) and international sources. Production relies on natural stocks, providing stability but remaining insufficient. Between 2005 and 2024, export volumes fluctuated between 523.8 and 1,026.8 tons, while imports rose from 1,558.8 tons in 2005 to 5,204.4 tons in 2022. Import expenditures increased from $2.6 million to $31.5 million, emphasizing Türkiye's reliance on external sources. The zero-tariff agreement with Malaysia supports imports, yet Malaysia's production capacity is insufficient to meet Türkiye's needs. China's large-scale harvesting and processing, particularly of *Dosidicus gigas* and *Todarodes pacificus*, likely contribute to these products reaching Türkiye via Malaysia. The COVID-19 pandemic caused imports to drop to 2,397.1 tons in 2020, recovering to 3,656.9 tons by 2023. Export revenues peaked at $4.8 million in 2021 but did not offset trade deficits. The study provides insights into market dependencies and trade imbalances. To achieve a sustainable balance, Türkiye must improve fisheries management, diversify supply sources, and reduce import dependency through strategic trade policies.

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1. Introduction

Squid and cuttlefish are economically valuable cephalopod species worldwide. Their production is generally based on harvesting from natural stocks, as aquaculture efforts remain limited (Ospina-Alvarez et al., 2022). Countries like China and Peru lead in production, while production has declined in countries such as Japan. The conservation of stocks, such as the Patagonian shortfin squid, is emphasized (Villasante et al., 2014). Fishing is conducted using advanced techniques. In Europe, these species are often caught as bycatch. Illegal, unreported, and unregulated (IUU) fishing poses a significant threat to stocks (FAO, 2020).

Globally, squid fishing involves approximately 30-40 commercially important species, with an increasing share in total marine product catches (Arkhipkin et al., 2015). *Dosidicus gigas* and *Todarodes pacificus* hold substantial commercial value in countries such as Peru, Japan, and Korea (FAO, 2012). In terms of fishing techniques, common methods include light-assisted jigging, bottom trawling, and netting. Nevertheless, the environmental impacts and pressure on stocks require ecosystem-based management approaches (Caddy & Rodhouse, 1998). Squid species adapt quickly to environmental changes due to their short life cycles, rapid growth, and high reproductive rates (Rodhouse et al., 2014). According to FAO

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(2012), data on cephalopod fisheries highlight significant biomass movements and cyclical effects on ecosystems.

In the Mediterranean, squid fishing is prominent, with species like *Loligo vulgaris* and *Sepioteuthis lessoniana* commonly found (Arkhipkin et al., 2015). In Türkiye, species such as *Loligo vulgaris* hold significant commercial importance. In Türkiye, commercially important species such as *L. vulgaris* are commonly caught using traditional fishing methods, including set nets, jigging, and bottom trawling. However, effective stock conservation and strict enforcement of fishing regulations are essential to ensure sustainability. As highlighted by Rodhouse et al. (2014), the short lifespan and annual fluctuations of squid populations require careful and adaptive management strategies.

Cuttlefish (*Sepia officinalis*) is widely caught for commercial purposes globally. It is found in the eastern Atlantic and Mediterranean Sea. Fishing is done using methods like trawling. Stocks are influenced by environmental factors such as temperature and food availability (Boletzky, 1983; Forsythe et al., 1994). In Türkiye, *S. officinalis* is an important species in the Mediterranean, Aegean, and Marmara Seas (Duysak et al., 2014)

The trade of these species involves the circulation of fresh, processed, and frozen products within a broad trade network. China and South Korea are leaders in fresh product markets. Spain and Italy are key trade hubs in Europe, while processed products are extensively exported from North Africa to Europe and Japan (Arkhipkin et al., 2015). Cold chain infrastructure plays a critical role in these processes. Consumption typically occurs in fresh or processed forms. Consumer habits drive the global demand for these species. However, increased demand raises the risk of stock depletion (Ospina-Alvarez et al., 2022).

To ensure sustainability, traceability systems should be improved, and transparency in trade processes should be increased. The origin and fishing methods of cephalopod products should be accurately documented and made accessible to consumers. Illegal, unreported, and unregulated (IUU) fishing must be addressed. Fishing quotas should be established through regional and international cooperation, and strategies to protect natural stocks should be implemented. Supporting aquaculture initiatives can increase sustainable production capacity for these species. Additionally, strengthening cold chain infrastructure can help maintain product quality and reduce waste (Gleadall et al., 2024).

This study aims to analyze the economic value and sectoral importance of squid and cuttlefish species in Türkiye by examining their production, export, and import parameters over the past 20 years. Within the scope of the research, the production volumes, export and import trends, annual changes, and trade balances of these species were thoroughly evaluated. Based on the findings, the contribution of these species to the national economy was assessed, and recommendations were proposed for the development of sustainable production and trade strategies.

2. Materials and Methods

This study utilized two primary datasets provided by the Turkish Statistical Institute (TÜİK):

1. Catch Production Figures: This dataset, obtained from fisheries statistics, covers the period from 2003 to 2023. It includes the annual production quantities of squid and cuttlefish recorded in Türkiye.

2. Foreign Trade Data: This dataset, sourced from the TÜİK Foreign Trade Platform, covers the years 2004 to 2024. It includes the total export and import quantities and values specifically for squid and cuttlefish.

The information derived from these datasets was analyzed using the following methods and formulas:

The annual averages of production and trade figures over the given period were calculated using the formula:

$$\text{Annual Average} = \Sigma X_i / n \quad (1)$$

Where, X_i represents the production or trade figure for the i-th year, and n is the total number of years.

Annual trends were analyzed using a simple linear regression model via Excel's LINEST or TREND functions:

$$Y_t = \beta_0 + \beta_1 t \quad (2)$$

Where, Y_t: Production or trade value for year t; t: Time (year); β_0: Intercept; β_1: Annual change coefficient.

The foreign trade balance was calculated to determine the trade deficit or surplus for the relevant products:

Trade Balance = Total Export Value - Total Import Value

This calculation was performed annually to evaluate Türkiye's trade performance in this product category.

Data were categorized based on the form of the products (fresh, frozen, processed and canned). The classification of squid and cuttlefish into fresh, processed, canned, and frozen categories is determined based on the customs codes used in foreign trade regulations, ensuring standardization in trade documentation and reporting. The impacts of different product forms on trade figures were compared by calculating the annual averages of imports and exports for each category.

All data were analyzed using Microsoft Excel. Visualizations such as charts and graphs were created to illustrate trends over time. Statistical analyses, including linear regression, were conducted using Excel's built-in tools to evaluate production and trade patterns.

3. Results

Türkiye’s squid and cuttlefish import-export data from 2005 to 2024 reveals fluctuations in export volumes, while unit prices have generally followed an increasing trend. In 2005, Türkiye exported 701.6 tons, generating $2 million in revenue, with an average unit price of $5.36/kg. By 2024, exports had risen to 848.4 tons, with revenue reaching $4.1 million and an average unit price of $5.90/kg. On the import side, there was a significant increase over the years, peaking in 2022 at 5,204.4 tons, costing $31.5 million. However, by 2024, import volumes had declined to 2,559.3 tons, while the unit price stood at $6.63/kg. These trends indicate shifting dynamics in Türkiye’s seafood trade, highlighting both export market developments and increasing import dependency over certain periods (Table 1).

Table 1. Türkiye’s squid and cuttlefish import-export volumes and average unit prices (2005-2024) (Data compiled from TÜİK foreign trade statistics).

Years	Export			Import		
	Tons	Million USD	Unit Price (Kg)	Tons	Million USD	Unit Price (Kg)
2005	701.6	2.0	5.36 ± 0.75	1.558.8	2.6	1.63 ± 0.18
2006	682.3	2.2	5.32 ± 0.71	1.364.9	1.9	1.94 ± 0.35
2007	650.9	2.9	6.23 ± 0.88	1.146.0	1.8	2.05 ± 0.36
2008	681.9	2.8	5.18 ± 0.71	1.660.0	2.5	2.35 ± 0.50
2009	791.6	2.7	4.74 ± 0.74	1.709.1	2.3	2.01 ± 0.40
2010	669.8	2.4	5.53 ± 0.71	2.713.0	3.2	1.40 ± 0.13
2011	752.4	3.5	6.25 ± 0.74	2.604.1	9.4	3.87 ± 0.14
2012	572.0	3.0	7.24 ± 0.66	3.624.7	14.3	3.64 ± 0.28
2013	675.0	2.5	7.66 ± 0.86	2.982.6	11.4	4.11 ± 0.15
2014	785.8	3.3	5.80 ± 1.07	2.436.1	9.1	4.03 ± 0.20
2015	523.8	2.0	5.99 ± 0.88	2.890.6	10.9	4.16 ± 0.21
2016	756.1	3.1	6.28 ± 0.98	2.397.2	9.3	4.36 ± 0.17
2017	785.6	3.4	5.07 ± 0.70	3.497.9	13.7	4.27 ± 0.18
2018	845.9	4.3	7.48 ± 0.81	3.350.6	11.6	4.79 ± 1.20
2019	758.4	2.9	5.91 ± 0.73	3.349.4	11.6	5.47 ± 1.47
2020	933.1	4.4	5.37 ± 0.51	1.842.6	8.1	4.12 ± 0.31
2021	1.026.8	4.8	6.70 ± 0.80	2.860.3	18.1	6.61 ± 0.59
2022	638.1	3.6	5.58 ± 0.69	5.204.4	31.5	6.59 ± 0.55
2023	784.3	3.9	6.16 ± 0.02	3.656.9	20.3	7.62 ± 1.65
2024	848.4	4.1	5.90 ± 0.68	2.559.3	12.0	6.63 ± 0.99

Import volumes increased throughout the years, starting from 1,558.8 tons in 2005 and reaching 5,204.4 tons in 2022. A steady rise in import expenditure was recorded, growing from $2.6 million in 2005 to $31.5 million in 2022. Annual data indicate that import volumes consistently exceeded export volumes. In 2005, imports amounted to 1,558.8 tons, while exports were recorded at 701.6 tons. By 2022, import volumes rose to 5,204.4 tons, whereas export volumes declined to 638.1 tons. The financial value of imports followed a similar pattern, increasing from $2.6 million in 2005 to $31.5 million in 2022. In contrast, export revenues grew at a much slower pace, rising from $2.0 million in 2005 to $4.1 million in 2024. Significant variations in unit prices per kilogram were identified. Export prices fluctuated over the years, ranging from $4.74 ± 0.74 in 2009 to $11.68 ± 3.02 in 2023. Import prices showed notable peaks, reaching $13.36 ± 9.01 in 2016 and remaining consistently above $6.59 ± 0.55 from 2022 onwards.

Table 2 provides Türkiye's foreign trade data for squid and cuttlefish, categorized by product type and analyzed over 5-year periods. For fresh products, export volumes ranged from 50.3±8.0 tons in 2005-2009 to 47.9±9.6 tons in 2020-2024, with a peak of 64.8±10.9 tons in 2010-2014. Imports decreased significantly, reaching 0.1±0.0 tons in 2020-2024. Export values reached their highest at 0.256 million USD in 2010-2014, while import values remained consistently low, with 0.001 million USD recorded in 2020-2024.

Table 2. Türkiye's squid and cuttlefish foreign trade data by product types: 5-Year periods and annual averages (Data compiled from TÜİK foreign trade statistics).

Product	**Period**	**Export**		**Import**	
		Tons	**Million USD**	**Tons**	**Million USD**
Fresh	2005-2009	50.3±8.0	0.183	5.4±1.0	0.009
	2010-2014	64.8±10.9	0.256	0.6±0.1	0.003
	2015-2019	51.1±8.1	0.171	2.6±0.4	0.011
	2020-2024	47.9±9.6	0.227	0.1±0.0	0.001
Processed	2005-2009	39.6±6.2	0.142	111.9±15.1	0.166
	2010-2014	30.6±5.7	0.134	290.0±43.4	0.950
	2015-2019	54.2±7.9	0.253	222.0±33.9	0.845
	2020-2024	16.8±2.6	0.093	70.9±12.8	0.246
Canned	2010-2014	6.1±0.8	0.073	36.6±0.9	0.185
	2015-2019	0.2±0.1	0.002	23.7±2.0	0.123
	2020-2024	0.2±0.1	0.002	4.9±0.6	0.025
Frozen	2015-2019	34.6±5.7	0.167	264.3±52.7	0.949
	2020-2024	35.3±8.2	0.176	327.9±89.8	1.845

As shown in Table 2, processed products had fluctuating export volumes, starting at 39.6±6.2 tons in 2005-2009, peaking at 54.2±7.9 tons in 2015-2019, and decreasing sharply to 16.8±2.6 tons in 2020-2024. Imports were highest in 2010-2014 at 290.0±43.4 tons but fell to 70.9±12.8 tons by 2020-2024. Export values were highest in 2015-2019 at 0.253 million USD, while import values saw a steady decline, dropping to 0.246 million USD in 2020-2024.

Table 2 also indicates that canned products recorded exports starting from 6.1±0.8 tons in 2010-2014, which decreased to 0.2±0.1 tons in both subsequent periods. Import volumes followed a similar declining trend, from 36.6±0.9 tons in 2010-2014 to 4.9±0.6 tons in 2020-2024. Export values dropped from 0.073 million USD in 2010-2014 to 0.002 million USD in later periods, while import values decreased from 0.185 million USD to 0.025 million USD during the same time frame.

Frozen products, as detailed in Table 2, were first recorded in 2015-2019, with export volumes increasing slightly from 34.6±5.7 tons to 35.3±8.2 tons in 2020-2024. Import volumes rose from 264.3±52.7 tons to 327.9±89.8 tons over the same periods. Export values showed a marginal increase from 0.167 million USD to 0.176 million USD, while import values saw a substantial rise from 0.949 million USD to 1.845 million USD in 2020-2024.

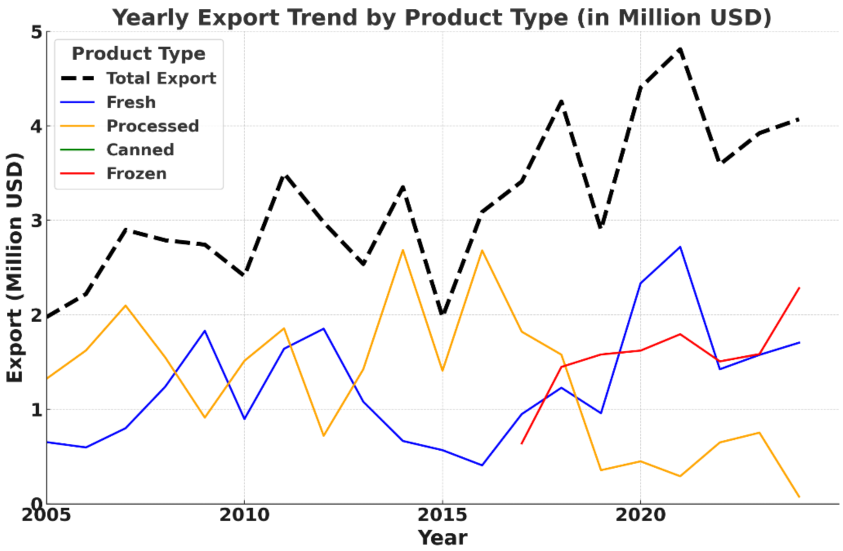

Figure 1. Export values of different products for Türkiye's squid and cuttlefish.

Figure 1 presents Türkiye's export performance for squid and cuttlefish across different product categories. Total export values increased over the years, reaching approximately 4.5 million USD in 2019. After this peak, exports declined slightly and stabilized around 4 million USD in the following years. Fresh squid and cuttlefish exports fluctuated between 2005 and 2020, ranging from 1 million USD to 2 million USD. After 2015, fresh exports increased, exceeding 1.5 million USD in 2022. Processed product exports varied significantly, reaching 2 million USD in 2015 and decreasing in the following years. By 2022, processed exports remained below 1 million USD. Canned exports remained consistently low, staying below 0.5 million USD throughout the observed period. Frozen squid and cuttlefish exports increased steadily, especially after 2018, surpassing 1.5 million USD in 2022.

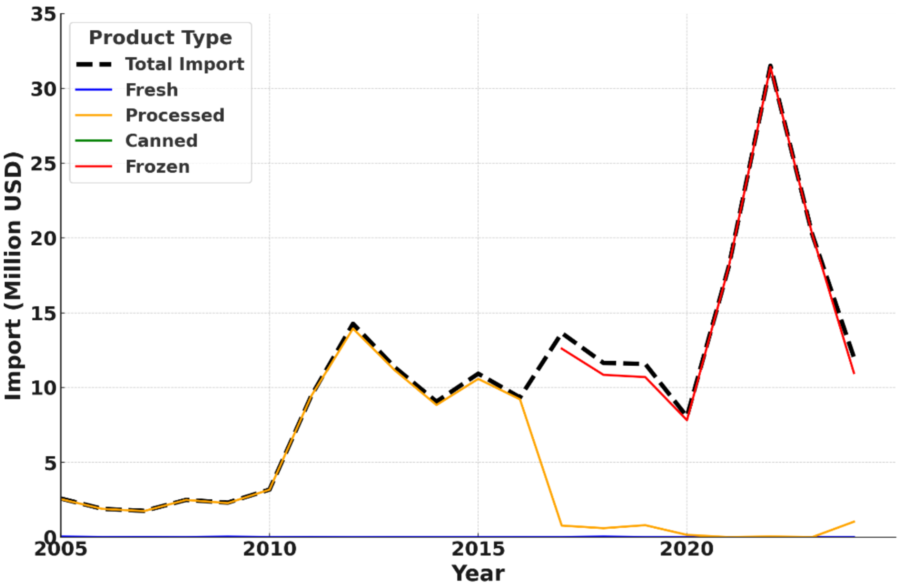

Figure 2. Import values of different products for Türkiye's squid and cuttlefish.

Türkiye's squid and cuttlefish production, export, and import data provide critical insights into the trade balance of these products. The chart indicates that while approximately half of the domestic production is directed toward exports, imports and the resulting trade deficit remain significant.

During the pandemic, particularly in 2020, a noticeable decline in imports was observed. This situation could be attributed to disruptions in global supply chains and logistical challenges. However, imports increased again in the following years, peaking in 2023. This increase likely reflects the easing of pandemic-related restrictions and a recovery in demand. In 2024, a reduction in imports was observed, possibly due to fluctuations in domestic demand or the availability of alternative supply sources.

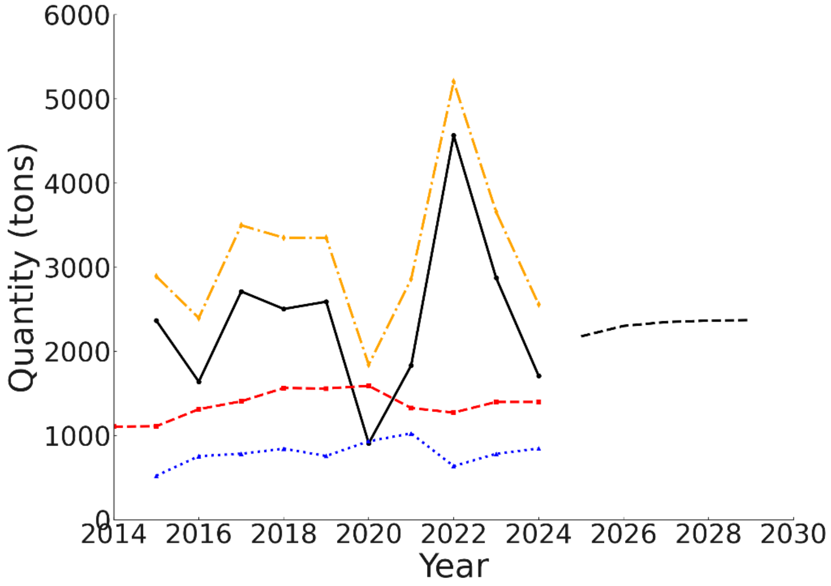

Figure 3. Türkiye's squid and cuttlefish production (red), export (blue), import (orange), trade deficit (black), and 5-year forecast.

In 2023, Türkiye's imports reached a peak of 4,566.3 tons, while exports remained at 845.9 tons. Domestic production was limited to 1,565.5 tons, resulting in a trade deficit of 3,720.4 tons. Although imports decreased to 2,890.6 tons in 2024, the trade deficit remained at a significant level of 2,366.8 tons. During the pandemic in 2020, imports dropped to 2,397.1 tons, while production and export levels remained stable, likely reflecting the impact of global supply chain disruptions on Türkiye’s trade.

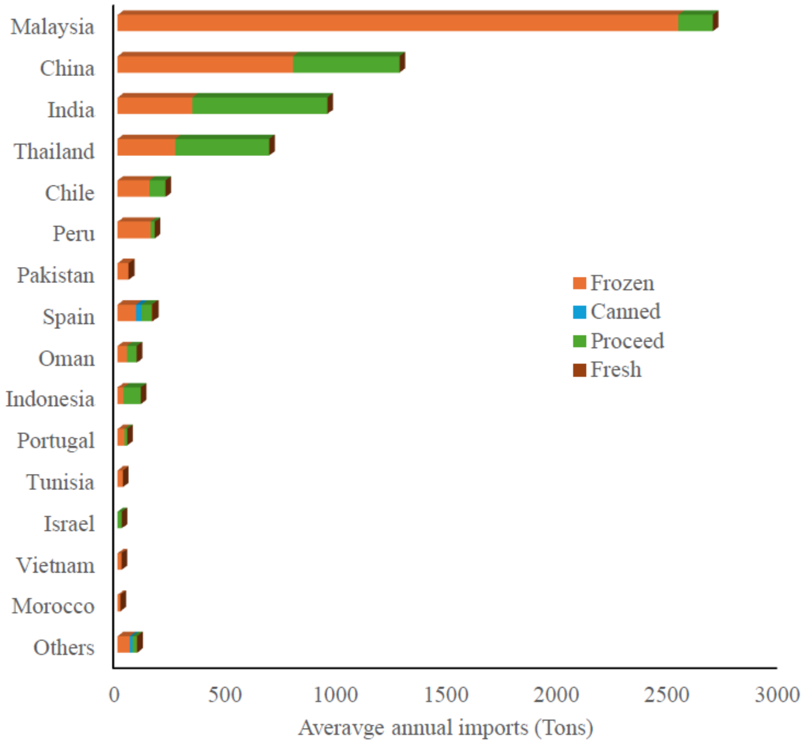

Figure 4. Annual import quantities of squid and cuttlefish by country in Türkiye over the last decades (Data compiled from TÜİK foreign trade statistics).

Türkiye’s squid and cuttlefish imports mainly come from Asian countries. The largest supplier is Malaysia, with an annual import volume of approximately 2,900 tons, mostly consisting of frozen products. China and India follow, with 1,700 tons and 1,200 tons, respectively, with the majority of their exports to Türkiye being frozen and processed products.

Thailand is also a significant supplier, contributing around 900 tons annually. Other Asian countries, including Pakistan, Indonesia, Vietnam, and Oman, also export squid and cuttlefish to Türkiye, but in smaller quantities (Figure 4).

4. Discussion

Türkiye’s production of squid and cuttlefish remains insufficient to meet domestic consumption demands. Addressing this gap by increasing production is considered a crucial step to reduce import dependency (Gazihan, 2017; Gökoğlu, 2021). However, since Türkiye’s production heavily relies on natural stocks, achieving a significant increase in production is constrained by the limited availability of these resources (Pierce et al., 2010). The country’s dependence on wild-catch methods, combined with the natural restrictions of its marine environment, makes large-scale production expansion challenging (Vidal et al., 2014). In this context, the cultivation of squid and cuttlefish locally emerges as a significant strategy to fulfill domestic demand sustainably. Such initiatives could reduce reliance on imports and simultaneously bolster both domestic consumption and potential export opportunities.

In the short term, however, imports remain a necessary solution to meet consumption demands. For years, Türkiye’s production and trade dynamics have shown a relatively stable pattern. Approximately half of the local production is allocated for export, demonstrating the necessity of imports to satisfy internal market needs. Nonetheless, the COVID-19 pandemic significantly disrupted these dynamics. During the initial stages of the pandemic, import volumes dropped substantially due to disruptions in global supply chains and challenges in sourcing products from Asian countries, which are key suppliers. Türkiye responded to these constraints by utilizing existing local stocks to meet domestic demand and fulfill export obligations, often relying on frozen products due to logistical limitations (Can et al., 2020; Demirci et al., 2020).

Following the pandemic, particularly in 2022 and 2023, imports experienced a notable surge, nearly doubling compared to previous years. This sharp increase was largely driven by accumulated demand that could not be met during the pandemic period. By 2024, import levels stabilized and returned to pre-pandemic levels, reflecting a balance between demand and supply. This stabilization was likely supported by the resumption of normal trade flows and the realignment of domestic and export needs.

Türkiye’s trade dynamics highlight the dependence on imports due to the limited capacity of local production. While local stocks were effectively managed during the pandemic, the heightened post-pandemic demand underscored the critical role of imports in sustaining market balance. This situation reinforces the need to develop local aquaculture initiatives to enhance production capacity and reduce reliance on external sources. Instead of reducing import dependency, the strategic management of imports and optimization of the trade balance are necessary. Examples of such strategies include:

High-Quality Imports: Increasing imports from countries like Malaysia, where production costs are low and zero-tariff agreements exist, can optimize quality and cost-effectiveness.

Strengthening Supply Chains: Improving logistics efficiency in import processes can reduce costs, especially by ensuring fresh and fast delivery to consumers in tourist areas.

Tourism-Integrated Models: Imported products can be marketed as part of the "Turkish cuisine" concept in tourist facilities, turning imports into an economic advantage.

International Partnerships: Establishing long-term agreements with suppliers like Malaysia can ensure consistent product availability and minimize price fluctuations.

When evaluating global squid and cuttlefish production volumes on a country basis, China and Peru lead the industry as the largest producers (Ospina-Alvarez et al., 2022). In contrast, Malaysia stands out as a key player in Türkiye’s squid and cuttlefish imports, likely due to the zero-tariff agreement between the two nations. However, Malaysia's production capacity alone may not suffice to meet Türkiye's demands, as a significant portion of squid and cuttlefish supplied through Malaysia likely originates from China and other Asian countries (Pierce & Portela, 2014).

China, as the world’s largest squid producer, predominantly harvests species such as *Dosidicus gigas* (Humboldt squid) and *Todarodes pacificus* (Pacific flying squid) (Chen et al., 2008; Liu et al., 2013). These species are caught in large volumes, with *Dosidicus gigas* being particularly abundant in the eastern Pacific (Pierce & Portela, 2014). China’s advanced fishing fleets and processing facilities allow it to dominate global trade in squid products. It is highly plausible that a portion of China’s production is exported indirectly to Türkiye, transiting through Malaysia to take advantage of the favorable trade agreement between Türkiye and Malaysia (Vieites et al., 2019). This arrangement underscores the interconnected nature of global squid and cuttlefish trade and highlights the strategic importance of trade policies in shaping market dynamics (Gleadall et al., 2024).

5. Conclusion

Türkiye’s squid and cuttlefish trade over the years has shown an increasing dependence on imports, leading to a widening trade deficit. Limited local production, reliant on natural stocks, has restricted growth, making imports essential to meet domestic demand. While import volumes have fluctuated, Türkiye continues to rely heavily on external suppliers. As the world's leading squid producer, China plays a central role in this trade, with a significant portion of its products reaching Türkiye through intermediary countries such as Malaysia. This highlights the importance of establishing direct trade agreements with China to minimize intermediary costs and enhance supply chain efficiency.

To reduce import dependency and improve trade balance, the following strategies are recommended:

- Enhancing Direct Trade with China: Shifting from indirect imports via intermediary countries to direct trade agreements with China could reduce extra costs and stabilize supply, improving Türkiye’s trade balance.
- Diversifying Alternative Suppliers: To decrease reliance on China, Türkiye should strengthen direct trade relations with other Asian countries, such as Indonesia, Thailand, and India, which also play a role in the global squid trade.
- Increasing the Value of Exports: While Türkiye’s production capacity is limited, investing in processed and value-added squid and cuttlefish products could increase export revenues and improve market competitiveness.
- Strengthening Logistics and Cold Chain Infrastructure: Improving efficiency in import logistics and enhancing cold chain facilities can reduce costs and ensure fresher, higher-quality products, especially for the tourism sector.
- Tourism-Integrated Consumption Strategies: Incorporating imported squid and cuttlefish into Türkiye’s gastronomic and tourism industries can transform imports into an economic advantage, particularly by promoting Turkish seafood cuisine to international visitors.
- Expanding International Trade Agreements: Securing long-term agreements with key supplier countries, such as Malaysia, could ensure stable supply and minimize price fluctuations, making Türkiye less vulnerable to market volatility.

Conflict of Interest

The author has no conflict of interest to declare.

References

Arkhipkin, A. I., Rodhouse, P. G., Pierce, G. J., Sauer, W., Sakai, M., Allcock, L., Arguelles, J., Bower, J. R., Castillo, G., Ceriola, L., … & Yamashiro, C. (2015). World squid fisheries. *Reviews in Fisheries Science & Aquaculture*, *23*(2), 92-252. https://doi.org/10.1080/23308249.2015.1026226

Boletzky, S. V. (1983). *Cephalopod life cycles*. Academic Press.

Caddy, J. F., & Rodhouse, P. G. (1998). Cephalopod and groundfish landings: Evidence for ecological change in global fisheries? *Reviews in Fish Biology and Fisheries*, *8*(4), 431-444. https://doi.org/10.1023/A:1008824208118

Can, M. F., Şimşek, E., Demirci, A., Akar, Ö., & Demirci, S. (2020). The evaluation of the early impacts of the COVID-19 pandemic on the export of fishery commodities of Turkey. *Marine and Life Sciences*, *2*(1), 18-27.

Chen, X., Liu, B., & Chen, Y. (2008). A review of the development of Chinese distant-water squid jigging fisheries. *Fisheries Research*, *89*(3), 211-221. https://doi.org/10.1016/j.fishres.2007.10.012

Demirci, A., Şimşek, E., Can, M. F., Akar, Ö., & Demirci, S. (2020). Has the pandemic (COVID-19) affected the fishery sector on a regional scale? A case study on the fishery sector in Hatay province, Turkey. *Marine and Life Sciences*, *2*(1), 13-17.

Duysak, Ö., Özcan, G., Çek, Ş., & Türeli, C. (2014). Reproductive biology of the common cuttlefish (*Sepia officinalis*) in İskenderun Bay (Northeastern Mediterranean Sea). *Indian Journal of Geo-Marine Sciences*, *43*(9).

FAO. (2012). *The state of world fisheries and aquaculture*. FAO.

FAO. (2020). *The state of world fisheries and aquaculture*. FAO. https://doi.org/10.4060/ca9229en

Forsythe, J. W., DeRusha, R. H., & Hanlon, R. T. (1994). Growth, reproduction, and life span of *Sepia officinalis* (Cephalopoda: Mollusca) cultured through seven consecutive generations. *Journal of Zoology London*, *233*(2), 175-192. https://doi.org/10.1111/j.1469-7998.1994.tb08582.x

Gazihan, A. (2017). *Investigations on ecosystem-based fisheries management strategies for the Turkish seas* (Doctoral dissertation, Middle East Technical University).

Gleadall, I. G., Moustahfid, H., Sauer, W. H. H., Ababouch, L., Arkhipkin, A. I., Bensbai, J., Elegbede, I., Faraj, A., Ferreiro-Velasco, P., González-Gómez, R., … & Yamaguchi, T. (2024). Towards global traceability for sustainable cephalopod seafood. *Marine Biology*, *171*, 44. https://doi.org/10.1007/s00227-023-04300-6

Gökoğlu, N. (2021). Molluscan shellfish. In N. Gökoğlu (Ed.), *Shellfish processing and preservation* (pp. 129-250). Springer. https://doi.org/10.1007/978-3-030-60303-8_3

Liu, B., Chen, X., Chen, Y., & Tian, S. (2013). Geographic variation in statolith trace elements of the Humboldt squid, *Dosidicus gigas*, in high seas of the Eastern Pacific Ocean. *Marine Biology*, *160*, 2853-2862. https://doi.org/10.1007/s00227-013-2276-7

Ospina-Alvarez, A., de Juan, S., Pita, P., Ainsworth, G. B., Matos, F. L., Pita, C., & Villasante, S. (2022). A network analysis of global cephalopod trade. *Scientific Reports*, *12*, 322. https://doi.org/10.1038/s41598-021-03777-9

Pierce, G. J., & Portela, J. (2014). Fisheries production and market demand. In J. Iglesias, L. Fuentes & R. Villanueva (Eds.), *Cephalopod culture* (pp. 41-58). Springer. https://doi.org/10.1007/978-94-017-8648-5_3

Pierce, G. J., Belcari, P., Bustamante, P., Challier, L., Cherel, Y., González, Á., Guerra, Á., Jereb, P., Koueta, N., Lefkaditou, E., … & Zumholz, K. (2010). *The future of cephalopod populations, fisheries, culture, and research in Europe*. ICES Cooperative Research Report.

Rodhouse, P. G., Pierce, G. J., Nichols, O. C., Sauer, W., Arkhipkin, A., Laptikhovsky, V., Lipiński, M. R., Ramos, J. E., Gras, M., Kidokoro, H., Sadayasu, K., Pereira, J., Lefkaditou, E., Pita, C., Gasalla, M., Haimovici, M., Sakai, M., & Downey, N. (2014). Environmental effects on cephalopod population dynamics: Implications for management of fisheries. *Advances in Marine Biology*, *67*, 99-233. https://doi.org/10.1016/B978-0-12-800287-2.00002-0

Vidal, E. A. G., Villanueva, R., Andrade, J. P., Gleadall, I. G., Iglesias, J., Koueta, N., Rosas, C., Segawa, S., Grasse, B., Franco-Santos, R. M., … & Wood, J. (2014). Cephalopod culture: Current status of main biological models and research priorities. *Advances in Marine Biology*, *67*, 1-98. https://doi.org/10.1016/b978-0-12-800287-2.00001-9

Vieites, J. M., Ruiz, C. S., Fernández, F., & Alonso, R. C. (2019). Importance of cephalopod health and welfare for the commercial sector. In C. Gestal, S. Pascual, Á. Guerra, G. Fiorito & J. M. Vieites (Eds.), *Handbook of pathogens and diseases in cephalopods* (pp. 15-30). Springer. https://doi.org/10.1007/978-3-030-11330-8_2

Villasante, S., Sumaila, R., & Antelo, M. (2014). Why cooperation is better: The gains of cooperative management of the Argentine shortfin squid fishery in South America. In S. Barrett, K.-G. Mäler & E. Maskin (Ed.), *Environment and development economics: Essays in honour of sir Partha Dasgupt* (pp. 270-294). Oxford university Press.

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RESEARCH ARTICLE

Effect of Grow Medium and Hydroponics Fertilizer on Iceberg Lettuce (*Lactuca sativa* var. *capitata*) Cultivation

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ABSTRACT

Iceberg lettuce (*Lactuca sativa* var. *capitata*), which belongs to the family Asteraceae, is a globally renowned vegetable, particularly in salads for its crisp texture, mild flavor and perceived health benefits. Iceberg lettuce remains relatively uncommon in Sri Lanka, and the cultivation is confined to cooler climates. However, there are gaps in the recommendations of fertigation protocols for hydroponics Iceberg lettuce cultivation. Therefore, this research was conducted to examine the influence of selected grow media and fertigation options on the vegetative growth and yield of Iceberg lettuce grown in the hydroponics system, "grow bag culture" in controlled environment agriculture. The research was carried out in a soft plastic-covered greenhouse under a mild climatic region in the higher attitudes (in Lindula), Sri Lanka. The experiment design was a single factor Completely Randomized Design (CRD) with six treatment combinations of grow media and fertigation options. Grow media compositions varied with different ratios of topsoil, coco-peat, rice hull charcoal, and compost. Vegetative growth was assessed during 1 to 7 weeks after transplanting (WAT). At the final vegetative stage, leaf area, chlorophyll concentration, leaf nitrogen content, plant fresh weight and dry weights were determined through destructive sampling. Percentage head formation was assessed at 9 – 11 WAT. At harvesting, yield parameters, head fresh weight, dry weight, head diameter and height were assessed. The results revealed significant variations in plant height, leaf parameters, and head characteristics among treatments, highlighting the effect of fertilizer mixtures and grow media on plant growth and head formation. Leaf color and chlorophyll content of Iceberg lettuce leaves indicated a lesser treatment effect. Substrate parameters, including pH, electrical conductivity, bulk density and water holding capacity, varied significantly among treatments and have a notable influence on the final yield. Treatment (T4) (which contained coco-peat: rice hull charcoal: topsoil at 1:1:2 ratio) and fed with half the strength of standard Alberts fertilizer dosage (F2) was the overall best combination for Iceberg lettuce cultivation. The information generated would be useful for revising the farmers' guide on hydroponics Iceberg lettuce with respect to the selection of grow media and management of fertigation.

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1. Introduction

With the continuous increase in the global population, food-crop supply is expected to increase intensely while other resources are gradually diminishing (Zhang et al., 2022). According to Jensen (2001) as cited in Sabir and Singh (2013) protected cultivation or controlled environment agriculture (CEA) is highly productive in terms of quality and quantity. In addition, it has a lesser environmental impact, compared to open-field cultivation (Chen et al., 2019).

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Lettuce (*Lactuca sativa*), is a leafy annual vegetable from the family Asteraceae. Iceberg lettuce, scientifically identified as *Lactuca sativa* var. *capitata*, Iceberg lettuce, or crisphead lettuce, is known for its tightly compacted head of crisp, pale green leaves with high water content. Nowadays Iceberg lettuce is being cultivated commercially worldwide as a salad crop (Meena & Kulakarni, 2022). In 2022, China ranked first in global lettuce and chicory production with 14.98 million metric tons, followed by the U.S. (3.30 million), Spain (0.97 million), Italy (0.64 million), Belgium (0.60 million), and Japan (0.56 million) (FAO, 2022). As a cool-season crop, lettuce is widely grown on all continents, primarily in temperate and subtropical regions. The growing cycle takes around 55 - 65 days depending on the climate of the production area (Ryder, 1999). The head weight of Iceberg lettuce ranges within 400 - 800 g, depending on the supermarket demand and season (Jenni & Bourgois, 2008; Kader, 2002).

Grow medium is a solid aggregate used in soilless culture systems to replace soil, providing anchorage for growing seedlings, and functioning as a source of plant nutrients, water and oxygen for the root system (Indriyani et al., 2011). An ideal grow medium should support seed germination, succeeding emergence, seedlings' growth, retaining moisture, draining excessive water, and providing sufficient plant nutrients for growth and development (Olaria et al., 2016). Grow media can include organic materials such as coir dust, rice hull charcoal, compost, biochar, and inorganic materials like sub-soil. This study aimed to develop a growing medium by mixing these substrates at different rates focusing on their physical characteristics, such as bulk density, and water-holding capacity.

Meanwhile, plants synthesize necessary carbohydrates through photosynthesis but require inorganic plant nutrients to diversify them structurally and functionally within the plant. Inadequate or unbalanced supply of these nutrients can negatively impact vegetable crop growth and productivity (El-Saady, 2016). However, excessive fertilizer supply may cause toxicity, nutrient uptake issues, reduced yields, environmental hazards, and increased costs. Proper balance of plant nutrients is essential to mitigate abiotic stresses such as low light, salinity, drought and heat (Söylemez, 2021).

Alberts hydroponics formula is the commonly used fully soluble fertilizer by Sri Lankan hydroponics growers but with the least adherence to recommended dosage (Weerakkody et al., 2007) or manufacturer's instructions. Due to its uncommon presence in Sri Lanka, there are no specific recommendations for fertilizer or suitable growing media. As a result, growers experience variable growth rates, yields and quality and due to issues in grow media and fertilizer management. Given the limited research on Iceberg lettuce in Sri Lanka, this study aimed to address the knowledge gap by exploring the effect of different grow media and fertilizer combinations on plant growth, yields, and head quality of Iceberg lettuce. It further aims to establishing proper fertigation protocols for grow-bag culture of Iceberg lettuce under semi-intensive climate control systems in the upcountry of Sri Lanka.

2. Materials and Methods

2.1. Experimental Setup

The experiment was conducted in a soft plastic-covered greenhouse in DIMO Agri Techno Park-Lindula located in the Agro-ecological region, WU1 (up country, wet zone) from November 2023 to February 2024. The mean day and night temperature was 23.4 °C and 18.0 °C. While the mean humidity was 76%. The percentage of fully sunny days during the season was 64%. The experiment was carried out as a grow bag culture (hydroponics) of Iceberg lettuce (*Lactuca sativa* var. *capitata*). Iceberg lettuce variety, Lettuce Tropical (Sakata Seed Corporation) was selected for the trial.

2.2. Experimental Design

The experimental design was single factor Completely Randomized Design (CRD) with six treatments (including the control) and three replicates. Each replicate was composed of five plants. Plants were grown individually in grow bags. The six treatment combinations made from four types of grow media and three fertilizer schedules (F1, F2 and F3) are given in Table 1. Plant density was maintained at 9 pots m^{-2}.

Table 1. Treatment combinations with grow media and fertilizer schedule.

Treatment	Grow media	Fertilizer schedule
T1 (Control)	Coco-peat: rice hull charcoal (1:1)	F1
T2	Coco-peat: rice hull charcoal: topsoil (1:1:2)	F1
T3	Coco-peat: rice hull charcoal: compost (1:1:2)	F1
T4	Coco-peat: rice hull charcoal: topsoil (1:1:2)	F2
T5	Coco-peat: rice hull charcoal: compost (1:1:2)	F2
T6	Coco-peat: topsoil (1:1)	F3

2.3. Media Preparation and Nursery Management

Coco peat was first subjected to excessive washing with water (super washing with adequate drainage), followed by calcium nitrate ($Ca(NO_3)_2$) treatment and excessive washing (Gbollie et al., 2021). This commodity treatment and repeated washing procedure ensured a low level of tannins and partial replacement of Na and Mg ions with Ca ions in coco peat, ensuring its readiness for attaining optimal plant growth. Subsequently, the ready-to-use media was utilized for filling the grow bags, creating a conducive environment for the cultivation of plants. Seedlings of Iceberg lettuce were raised in properly rehydrated jiffy pellets after. They were covered with paper for three days to facilitate seed germination. Then the seedlings were maintained under greenhouse conditions with proper water and plant nutrient management.

2.4. Transplanting and Fertigation

Transplanting was done at four true leaf stage (3 weeks after seeding). Grow bags were disinfected with Topsin (a.i. Thiophanate-methyl 70%) before transplanting, as a preventive measure against fungal infections (Usman et al., 2013). Seedlings were irrigated according to the manufacturer's instructions (Table 2) and the farm practice at daily basis and at a fixed time, manually. For Fertilizer schedule 2 (F2), half the dosage of F1 was applied.

Table 2. Fertilizer schedule 1 (F1).

Growth stage (WAT)	Alberts fertilizer (g/plant/day)	Water (ml/plant/day)	Approximate EC (mS/cm)
0 – 2/3	0.2	600	0.8
2/3 – 4/5	0.4	600-1000	1.2
4/5 – 7/8	0.6	1000-1500	1.5
7/8 – 10/12	0.8	1500-2000	1.9
After 10/12	0.4-0.6	1000-2000	1.5

Sources: CIC Colombo, Unipower, Colombo. WAT: Weeks after transplanting.

The fertilizer schedule 3 (F3) was the regular farm practice. In F3, the weekly fertigation started on the 1st day of the week with a dosage of 0.25 g of Alberts fertilizer per plant/day, dissolved in 300 ml. It was followed by applying the same dosage of NPK fertilizer (at the ratios of 10:52:10 and 30:10:10) and $Ca(NO_3)_2$ in subsequent days, respectively. Grow medium was subjected to excessive drainage (for removing accumulated salts) by irrigating without fertilizer application (with or without $MgSO_4$) two days a week. Meanwhile, EC of the supply solution was increased weekly by increasing the fertilizer dosage by 0.25 g/ plant increments at weekly basis up to the fourth week, keeping the same dilution rate. Fifth week onwards, the dosage was increased to 1.25 g/plant, dissolved in 600 ml of water.

Daily observations based pest and disease identification, followed by pesticide application were practiced, starting from the transplanting. Lettuce Drop, caused by *Sclerotinia sclerotiorum* was observed and controlled by applying Cabrio Top (a.i. Pyraclostrobin 5%+metiram) 55% (w/w) WG. Some lettuce heads were damaged by cabbage semilooper, *Tircihoplusia ni* (Lepidoptera; Noctuidae), and were controlled by applying Croagen (a.i. chlorantraniliprole) 200 g L^{-1} SC.

2.5. Data Collection and Analysis

Data collection on plant height and leaf number was done up to six weeks after transplanting (WAT). Counting leaves after 4 WAT was difficult due to their spiral arrangement in a dense rosette. Destructive sampling was done at peak vegetative growth (at 7 WAT) for dry weight and chlorophyll measurements. At harvesting, head formation, fresh weight, dry weight and dimensions of the head were determined using standard laboratory procedures. Leaf area was measured in destructive samples, using a leaf area meter (model AM350 ADC BioScientific Ltd.), and leaf chlorophyll content was measured using a SPAD meter (model 502Plus, Konica Minolta, Japan).

Meanwhile, leaf nitrogen content was determined using the Kjeldahl procedure (Martín et al., 2017). pH and electrical conductivity (EC) of the supply solutions and grow media were determined using a digital tabletop, pH- EC meter. The bulk density of the initial material and grow media were calculated by obtaining the weight of a known volume of air-dried and oven-dried samples (at 80 °C for 48 hours) by inserting them into moisture cans. Meanwhile, excessively moistened media samples were brought to field capacity (FC) by holding overnight. Subsequently, they were used to determine water holding capacity (WHC) using the following equation (Wilke, 2005).

$$WHC\ (\%) = \frac{Wsm - Wodm}{Wodm}\ x\ 100 \tag{1}$$

Where, W_{sm}: Weight of saturated medium and W_{odm}: Weight of oven dried medium.

The parametric data of plant growth and yield parameters were analyzed using ANOVA of the statistical software, SAS (version 9.0). Mean separation was done using DnMRT to compare the treatment combinations. Non-parametric data were analyzed through chi-square test using Minitab.

3. Results and Discussion

3.1. Vegetative Growth

The effect of different grow media and fertilizer combinations on the vegetative growth of Iceberg lettuce is illustrated in Figure 1. The treatment effect was significant with respect to plant height ($p<0.0002$), except for the first week after transplanting (WAT). Plant height of T6 showed a significant increase at 3 WAT and continued to dominate throughout the vegetative phase, reaching 28.53±0.60 cm at 7 WAT, and was followed by T1 (25.5±1.6 cm) and T4 (21.8±1.2 cm), respectively. There was no significant difference ($p>0.05$) between T2 and T4 as well as T3 and T5 which were having the same grow media but different fertilizer schedules (Figure 1a). Farid et al. (2022) found that while variations in the composition of grow media significantly enhanced shallot growth, different dosages of NPK fertilizer did not yield significant changes. In here as the control treatment, coco-peat and rice hull charcoal were used to observe the direct effects of fertilizer treatments, as they are almost inert and have minimum nutrient content, compared to other treatments. The mixture of rice hull charcoal and compost provided superior results, may be due to better water retention and nutrient availability, and also aeration in charcoal, promoting healthier plant development. Meanwhile, lack of significant response to variable fertilizer dosages may be due to environmental factors and limited nutrient absorption during the growth phase. These findings emphasize that improving of the grow media plays a more crucial role in plant productivity than merely adjusting fertilizer levels.

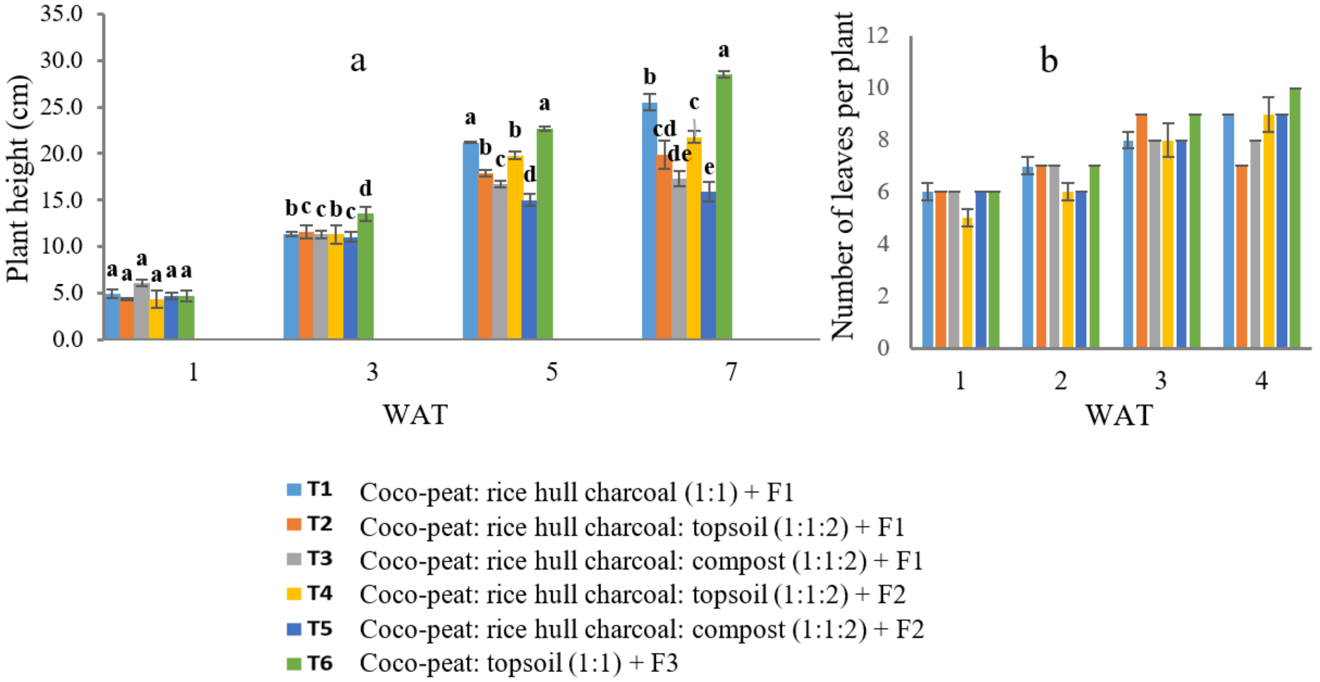

Figure 1. Effect of grow media and fertilizer treatments on plant height (a) and number of leaves (b) of Iceberg lettuce. Error bars indicate standard error (SE) of means, and the treatment means labeled with different letters are significantly different (at $p<0.05$); WAT: Weeks after transplanting.

The leaf number was not significantly different ($p>0.05$) among grow media and fertilizer treatments during 1- 4 WAT (Figure 1b). Generally, growth and development of leaves are affected by hormones, plant nutrition, environmental conditions (soil water content, incident light, and leaf temperature), and genetic factors (Cookson et al., 2005). However, in this experiment, the treatment effect was not evident in terms of leaf number but significantly evident with leaf area (Table 3). The highest total leaf area was shown by T6, followed by T1, reaching the upper range reported by Afton (2018) for Crisp head lettuce cultivars (3579.7 - 5449.7 cm² per plant).

Healthier and vigorous leaf growth is also reflected by leaf colour (SPAD reading). Media treatments, coco-peat and rice hull charcoal under F1 (higher fertilizer dosage) (T1) and the same medium with topsoil under F2 (lower fertilizer dosage) (T4) have shown a significantly lower SPAD reading (Table 3). Hence it reflects the incapability of the grow medium to sustain the supplied fertilizer, leading to a deficiency or imbalance of plant nutrients. This observation is supported by the fact that T5 has shown a higher SPAD reading among the treatments, under the lower fertilizer dosage (F2) where it contained an equal amount of compost to match with coco-peat and rice hull charcoal (1:1:2). This could be either due to higher N content in the compost supplement or its improved water and nutrient retention capacity. The first possibility is further supported by the results of leaf N analysis which indicates a similar treatment difference to SPAD reading (T6 and T3) (Table 3).

Table 3. Plant growth and physiological parameters of Iceberg lettuce at the final vegetative stage (7 WAT).

Trt.	Leaf area (cm^2 plant^{-1})	Chlorophyll concentration- SPAD reading	leaf N content (mg g^{-1})	Plant fresh weight (g)	plant dry weight (g)
1	4728^{ab}	27.0^{b}	24.54^{b}	328^{ab}	13.16^{ab}
2	2377^{c}	38.6^{a}	24.36^{b}	131^{cd}	4.30^{c}
3	1413^{c}	45.8^{a}	29.12^{a}	87^{d}	4.85^{c}
4	3117^{bc}	26.1^{b}	24.45^{b}	227^{bc}	7.84^{bc}
5	1177^{c}	41.5^{a}	30.70^{a}	62^{d}	3.32^{c}
6	5664^{a}	38.0^{a}	28.98^{a}	370^{a}	13.61^{a}

Trt.: Media and fertilizer combinations. The means that do not share the same letter in superscripts are significantly different (DMRT/$p<0.05$).

Meanwhile, significantly higher leaf growth (indicated by leaf area) in T6 yielded the highest dry weight at the end of vegetative growth of Iceberg lettuce, and it was followed by T1 and T4, respectively (Table 3). The treatment effect on the plant fresh weight at that stage was also significant, following the same pattern of treatment differences. Both fresh and dry weights of T1 and T6 were significantly higher than the other media and fertilizer combinations ($p=0.0010$) (Table 3).

Based on the overall results, T6 (coco peat and topsoil with F3) has shown a dominant effect, indicating the superiority of the grow medium and fertilizer combination for maintaining the most favorable conditions for overall plant growth. This treatment also maintained a fairly high chlorophyll concentration and leaf nitrogen content, though it was not the highest for these parameters. T1 (coco peat and rice hull charcoal with F1) exhibited to be the closest competitor to T6. Even though T5 contributed to a relatively high level of leaf color (SPAD reading) and leaf nitrogen content, it has not been able to effectively convert these advancements into vegetative growth. Landis et al. (1989) (cited by Tripathi & Raghubanshi, 2014) reported decline in root: shoot ratio with accumulation of N in leaves under high nitrogen input. Hence, shoot growth retardation in T5 could be a negative influence of excessive plant uptake of nitrogen. This condition can damage the root tips, restricting root development and ultimately hindering the overall growth of the plant. The different stages of growth of Iceberg lettuce during the experiment are shown in Figure 2.

Figure 2. Iceberg lettuce plants at different growth stages: (A) three weeks after transplanting; (B) head formation stage; (C) harvested heads.

3.2. Head Formation

There was a statistically significant treatment effect on the time of head formation, which was assessed by the percentage of plants with a visible head formed at 9 WAT (P=0.03) but no significant difference was observed at 11 WAT (p = 0.981). Based on the results of both assessments the earliness of head formation was in the order of T1=T4>T2=T6>T3>T5. Meanwhile, the same results clearly indicate a greater uniformity of the time of head formation by T1, T2, T4 and T6, compared to T3 and T5 (Figure 3).

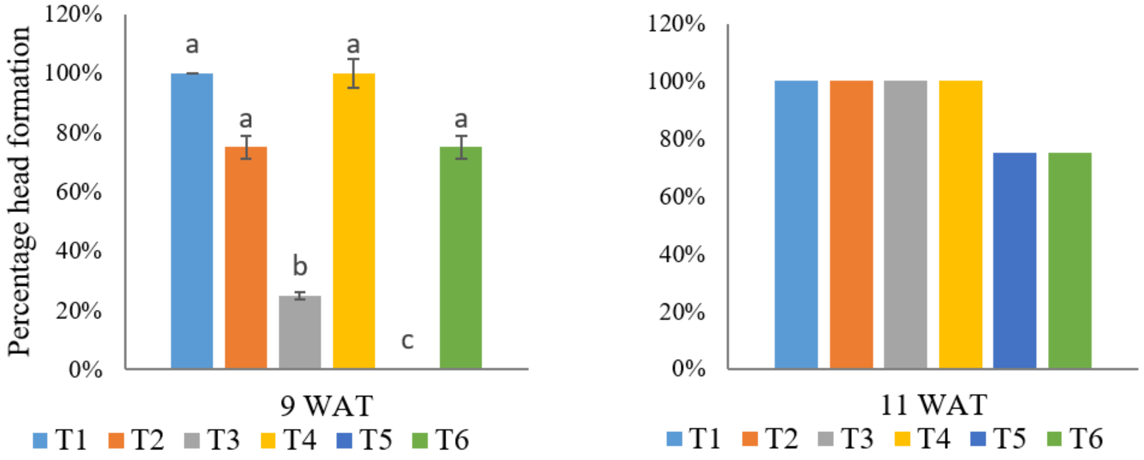

Figure 3. Effect of media and fertilizer combinations on the percentage head formation of Iceberg lettuce at 9 and 11 weeks after transplanting (WAT). Error bars indicate standard error (SE) of means, and the treatment means labeled with different letters are significantly different (at $p<0.05$).

The treatment effects on the head fresh weight (yield) at the harvesting stage were statistically significant ($p = 0.028$) where T1, T2, T4, and T6 gave higher yields (260 – 329 g per head) compared to the yields given by T3 and T5 (131 – 161.33 g per head) (Table 4). Meanwhile, the highest head dry weight among treatments was attained by T4 (14.8±1.54 g) which was significantly higher than all the other treatments (7.6 – 10.6 g head). Among the treatments having significantly higher head fresh weight, only T2, and T4 had the highest head height as well as head diameter, whereas the other treatments with higher head fresh weight (T1 and T6) had either higher head height or diameter (Table 4).

Table 4. Yield components and head dimensions of Iceberg lettuce treated with different media and fertilizer combinations, at harvesting.

Trt.	Head fresh weight (g/plant)	Head dry weight (g/plant)	Head diameter(cm)	Head height (cm)
1	271.67^{ab}	10.59^{b}	13.3^{a}	12.3^{b}
2	312.00^{a}	9.91^{b}	13.7^{a}	14.3^{a}
3	131.00^{c}	7.75^{b}	9.2^{c}	10^{c}
4	329.00^{a}	14.80^{a}	13.6^{a}	15.4^{a}
5	161.33^{bc}	7.55^{b}	9.8^{c}	10.4^{c}
6	260.33^{abc}	9.42^{b}	11.8^{b}	14.4^{a}

Trt.: Media and fertilizer combinations; The means that do not share the same letter in superscripts are significantly different (DMRT/$p<0.05$).

3.3. Substrate Parameters

The most used and optimal pH range in the grow medium or substrate for growing leafy greens is 5.5 - 6.6 (Kudirka et al., 2023). However, grow medium in T2 was in the acidic range while T3 and T5 were in the alkaline range (Table 5). Drifting pH out of the optimal range causes partial unavailability of plant nutrients, particularly micronutrients, in the grow medium (Nakano et al., 2006). The reason for lower plant fresh weight and dry weight in T2, T3, and T5 found at 7 WAT may be due to these unfavorable pH conditions which makes unavailability of some of the plant nutrients in the substrate. Meanwhile, EC of the supply solution when applying the recommended dosage of Alberts fertilizer (CIC Colombo, Unipower, Colombo) to crops at 2-7 WAT is 0.8-1.9 mS cm^{-1}. However, the substrate samples taken from T3 and T5 24 hours after fertigation show much higher EC values (1.651 – 4.681 mS cm^{-1}), indicating a lower rate of nutrient uptake, compared to the rate of water uptake by the root system. Unfavourable pH discussed above could be the root cause for a lower rate of nutrient uptake in these treatments, ultimately accumulating plant nutrients largely in the grow medium. Apart from this, higher EC itself reduces nutrient uptake rate by increasing osmotic pressure whereas lower EC may severely affect plant health and yield (Samarakoon et al., 2006). The reason for the lower plant fresh weight and dry weight in T3 and T5 at 7 WAT may be sub-optimal plant nutrient uptake under pH driven low availability of mineral ions.

Meanwhile, usual range of bulk density maintained in most grow media in solid media-based (aggregate type) hydroponics falls within the range of 0.1 - 0.7 g cm^{-3} (Khomami et al., 2019). All the grow media used in this experiment falls into this range. The highest bulk density was observed in T6 and the lowest bulk density was observed in T1 but their vegetative growth parameters and head parameters were satisfactory, when compared to the substrates having normal levels of bulk density (i.e. T3 and T5). Meanwhile, the influence of the grow media treatments on the WHC at the harvesting stage was significantly different (p=0.04). The differences among T2, T3, T4, and T6 were insignificant (p>0.05) but the WHC of T1 and T5 were significantly higher than the other treatments. The highest WHC was observed in T1 (0.75 ±0.07), and the lowest was observed in T6 (0.34 ± 0.02 gg-1) Meanwhile, the observations indicated a frequently dry substrate condition in T6, leading to rapid wilting of plants, compared to all other treatments. As shown in Table 3, the leaf area (per plant) of T6 was notably higher. Anticipated higher evapotranspiration (ET) through a relatively large leaf area while the WHC of grow media was low could have caused the plants in T6 to run into water stress conditions frequently. Eriksen et al. (2016) and Baslam and Goicoechea (2012) reported that under water stress, plants reduce growth and redirect developmental resources toward rapid maturation. At 11 WAT only T6 had begun to bolt in some replicates, probably due to this influence. Contrary to this, T6 has shown the highest plant dry weight during the vegetative stage at 7 WAT and achieved a relatively high head dry weight (9.42 g) at harvesting.

Table 5. pH, electrical conductivity (EC), bulk density (BD), water holding capacity (WHC) of different media and fertilizer combinations.

Trt.	**pH**	**EC (mS cm^{-1})**	**Bulk density (g cm^{-3})**	**WHC (g g^{-1})**
T1	6.82 ± 0.17^{a}	0.457 ± 0.157^{b}	0.18 ± 0.03^{c}	0.75 ± 0.07^{a}
T2	4.64 ± 0.14^{c}	0.302 ± 0.116^{b}	0.65 ± 0.01^{ab}	0.44 ± 0.03^{c}
T3	7.49 ± 0.11^{a}	2.157 ± 0.506^{a}	0.62 ± 0.07^{b}	0.42 ± 0.06^{c}
T4	6.04 ± 0.62^{b}	0.161 ± 0.053^{b}	0.68 ± 0.07^{ab}	0.4 ± 0.05^{c}
T5	7.28 ± 0.43^{a}	3.158 ± 1.523^{a}	0.67 ± 0.03^{ab}	0.56 ± 0.12^{b}
T6	5.49 ± 0.54^{b}	0.326 ± 0.018^{b}	0.74 ± 0.07^{a}	0.34 ± 0.02^{c}

Trt.: Media and fertilizer combinations; The means that do not share the same letter in superscripts are-significantly different (DMRT/p<0.05).

Overall results of this experiment indicate that the substrate parameters have significantly influenced the final yield of Iceberg lettuce. Treatments T2 and T4, with optimal pH, bulk density (BD), and water holding capacity (WHC), produced the highest head fresh weights. Conversely, T3 and T5, undesirable medium pH and higher nutrient accumulation in the medium (high EC), resulted in lower head weight and head size. T6 exhibited the highest BD and lowest WHC, leading to frequent wilting and reduced yields. T1, characterized by low BD and high WHC, yielded moderately. As a whole, optimal pH, moderate EC, suitable BD, and high WHC are critical for maintaining the plant growth and yield of Iceberg lettuce in solid media based hydroponics culture.

4. Conclusion

Grow media and fertilizer combinations significantly affect the vegetative growth and yield parameters of Iceberg lettuce grown in solid media based hydroponics under semi-intensive greenhouse conditions. Comparable vegetative growth parameters indicate the possible replacement of the grow media and fertigation schedules used in the industry (T6) where 1:1 ratio of coco peat and topsoil is applied with rather complicated (daily changing) fertigation schedule (F3) with equally effective grow media and less complicated fertigation schedules. Treatment combination, T1 (coco peat and rice hull charcoal at 1:1 with standard dosage of Albrets fertilizer; F1) was found the best contender in this regard. However, despite its moderate vegetative growth rate, T4 (coco peat, rice hull charcoal and topsoil at 1:1:2, with half the standard dosage of Albrets fertilizer; F2) was found to be resulting in superior head dry weight and comparable yield and head quality (dimensions) to T1 and T6. Therefore, with proper water management, coco peat, rice hull charcoal and topsoil at 1:1:2 can be recommended as the most effective grow medium or substrate, to be used with half of the standard dosage of hydroponics fertilizer (Alberts) for the grow bag culture of Iceberg lettuce under semi-intensive greenhouse conditions in the mild weather regions in the tropics. Upgrading the fertigation schedule up to full strength of the standard dosage (EC at 0.8 – 1.2 mS cm^{-1} and pH at 5.5 – 6.5) during 1-5 WAT might be a better option for enhancing vegetative growth furthermore. Meanwhile, based on the relationship between plant growth/ yield performance and physical properties of grow media, the bulk density at 0.64-0.81 g cm^{-3} and WHC at 0.32- 0.47g g^{-1} (found in T2, T4, and T6) could be used for selecting grow media for Iceberg lettuce cultivation in fertigated grow bag culture.

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Conflict of Interest

The authors declare no conflict of interest related to this research.

References

Afton, W. D. (2018). *Evaluation of growth characteristics, yield, marketability and nitrate levels of lettuce (Lactuca sativa L) cultivars produced in south Louisiana* (Master's thesis, Louisiana State University).

Baslam, M., & Goicoechea, N. (2012). Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. *Mycorrhiza*, *22*, 347-359. https://doi.org/10.1007/s00572-011-0408-9

Chen, Z., Han, Y., Ning, K., Luo, C., Sheng, W., Wang, S., & Wang, Q. (2019). Assessing the performance of different irrigation systems on lettuce (*Lactuca sativa* L.) in the greenhouse. *PLOS One*, *14*(2), e0209329. https://doi.org/10.1371/journal.pone.0209329

Cookson, S. J., Van Lijsebettens, M., & Granier, C. (2005). Correlation between leaf growth variables suggests intrinsic and early controls of leaf size in *Arabidopsis thaliana*. *Plant, Cell & Environment*, *28*(11), 1355-1366. https://doi.org/10.1111/j.1365-3040.2005.01368.x

El-Saady, W. A. (2016). Spinach (*Spinacia oleracea* L.) growth, yield, and quality response to the application of mineral NPK fertilizer ratios and levels. *Middle East Journal of Agriculture*, *5*(4), 908-917.

Eriksen, R. L., Knepper, C., Cahn, M. D., & Mou, B. (2016). Screening of lettuce germplasm for agronomic traits under low water conditions. *HortScience*, *51*(6), 669-679. https://doi.org/10.21273/HORTSCI.51.6.669

FAO. (2022). *FAOSTAT: Food and agriculture data*. https://www.fao.org/faostat/en/#rankings/countries_by_commodity

Farid, A. I. N., Helilusiatiningsih, N., & Handayani, T. (2022). Effect of planting media composition and NPK dosage on the growth and production of shallots (*Allium Cepa* L.) Thailand Varieties. *Journal of Soilscape and Agriculture*, *1*(1), 22-31. https://doi.org/10.19184/jsa.v1i1.128

Gbollie, S. N., Mwonga, S. M., & Kibe, A. M. (2021). Effects of calcium nitrate levels and soaking durations on cocopeat nutrient content. *Journal of Agricultural Chemistry and Environment*, *10*(3), 372-388. https://doi.org/10.4236/jacen.2021.103024

Indriyani, N. L. P., Hadiati, S., & Soemargono, A. (2011). The effect of planting medium on the growth of pineapple seedling. *Journal of Agricultural and Biological Science*, *6*(2), 43-48.

Jenni, S., & Bourgeois, G. (2008). Quantifying phenology and maturity in crisphead lettuce. *Hort Technology*, *18*(4), 553-558. https://doi.org/10.21273/HORTTECH.18.4.553

Jensen, M. H. (2001). *Controlled environment agriculture in deserts, tropics and temperate regions-A world review.* International Symposium on Design and Environmental Control of Tropical and Subtropical Greenhouses. Taiwan

Kader, A. A. (2002). *Postharvest technology of horticultural crops*. University of California Agriculture and Natural Resources.

Khomami, A. M., Padasht, M. N., Lahiji, A. A., & Mahtab, F. (2019). Reuse of peanut shells and Azolla mixes as a peat alternative in growth medium of *Dieffenbachia amoena* 'tropic snow'. *International Journal of Recycling of Organic Waste in Agriculture*, *8*, 151-157. https://doi.org/10.1007/s40093-018-0241-7

Kudirka, G., Viršilė, A., Sutulienė, R., Laužikė, K., & Samuolienė, G. (2023). Precise management of hydroponic nutrient solution Ph: The effects of minor pH changes and MES buffer molarity on lettuce physiological properties. *Horticulturae*, *9*(7), 837. https://doi.org/10.3390/horticulturae9070837

Landis, T. D., Tinus, R. W., McDonald, S. E., et al. (1989). Seedling nutrition and irrigation. In T. D. Landis, R. W. Tinus, S. E. McDonald & J. P. Barnett (Eds.), *The container tree nursery manual* (pp. 1-67). USDA Forest Service.

Martín, J., Sarria, L. F., & Asuero, A. G. (2017). The Kjeldahl titrimetric finish: On the ammonia titration trapping in boric acid. In V. D. Hoang (Ed.), *Advances in titration techniques* (pp. 23-58). IntechOpen. https://doi.org/10.5772/intechopen.68826

Meena, S. S., & Kulakarni, A. (2022). Management of iceberg lettuce quality. *Asian Journal of Agricultural Extension, Economics & Sociology*, *40*(10), 593-602. https://doi.org/10.9734/ajaees/2022/v40i1031119

Nakano, Y., Watanabe, S., Kawashima, H., & Takaichi, M. (2006). The effect of daily nutrient applications on yield, fruit quality, and nutrient uptake of hydroponically cultivated tomato. *Journal of The Japanese Society for Horticultural Science*, *75*(5), 421-429. https://doi.org/10.2503/JJSHS.75.421

Olaria, M., Nebot, J. F., Molina, H., Troncho, P., Lapena, L., & Lorens, E. (2016). Effect of different substrates for organic agriculture on seedling development of traditional species of Solanaceae. *Spanish Journal of Agricultural Research*, *14*(1), 8001-8013. https://doi.org/10.5424/sjar/2016141-8013

Ryder, E. J. (1999). *Lettuce, endive and chicory*. CABI Publishing.

Sabir, N., & Singh, B. (2013). Protected cultivation of vegetables in the global arena: A review. *Indian Journal of Agricultural Sciences*, *83*(2), 123-135.

Samarakoon, U. C., Weerasinghe, P. A., & Weerakkody, W. A. P. (2006). Effect of electrical conductivity [EC] of the nutrient solution on nutrient uptake, growth and yield of leaf lettuce (*Lactuca sativa* L.) in stationary culture. *Tropical Agricultural Research*, *18*, 13-21.

Söylemez, S. (2021). The impact of different growth media and ammonium-nitrate ratio on yield and nitrate accumulation in lettuce (*Lactuca sativa* var. longifolia). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *49*(4), 12540. https://doi.org/10.15835/nbha49412540

Tripathi, S. N., & Raghubanshi, A. S. (2014). Seedling growth of five tropical dry forest tree species in relation to light and nitrogen gradients. *Journal of Plant Ecology*, *7*(3), 250-263. https://doi.org/10.1093/jpe/rtt026

Usman, M., Shah, M. H., & Zaman, Q. (2013). *Citrus and guava nursery raising: A practical approach*. University of Agriculture Faisalabad Press.

Weerakkody, W. A. P., Mayakaduwa, M. A. P., & Weerapperuma, K. N. (2007). Effect of supply volume and weather-based EC adjustments on the growth and yield of greenhouse tomato and bell pepper. *Acta Horticulturae*, *742*, 105-111. https://doi.org/10.17660/ActaHortic.2007.742.15

Wilke, B. M. (2005). Determination of chemical and physical soil properties. In R. Margesin & F. Schinner (Eds.), *Manual for soil analysis - monitoring and assessing soil bioremediation* (pp. 47-95). Springer. https://doi.org/10.1007/3-540-28904-6_2

Zhang, H., He, L., Di Gioia, F., Choi, D., Elia, A., & Heinemann, P. (2022). LoRaWAN based internet of things (IOT) system for precision irrigation in plasticulture fresh-market tomato. *Smart Agricultural Technology*, *2*, 100053. https://doi.org/10.1016/j.atech.2022.100053

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RESEARCH ARTICLE

Assessment of Catena Relationships in Soil Properties within a Transitional Landscape: A Case Study from Northwestern Türkiye

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ABSTRACT

This research was carried out to classify soils located between Gökyaka and Sarpdere villages in the Demirköy district of Kırklareli province (NW Türkiye). Soil profile definitions were made by determining the morphological, chemical and physical properties of 7 profiles. In the HS-1 and HS-4 profiles located around the Balaban stream, the upper part of the sampling area is sloping, and the slope continues at the bottom (slope land). In these profiles, the soil order was Inceptisol. The subgroup of HS-1 was Typic Humixerept due to its high organic matter content, and HS-4 was Typic Haploxerept. The sample points around the HS-2 and HS-5 profiles were at the head of the sloping land. The pH of the soil is generally slightly acidic, no salinity problem is observed, the lime content is usually very low, the organic matter is found to be high in the upper layers, and the textures are clay, clay loam and silty clay loam. According to Soil Taxonomy of Soil Survey Staff, soils are classified as Entisol, Inceptisol and Alfisol. Thus, topography seems to be the main pedologic factor for the formation of soils in a short distance and under the same climatic conditions, in Gökyaka and Sarpdere villages of the Yıldız Mountain in NW Türkiye.

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1. Introduction

Rapidly increasing urbanization and industrialization also increase the use of natural resources (İkiz, 2020; Ozsahin & Ozdes, 2022). This rising consumption causes the depletion or decrease of natural resources (Tufan et al., 2023).

Soil formation does not occur in a short time. First, rocks weathers to soil parent material and then into soil. Soils are formed over many years under the influence of many soil formation processes (Arrouays et al., 2020; Yüksel & Ekinci, 2021).

Başayiğit et al. (2004) stated that soil is a natural resource formed by complex interactions and emphasised that soil is a living, dynamic, three-dimensional system. Actors such as mineral weathering/alteration, displacement and losses play a major role in soil formation from the parent material, and because of these processes, horizon differentiation occurs in the soil (Camacho et al., 2020). For this reason, it is emphasised that the effects and contributions of biological, chemical, and physical events in different environments should be considered when determining soil properties (Pacci et al., 2021).

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Soil formation varies over time depending on the effects of soil formation stages. Russian scientist Dokuchaev stated that different soil types are formed depending on climate, living organisms, topography, and time (Pritchett, 1980).

Changes in the geochemical properties of soils, mineral weathering, and soil-plant-water cycle are discussed, and the changing characteristics of soil formation over time are emphasised (Alaboz et al., 2021). Weathering rates of minerals can vary widely due to changes in soil properties and differences in environmental conditions. Also time affected soil properties and weathering/alteration rates and that changes occur in physical, chemical, and mineralogical properties during soil formation (Soluk & Özcan, 2020), In these processes, the chemical structure of soils is controlled by the parent material, while the chemical properties of mature soils reflect the effects of the weathering environment.

Determining agricultural lands' morphological, physical, and chemical properties is essential for sustainable management of soils (Nalan & Ekberli, 2020). The productivity of agricultural lands is not infinite (Özden et al., 2022). Therefore, it is necessary to interpret the properties of the soil well to enhance the productivity and sustainability of agricultural lands. For this reason, it is essential to determine the morphological, physical, and chemical properties of the soil well, and to produce detailed soil maps for their better management (Ormancı et al., 2023; Sokolov et al., 2021). In addition, classification and mapping of land use, erosion, and productivity for particular purposes can be carried out by referencing soil maps (Akgül, 1992; Tağil & Danacioğlu, 2021).

The properties of soils may meet only slightly the requirements of some types of uses while meeting all the requirements of other types of uses. To efficient use of soils with different characteristics, it is essential to plan by considering different types of management (Dent & Young, 1981; FAO, 1976).

The study on seven soil profiles in an approximately 50 km^2 area between Bayramiç and Çan (W. Türkiye) examined the profiles' physicochemical and mineralogical properties. Researchers found the highest clay content (47.75%) and the highest cation exchange capacity (CEC) (47 mmol.kg^{-1}) in the Vertisol profile in the soils formed on the old lake terrace. In addition, the soils of the research area were classified as Alfisol, Mollisol, Inceptisol, Entisol and Vertisols of the Soil Taxonomy (Soil Survey Staff, 2022), and Phaozems, Luvisols, Calcisols, Cambisols, Fluvisols and Vertisols reference groups in the IUSS Working Group (WRB, 2022) classification system (Başarlar & Ekinci, 2019).

Martin (2016) examined the integration of local and scientific knowledge in soil classification. This research investigates how farmers in Imugan, Philippines, use their traditional knowledge to classify soils and compares this with soil classification systems. The study highlights the importance of incorporating local knowledge into soil classification frameworks.

Catena is defined as a series of soils formed from the same parent material, in the same climate and over a similar period of time, but with different characteristics due to differences in relief and drainage. (Conforti et al., 2020).

FAO (1976) and Dent and Young (1981) state that some lands cannot adequately meet the requirements of all types of management but can suitable for specific type of use. For example, some soils may be suitable for agricultural production, while others are better suited for growing forests or for natural habitats (Çalişkan & Göl, 2022). For this reason, they stated that planning should consider soil properties and usage types for sustainable use of lands efficiently.

The Yıldız Mountains in the study area are the highest point of the region and the height of Mahya Mountain, which is the summit, is 1035 metres. The research area was selected in the northeastern part of the Yıldız Mountains and the aim was to determine the soil types in this region. Although some studies have been carried out in the eastern part of the region, there is no study on this part. This study was carried out in order to fill this gap and contribute to the literature.

2. Materials and Methods

The study area was located at a distance of 15 km between Sarpdere village of Demirköy district, Kırklareli province, and the total study area is 68 km^2. The location of the study area and the sample points displayed on the Google Earth Satellite image are given in Figure 1. The coordinates of the sample points are given in Table 1.

Table 1. Coordinates of sample points.

Coordinates of Sample Points		
Sample	**Latitude**	**Longitude**
HS-1	41°51'16.83"N	27°39'17.60"E
HS-2	41°50'23.16"N	27°39'35.73"E
HS-3	41°52'36.04"N	27°37'5.04"E
HS-4	41°52'20.37"N	27°36'35.59"E
HS-5	41°51'24.63"N	27°33'54.10"E
HS-6	41°51'35.47"N	27°34'18.17"E
HS-7	41°51'19.95"N	27°38'22.86"E

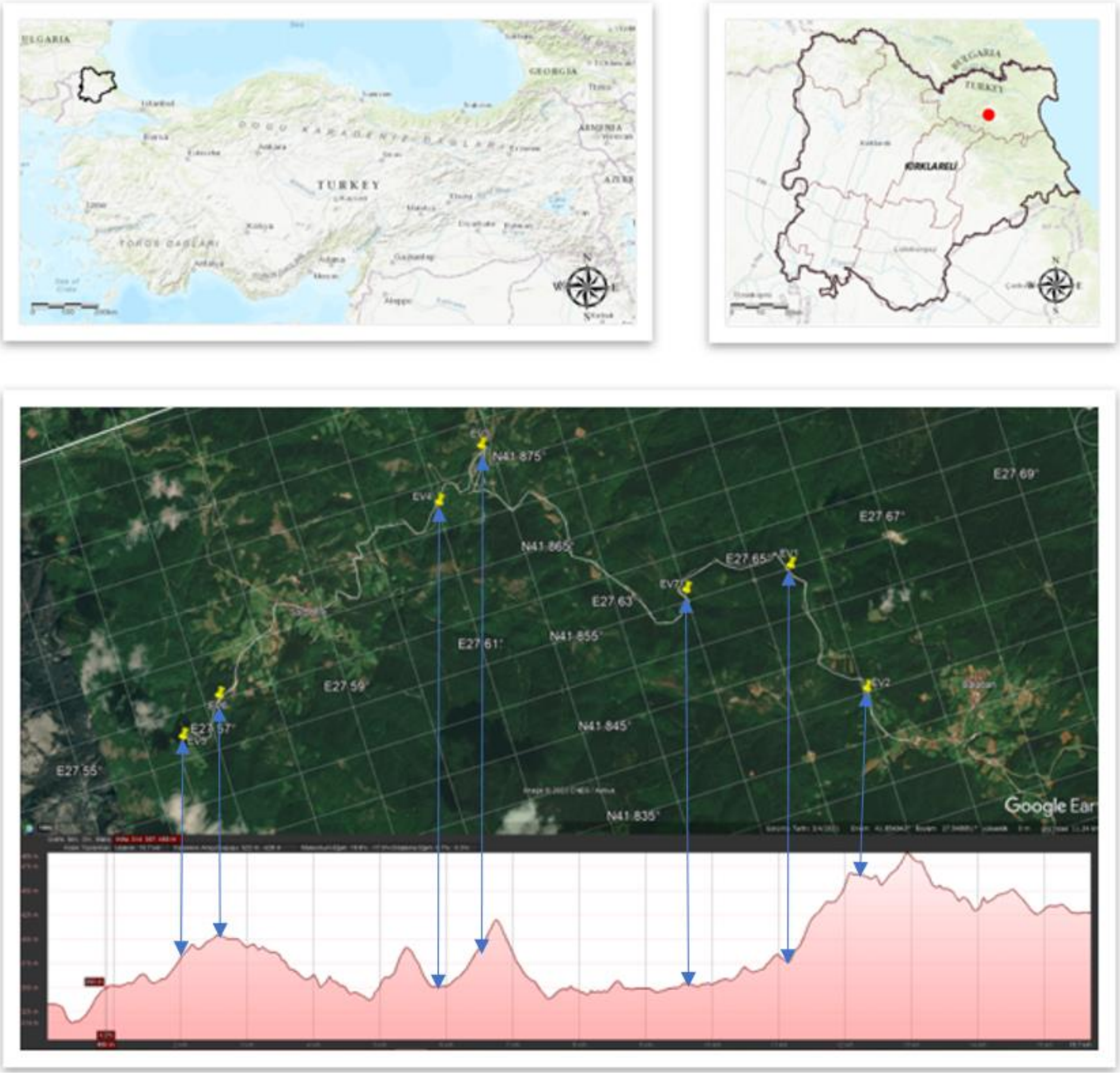

Figure 1. Map showing the study area and Remote Sensing Image Showing Balaban-Sarpdere Surroundings, sample points and elevation profiles of sample points according to Google Earth (2020 Image).

The 7 points determined between Demirköy and Sarpdere correspond to a section extended approximately 15 kilometres. Several soil types are determined at such a short distance most probably due to drainage, accumulation, and transportation events. These soils, which are formed as a function of topography, were evaluated using the Thorp and Smith (1949), Soil Taxonomy (Soil Survey Staff, 2022) and IUSS Working Group (WRB, 2022) classification systems.

Located in the south-eastern part of the Balkan peninsula, the Thrace region offers a wide variety of hilly landscapes. This diversity is created by mountains and hills with different elevations, plateaus with lower elevations, and plains have different sizes. Topographically, the mountainous are the Istranca mountain range in the north and northeast and the Ganos and Koru mountains in the south and southeast. The gently sloping, slightly hilly lands between these two mountainous lands, split by the branches of the Ergene River, constitute the basis of the Thrace peneplain (Kibaroğlu & Garipağaoğlu, 2022).

The soil samples, taken for the descriptive definition of the horizons defined in the study area and for the genetic characterisation of the soils, were air dried in the laboratory, crushed with a wooden mallet, sieved through a 2 mm sieve and prepared for analysis. Particle size distribution (texture) was determined using the hydrometer method (Bouyoucos, 1951). The texture triangle was used for nomenclature of texture classes (United States Division of Soil Survey, 1996).

Soil reaction pH was classified according to Jackson (1958) and salinity (%) according to Richards (1954). Electrical Conductivity (EC) was measured in the prepared saturation sludge using a Wheatstone Bridge conductivity meter (Tüzüner, 1990). Salinity (%) was determined by the equation provided by Tüzüner (1990). pH and salinity (%) were classified according to Richards (1954). Lime (%) was determined by the volumetric calcimeter method (Anonymous, 1988). Organic matter (%) was determined by the modified Walkley Black wet combustion method (Walkley, 1947) (Anonymous, 1988). Lime and organic matter contents of the soils were interpreted according to the limits of Sağlam (2008).

Nitrogen content (%) of soil samples was determined by calculation according to FAO (1976). Phosphorous (ppm) was measured by Olsen method (Olsen et al., 1954) and a spectrophotometer (FAO, 1976). K, Ca, Mg and Na (ppm) were extracted with ammonium acetate and determined by ICP (FAO, 1976). Fe, Cu, Mn and Zn (ppm) were analysed by extraction with DTPA followed by ICP (FAO, 1976; Follet 1969; Lindsay & Norvell, 1969). Nitrogen, phosphorus, potassium, calcium, magnesium and sodium were classified according to FAO (1976). Iron and copper were classified according to Lindsay and Norvell (1969). Zinc and manganese contents were also classified according to FAO (1976). Soil color was assessed as dry and moist (Nemcsics, 1994). IBM SPSS Statistics 22 software was used for correlation analysis of the results of the soil property analyses.

3. Results and Discussion

Seven soil profiles were opened in the study area. The topographic location and coordinates of the profiles given in Figure 1. The profile images are given in Figure 2. Profile descriptions of laboratory analyses of the profiles and morphological and other observations made during field studies are given in Tables 2 and 3 for each profile.

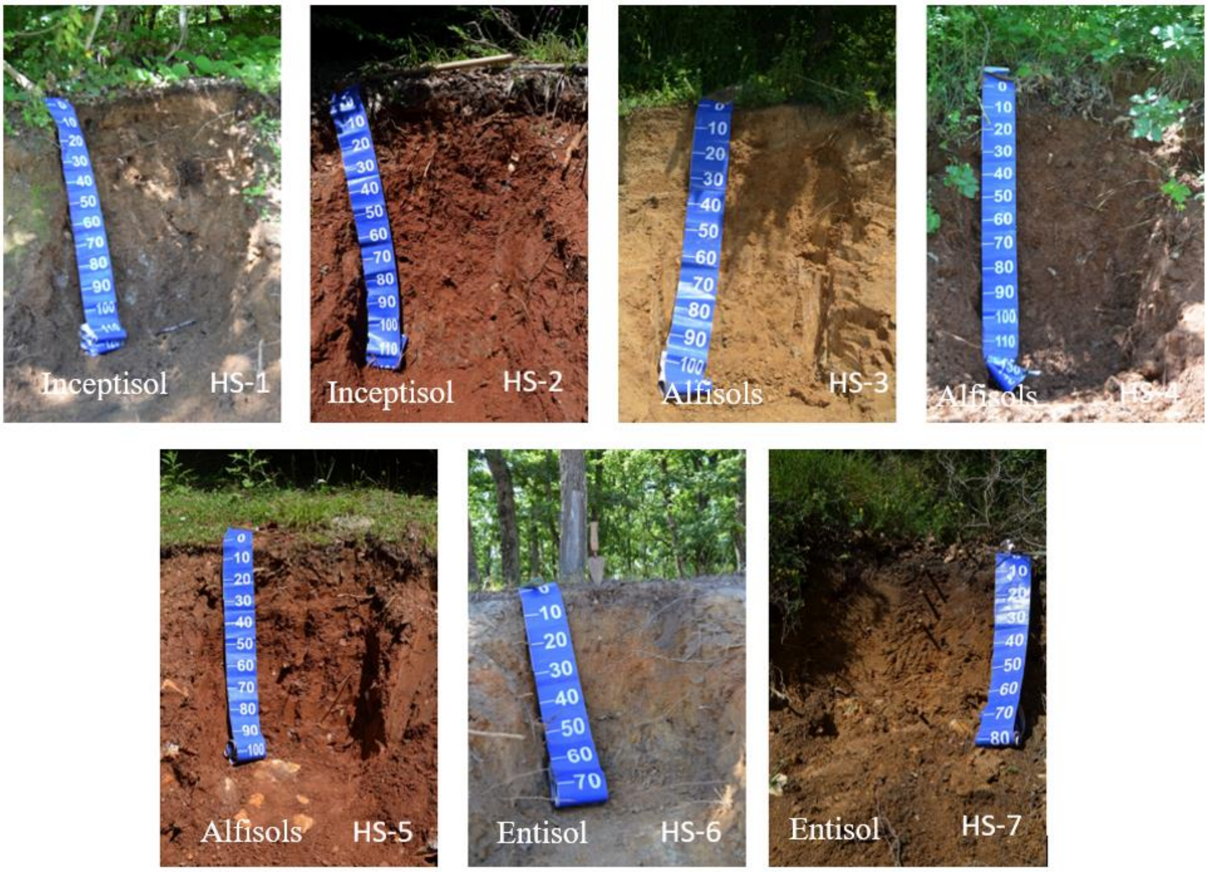

Figure 2. Photos of profiles.

3.1. Profile: HS-1

Region: Demirköy Dupnisa Cave Road, Location: Balaban-Gökyaka road junction 3rd km, Coordinates: 41°85'46.76' N 027°65'48.89' E, Altitude above sea level: 371, Vegetation: Hornbeam forest (forest undergrowth: short surface cover), Parent material: Granite, Physiography: valley ridge slope, Landform: Convex, Slope: 30% +, Drainage: good, Groundwater depth: Deep, Rocky soil: None, Land Use: Forest, Previous classification: Brown Forest Soil, Soil Taxonomy: Typical Humixerept.

3.1.1. Profile description

Ah 0-6 cm; Brownish black (10YR 4/2 dry), black (10YR 2/2 moist), loam, strong small furrows, very abundant micro mesopores, straight, fine and very fine roots.

A 6-21 cm; Dull yellowish-brown (10YR 6/3 dry), Dull yellowish-brown (10YR 4/3 moist), sandy loam, weak medium half-angular block, abundant micro-abundant medium mesopores, straight and open, very medium and very fine roots, large medium 2-3 cm diameter cavities.

Table 2. Some analysis results of soil samples.

Horizon	**Texture**				**%Organic Material**	**pH (1:2,5) Soil-Water Solution**	**%Salt**	**%$CaCO_3$**	**Colour**	
	%Sand	**%Silt**	**%Clay**	**Texture**					**Dry**	**Moist**
HS-1 - Ah 0-6	48.54	33.44	18.02	L	10.15	6.59	0.25	0.78	10YR 4/2	10YR 2/2
HS-1 - A 6-21	54.89	22.48	22.63	SCL	1.89	6.26	0.04	0.39	10YR 6/3	10YR 4/3
HS-1 - Bw 21-49	57.10	22.38	20.52	SCL	0.91	6.30	0.04	0.39	10YR 6/4	10YR 4/4
HS-1 - C1 49-78	50.94	24.45	24.61	SCL	0.49	6.23	0.04	0.78	10YR 6/4	10YR 4/4
HS-1 - C2 78-125	61.17	18.31	20.52	SCL	0.21	6.34	0.03	0.39	10YR 7/4	10YR 4/4
HS-1 CR 125+	61.06	16.33	22.61	SCL	0.91	6.21	0.04	0.00	10YR 7/6	10YR 5/6
HS-2 Ah 0-6	38.84	27.34	33.82	CL	10.85	6.43	0.32	0.39	5YR 4/6	5YR 2/4
HS-2 A 6-24	22.67	31.28	46.05	C	3.01	6.20	0.06	0.78	5YR 4/8	5YR 3/6
HS-2 Bt 24-60	21.15	19.14	59.71	C	0.91	6.48	0.03	0.00	2.5YR 4/6	2.5YR 3/6
HS-2 BC 60-103	26.79	22.96	50.25	C	0.77	6.45	0.03	1.17	2.5YR 4/6	2.5YR 3/6
HS-2 C 103+	58.03	2.09	39.88	SC	0.21	6.38	0.03	0.00	2.5YR 4/6	2.5YR 3/6
HS-3 A 0-17	44.53	26.63	28.84	CL	2.73	6.35	0.10	0.00	10YR 6/4	10YR 4/4
HS-3 AC 17-46	42.66	30.63	26.71	CL	1.05	6.15	0.04	0.39	10YR 7/4	10YR 4/6
HS-3 C 46+	44.26	28.81	26.93	CL	0.91	6.26	0.04	0.00	10YR 7/6	10YR 5/6
HS-4 A 0-16	9.73	25.14	65.13	C	3.01	6.45	0.15	0.39	5YR 4/6	5YR 3/4
HS-4 AB 16-31	8.97	23.24	67.79	C	1.89	6.63	0.09	0.00	5YR 5/6	5YR 3/6
HS-4 Bw 31-76	6.46	19.10	74.44	C	1.05	6.41	0.08	0.39	5YR 4/6	5YR 3/6
HS-4 C 76+	6.94	21.11	71.95	C	0.35	6.72	0.08	0.39	5YR 4/8	5YR 3/6
HS-5 A1 0-5	42.92	23.19	33.89	CL	6.09	6.10	0.11	0.00	7.5YR 6/6	7.5YR 4/6
HS-5 A2 5-20	40.25	20.55	39.20	CL	1.47	6.25	0.04	0.39	7.5YR 6/6	7.5YR 4/6
HS-5 Bt 20-57	23.45	10.61	65.94	C	1.19	6.46	0.04	0.39	2.5YR 4/6	7.5YR 3/6
HS-5 C 57+	26.59	16.74	56.67	C	0.77	6.43	0.06	0.39	2.5YR 4.6	7.5YR 3/6
HS-6 A 0-9	39.89	37.21	22.90	L	8.61	5.57	0.11	0.39	10YR 6/2	10YR 3/4
HS-6 CA 9-22	44.64	28.62	26.74	CL	1.61	5.51	0.08	0.00	10YR 7/4	10YR 5/6
HS-6 C1 22-45	26.43	27.26	46.31	C	1.75	5.28	0.05	0.39	10YR 7/6	10YR 5/8
HS-6 C2 45+	26.74	33.41	39.85	C	1.05	5.38	0.04	0.39	10YR 7/6	10YR 6/8
HS-7 Ah 0-6	51.29	31.66	17.05	L	11.14	5.51	0.13	0.39	10YR 4/3	10YR 2/3
HS-7 A 6-15	51.93	26.13	21.94	SCL	4.55	5.80	0.05	0.39	10YR 5/3	10YR 3/3
HS-7 AC 15-30	56.84	22.52	20.64	SCL	2.73	5.68	0.04	0.00	10YR 7/4	10YR 4/3
HS-7 C 30-74	57.04	22.42	20.54	SCL	1.61	5.94	0.04	0.39	10YR 7/4	10YR 4/3

Bw 21-49 cm; Brown (10YR 6/4 dry), Brown (10YR 4/4 moist), sandy loam, medium coarse semi-angular block, micro and mesopores, wavy and open, abundant medium roots, abundant 1-3 cm diameter insect burrows, 10-15 cm diameter surface material accumulated in cracks.

C1 49-78 cm; brown (10YR 6/4 dry), brown (10YR 4/4 moist) sandy loam, massive, very few micropores, wavy and open, medium roots.

C2 78-125 cm; Dull yellow orange (10YR 7/4 dry), Brown (10YR 4/4 moist) sandy loam, massive structure, porosity very few micropores, boundary wavy and open, base sat roots very few and very fine roots.

CR 125+cm; Light yellowish brown (10YR 7/6 dry), yellowish brown (10YR 5/6 moist), sandy clay loam, massive.

The A horizon in the HS-1 Profile is determined as the loam texture class, and the other horizons are determined as sandy clay loam. Soil reactions (pH) are neutral in the A horizon (6,59), slightly acidic (6,21-6,34) in the other horizons and contain few carbonate. While the amount of organic matter is high in the A horizons of this profile, which is in the saline class, it decreases towards the bottom (Table 2). When examined in terms of nutritional elements, P and Ca are in low amounts, while Na, Ca, Mg, K, Fe, Cu, Mn, and Zn are in sufficient amounts. These amounts decrease towards the lower horizons. Total nitrogen is present at sufficient levels (Table 3). The main reasons for low P and Ca levels are that the slightly acidic environment increases phosphorus fixation and calcium solubility. The sandy clay loam texture can reduce plant uptake by facilitating the binding of phosphorus to clay minerals (Zheng at al., 2003). The reduction of organic matter in the lower horizons limits the biological mineralization of phosphorus and has a negative effect on the retention of calcium. Adequate amounts of Na, Mg, K, Fe, Cu, Mn and Zn are favoured by the soil mineral structure rich in these elements, the high CEC and the A horizon rich in organic matter. However, the decrease of these elements in the lower horizons can be attributed to the loss of organic matter, poor microbial activity, the presence of an accumulation horizon and the washing effect of the drainage system.

3.2. Profile: HS-2

Region: Demirköy Dupnisa Cave Road, Location: Balaban - Gokyaka 1st Km, Coordinates: 41° 83'97.67' N 27°65'99.25' E, Sea level: 425, Vegetation: Beech, Parent material: Claystone (colluvial quartzite), Physiography: Valley ridge slope, Shape of the surrounding land: Convex, Slope: 35%+, Drainage: good, Groundwater depth: Deep, Rocky soil: None, Land Use: Forest, Humidity: Low, Former classification: Brown Forest Soil, Soil Taxonomy: Inceptic Haploxeralf.

3.2.1. Profile description

Ah 0-6 cm; reddish brown (5YR 4/6 dry), very dark reddish brown (5YR 2/4 moist), clay loam, very abundant micro and fine pores, straight and well defined, fine and very fine roots.

A 6-24 cm; reddish brown (5YR 4/8 dry), dark reddish brown (5YR 3/6 moist), clay, medium small semi-angular, medium macro and mesopores, wavy and open, less thick, medium thick less thin roots.

Bt 24-60 cm; reddish brown (2.5YR 4/6 dry), dark reddish brown (2.5YR 3/6 moist), clay, strong to medium prismatic, few micro-pores, wavy and open, medium roots abundant fine roots, 1.5 cm scattered clay stones.

BC 60-103 cm; reddish brown (2.5YR 4/6 dry), dark reddish brown (2.5YR 3/6 moist), clay, strongly small prismatic, slightly micropolar, rim wavy and clear, 1-7 cm diameter scattered clay pebbles.

C 103+ cm; reddish brown (2.5YR 4/6 dry), dark reddish brown (2.5YR 3/6 moist), sandy clay.

The texture class of the HS-2 profile is clay loam, and the pH is slightly acidic. The amount of lime is low, and the amount of organic matter is high in the upper horizon and decreases to a low level towards the bottom. In the case of salt, it is classified as salt-free soil (Table 2). Regarding nutritional elements, P and Ca are in low amounts, while Na, Mg, K, Fe, Cu, Mn and Zn are in sufficient amounts. The main reasons for the low P and Ca levels in the soil are the decalcification of these elements due to slightly acidic pH, the tendency of clay tissue to fix phosphorus and the decrease in soil organic matter in the lower horizon. On the other hand, adequate levels of Na, Mg, K, Fe, Cu, Mn and Zn can be explained by the rich mineral structure of the soil, the high CEC and the organic matter rich conditions in the upper horizon. The decline in nutrients in the lower horizons can be attributed to processes such as reduced organic matter and microbial activity, drainage-induced leaching and clay fixation. High total nitrogen in the upper horizon is associated with the accumulation of organic matter and the effective functioning of nitrogen-fixing microorganisms. These amounts decrease towards the lower horizons. Total nitrogen is present in high levels in the upper horizon (Table 3).

3.3. Profile: HS-3

Region: Demirköy Dupnisa Cave Road, Location: Gökyaka village entrance, Coordinates: 41° 87'66.78' N 27°61'80.66' E, Sea level: 414 m. Vegetation: Oak forest (the surface of the ground is covered with forest plants), Parent material: Granite, Physiography: High slope (mountain slope), Landform: Convex, Slope: 25%, Drainage: Deep, Groundwater depth: Deep, Rocky soil: None, Land Use: Forest, Humidity: Low, Former classification: Brown forest soil, Soil taxonomy: Typical Xereorthent.

3.3.1. Profile description

A 0-17 cm; Brown (10YR 6/4 dry), brown (10YR 4/4 moist), clay loam, weak small furda structure, few macro very micro polar, straight and open, few medium roots.

AC 17-46 cm; pale yellow-orange (10YR 7/4 dry), brown (10YR 4/6 moist), clay loam, weak small, semi-angular block structure, few macro and mesopores, border straight and open, very medium and very fine roots.

C 46 cm+; glossy yellowish brown (10YR 7/6 dry), yellowish brown (10YR 5/6 moist), clay loam, massive, less fine and very fine roots.

The texture of the HS-3 profile falls into the clay loam class. The pH is slightly acidic, with very few lime. In this non-saline soil, organic matter is low in the upper horizons and low in the middle bottom (Table 2). Colour varies between yellowish-brown and brown. While K, P, Zn and Ca are in low amounts, Na, Mg, Fe, Cu and Mn are in sufficient amounts. These amounts decrease towards the lower horizons. Total nitrogen is present at very good levels (Table 3). Ca, K, P and Zn levels were found to be low. The low organic matter content prevents the biological release of these nutrients. The reason for the sufficient levels of Na, Mg, Fe, Cu and Mn is the mineral richness of the soil parent material (granite) and the high CEC. The decrease of these elements in the lower horizons can be explained by the loss of organic matter and leaching by water movement. Total nitrogen is well represented in the upper horizons due to efficient mineralisation processes.

Table 3. Analysis results of soil samples.

HORIZON	**Available Phosphorus (ppm)**	**Available Calcium (ppm)**	**Available Potassium (ppm)**	**Available Magnesium (ppm)**	**Available Sodium (ppm)**	**Available Copper (ppm)**	**Available Iron (ppm)**	**Available Manganese (ppm)**	**Available Zinc (ppm)**	**Total N (%)**
HS-1 - Ah 0-6	7.63	150.80	62.50	29.34	2.70	0.62	22.64	12.64	2.09	0.50
HS-1 – A2 6-21	9.04	67.15	18.80	12.10	2.80	0.75	40.64	22.52	0.39	0.09
HS-1 - Bw 21-49	7.24	65.08	15.50	12.08	2.50	0.69	49.40	11.01	0.35	0.04
HS-1 - C1 49-78	3.81	55.85	12.40	12.67	2.10	0.59	35.07	5.25	0.33	0.02
HS-1 - C2 78-125	2.32	52.53	13.10	8.58	2.30	0.71	23.61	1.90	0.24	0.01
HS-1 CR 125+	4.55	61.97	14.40	12.35	2.40	0.75	20.15	4.64	0.25	0.04
HS-2 Ah 0-6	7.45	119.92	70.50	20.47	2.90	1.84	36.45	128.55	3.82	0.54
HS-2 A2 6-24	12.66	80.10	52.10	13.04	2.30	2.65	16.78	18.95	1.12	0.15
HS-2 Bt 24-60	12.24	80.67	19.00	15.36	2.80	1.22	5.51	1.08	0.34	0.04
HS-2 BC 60-103	10.42	71.49	16.70	13.05	2.20	1.51	6.53	1.07	0.46	0.03
HS-2 C 103+	10.72	54.86	14.90	11.26	2.40	1.26	3.35	1.16	0.36	0.01
HS-3 A 0-17	10.95	52.20	49.60	9.78	3.10	1.15	14.02	36.25	0.54	0.13
HS-3 AC 17-46	10.73	75.75	16.90	14.51	2.50	1.36	27.60	79.84	0.52	0.05
HS-3 C 46+	11.62	11.17	15.40	2.16	3.60	0.94	14.80	4.16	0.54	0.04
HS-4 A 0-16	10.29	107.56	70.30	19.28	2.60	3.10	10.95	54.26	1.28	0.15
HS-4 AB 16-31	11.47	132.74	44.20	23.00	3.70	3.42	13.96	50.23	1.37	0.09
HS-4 Bw 31-76	11.39	163.21	19.40	27.90	3.50	2.49	11.22	51.88	1.69	0.05
HS-4 C 76+	13.72	140.58	17.20	27.67	2.90	2.42	17.96	82.67	4.38	0.01
HS-5 A1 0-5	10.26	108.83	56.00	20.42	2.10	1.16	60.05	40.26	1.15	0.30
HS-5 A2 5-20	12.31	83.40	18.10	15.92	1.70	1.84	29.30	27.48	0.65	0.07
HS-5 Bt 20-57	10.85	148.34	18.70	25.84	3.40	1.95	9.06	19.83	0.49	0.05
HS-5 C 57+	12.26	131.12	15.80	28.26	2.50	2.03	14.79	35.56	0.72	0.03
HS-6 A 0-9	13.34	119.20	49.30	24.94	3.00	1.03	85.42	7.54	1.32	0.43
HS-6 CA 9-22	12.29	68.31	14.20	12.91	2.20	0.86	46.98	4.70	0.44	0.08
HS-6 C1 22-45	13.78	160.05	19.00	27.41	3.30	0.87	14.83	1.60	0.30	0.08
HS-6 C2 45+	12.82	178.80	17.40	34.58	3.80	0.83	17.83	1.65	0.34	0.05
HS-7 Ah 0-6	14.93	141.15	46.50	25.07	2.50	0.87	81.11	12.52	2.97	0.55
HS-7 A2 6-15	12.25	105.57	15.00	19.66	1.80	0.81	87.36	1.07	0.72	0.22
HS-7 AC 15-30	10.91	36.48	12.40	6.21	1.10	0.82	79.05	0.80	0.44	0.13
HS-7 C 30-74	11.78	43.82	13.00	7.32	0.90	0.77	10.17	0.40	0.21	0.08

3.4. Profile: HS-4

Region: Demirköy Dupnisa Cave Road, Location: Balaban-Sarpdere, 13 km after the junction, Coordinates: 41° 87'23.26' N 27°60'98.86' E, Altitude above sea level: 377, Vegetation: Forest (oak wood), Parent material: Clay stone, Physiography: Slope terrain (mountain slope), Shape of the surrounding terrain: Convex, Slope: 25-30%, Drainage: Deep, Groundwater depth: Deep, Rocky soil: None, Land Use: Roadside, Humidity: Low, Former classification: Brown forest soil, Soil taxonomy: Typical Haploxerept.

3.4.1. Profile description

A 0-16 cm; reddish brown (5YR 4/6 dry), dark reddish brown (5YR 3/4 moist), clay, weak small furrows, very good macro and mesopores, fracture open.

AB 16-31 cm; light reddish brown (5YR 5/6 dry), light reddish brown (5YR 3/6 moist), clay, strong medium sized block, medium micro and mesopores, 1-2 cm diameter clays, slightly wavy.

Bw 31-76 cm; reddish brown (5YR 4/6 dry), dark reddish brown (5YR 3/6 moist), clay, strong coarse semi-angular block, few micro and mesopores, 1-5 cm diameter clays, wavy light.

C 76+ cm; Reddish brown (5YR 4/8 dry), dark reddish brown (5YR 3/6 moist), clay, massive structure, with black spots covering the surface of clay structures between 0.2-5 cm.

The texture of the HS-4 profile is clay. The pH is slightly acidic, and they are salt-free soils. While organic matter is enough at the top, it is at lower horizons (Table 2). Colour varies from dark greyish brown to bright reddish brown. When examined in terms of nutritional elements, P and Ca are in low amounts, while Na, Mg, K, Fe, Cu, Mn, and Zn are in sufficient amounts. These amounts decrease towards the lower horizons. Total nitrogen is present at high (Table 3).

3.5. Profile: HS-5

Region: Demirköy Dupnisa Cave Road, Location: Sarpdere - Dupnisa 2 km, Coordinates: 41° 85'68.42' N 27'56 50.27' E, Altitude above sea level: 344, Vegetation: Forest (hornbeam - oak), Parent material: Marble, Physiography: Sloping, Shape of the surrounding land: Convex, Slope: 25%, Drainage: Deep, Groundwater depth: Deep, Rocky soil: None, Land Use: Forest, Humidity: Low, Former classification: Brown Forest Soil, Soil Taxonomy: Typical Haploxeralf.

3.5.1. Profile description

A1 0-5 cm; Orange (7,5YR 6/6 dry), Brown (7,5YR 4/6 moist), Clay loam, weak small furrows, abundant macro, micro and mesopores, straight open, very fine and very fine roots.

A2 5-20 cm; orange (7,5YR 6/6 dry), brown (7,5YR 4/6 moist), clay loam, weak to medium semi-angular, porosity less meso and micro pores, wavy open, less to medium very fine roots very few very fine roots.

Bt 20-57 cm; reddish brown (2.5YR 4/6 dry), dark reddish brown (2.5YR 3/6 moist), clay, very coarse, semi-angular, few meso and micro pores, wavy open, less to medium thick very fine to very fine to very fine roots.

C 57+ cm; reddish brown (2.5YR 4/6 dry), dark reddish brown (2.5YR 3/6 moist) clay, strong small block structure when broken into massive fragments, very few micropores, few fine roots, bedrock fragments 2+ cm in diameter.

The texture classes of the HS-5 profile are clay loam in the upper horizons and clay in the lower parts. The pH is slightly acidic, and there is very few lime. Organic matter is high in the upper horizon and is in the non-saline soil class (Table 2). While P and Ca are in low amounts, K and Mg are in medium amounts, Na, Zn, Fe, Cu and Mn are in sufficient amounts. These amounts decrease towards the lower horizons. Total nitrogen is high in the A1 horizon (0.3), sufficient in the A2 and Bt horizons (0.07-0.06) and low in the C horizon.

3.6. Profile: HS-6

Region: Demirköy Dupnisa Cave Road, Location: Sarpdere Dupnisa 1st Km, Coordinates: 41° 85'98.53' N 27°57'17.14' E, Sea level: 357, Vegetation: Forest (oak), Parent material: Chalcchist, Physiography: Hilly, Shape of the surrounding terrain: Convex, Slope: Flat, Drainage: Good, Groundwater depth: Deep, Rocky soil: None, Land Use: Road cut, Humidity: Low, Previous classification: Brown forest soil, Soil taxonomy: Typical Xereorthent.

3.6.1. Profile description

A 0-9 cm; Greyish-yellowish brown (10YR 6/2 dry), dark brown (10YR 3/4 moist), loam, weak, small, semi-angular block, abundant micro- and mesopores, straight cut, very abundant and very fine roots.

CA 9-22 cm; Dull yellow-orange (10YR 7/4 dry), yellowish brown (10YR 5/6 moist), loam, massive, few micro-pores, wavy and open, special features few fine and very fine roots.

C1 22-45 cm; light yellowish brown (10YR 7/6 dry), yellowish brown (10YR 5/8 moist), clay, massive, wavy and open, many medium roots.

C2 45+ cm; light yellowish brown (10YR 7/6 dry), light yellowish brown (10YR 6/8 moist), clay, massive, few medium roots, may have traces of iron reduction.

In the HS-6 profile, the texture is loam in the upper horizon and changes between clay loam and clay towards the bottom. The pH is slightly acidic to moderately acidic, and the soil is salt-free. While the amount of organic matter is high at the top, it decreases towards the bottom (Table 2). Colour varies between bright yellowish brown and dark brown. Regarding nutritional elements, P and Ca are in low amounts, K and Mg are in medium amounts, while Na, Ca, Zn, Fe, Cu and Mn are in sufficient amounts.

These amounts decrease towards the lower horizons. Total nitrogen is present at high (Table 3).

3.7. Profile: HS-7

Region: Demirköy Dupnisa Cave Road, Location: Balaban-Gokyaka 4th Km, Coordinates: 41° 85'55.43' N 27°63'96.82' E, Sea level: 369, Vegetation: Forest (oak), Parent material: Granite, Physiography: valley slope, slope: 20-25%, Drainage: Deep, Groundwater depth: Deep, Rocks: None, Land Use: Forest, Humidity: Less, Old classification: Brown forest soil, Soil taxonomy: Typical Xereorthent.

3.7.1. Profile description

Ah 0-6 cm; dull yellowish brown (10YR 4/3 dry), brownish black (10YR 2/3 moist), loam, weak structure, small furrows, many macro and micro pores, straight open, very abundant and very fine roots.

A 6-15 cm; Dull yellowish-brown (10YR 5/3 dry), dark brown (10YR 3/3 moist), sandy loam, weak small half-spherical block, few macro, few micro, border wavy and clear, few medium and very fine roots.

AC 15-30 cm; dull yellow-orange (10YR 7/4 dry), dull yellow-brown (10YR 4/3 moist), sandy loam, structure massive, few macro, border wavy and open, special features very many medium and thick roots.

C 30-74 cm; dull yellow-orange (10YR 7/4 dry), dull yellow-brown (10YR 4/3 moist), sandy loam, massive, broken and transitional, anthills 2-5 cm diameter, insect and animal tracks 1-5 cm diameter.

In the HS-7 profile, the texture varies between clay loam and silt loam. In this profile, where the pH is slightly acidic, there is a high amount of organic matter at the top and a small and medium amount at the bottom (Table 2). In terms of colour, it varies between dull yellowish brown and brownish black. Regarding nutritional elements, P and Ca are in low amounts, K is in medium amounts, while Mg, Na, Ca, Zn, Fe, Cu and Mn are in sufficient amounts. These amounts decrease towards the lower horizons. Total nitrogen is present at high (Table 3).

The distribution of low and sufficient nutrients in the HS-4, HS-5, HS-6 and HS-7 profiles varies according to the pH, organic matter content and textural characteristics of the soils. The main reason for low phosphorus (P) and calcium (Ca) levels is that the slightly acidic pH causes phosphorus to bind to soil minerals and calcium causes decalcification. The clay content of soils can also reduce the availability of these elements for plants. The moderate levels of potassium (K) and magnesium (Mg) are due to the mineral structure and calcium-magnesium balance of the soils. Sodium (Na), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) are present in sufficient quantities because these elements are naturally present in sufficient quantities in the soil and the soils have a high cation exchange capacity (CEC). However, the decrease of these elements in the lower horizons can be explained by the decrease of organic matter and the effect of leaching by water movement. The very good total nitrogen levels are due to the high organic matter content of the upper horizons and efficient biological mineralisation.

Figure 2 shows photos of all profiles. All profiles show characteristics suitable for the growth of plants that can grow in slightly acidic environments, except for cultivated plants with extreme preferences in terms of productivity. Their current situation is a forest with oak and beech trees.

When the soils of the research area are classified according to the classification of Thorp and Smith (1949), "Brown Forest Soil" falls into the Great Soil Group. When classified according to Soil Taxonomy (Soil Survey Staff, 2022), it is classified in 3 orders: Entisol, Inceptisol and Alfisols. Profiles numbered HS-1 and HS-4 are in the Inceptisol order and are classified in the HS-4 Typic Haploxerert subgroup due to their being in the xeric moisture regime and their typical feature. HS-1 is included in the Typic Humixerept subgroup with its dark colour and high organic matter content. HS-2 and HS-5 profiles are in the Alfisols order and are in the Inceptic Haploxeralf subgroup due to high clay accumulation. The soils in the Alfisol order are in a xeric moisture regime. Profiles numbered HS-3, HS-6, and HS-7 are in the Entisol order, and all profiles are in the xeric moisture regime. These profiles are included in the Typic Xereorthent subgroup. In addition, according to the WRB (2022) classification system, HS-1 and HS-4 are Humic Cambisol, HS-2 and HS-5 are Haplic Luvisol, HS-3, HS-6, HS-7 are Haplic Regosol. Table 4 shows the classification of the soils of the research area according to Soil Taxonomy (Soil Survey Staff, 2022), WRB (2022) and Thorp and Smith (1949) Systems.

Table 4. Classification of research area soils according to Soil Taxonomy (Soil Survey Staff, 2022) and Thorp and Smith (1949) systems.

Thorp and Smith (1949) (Great Soil Group)	**Soil Taxonomy (Soil Survey Staff, 2022)**				**WRB (2022)**	**Profile Number**
	Order	Sub Order	Great Group	Subgroup		
Brown Forest Soil	Inceptisol	Xerepts	Humixerept	Typic Humixerept	Humic Cambisol	1
Brown Forest Soil	Inceptisol	Xerepts	Haploxerept	Typic Haploxerept	Haplic Cambisol	4
Brown Forest Soil	Alfisols	Xeralfs	Haploxeralf	Inceptic Haploxeralf	Haplic Luvisol (Inceptic)	2
Brown Forest Soil	Alfisols	Xeralfs	Haploxeralf	Typic Haploxeralf	Haplic Luvisol	5
Brown Forest Soil	Entisol	Orthents	Xereorthent	Typic Xereorthent	Haplic Regosol	3
Brown Forest Soil	Entisol	Orthents	Xereorthent	Typic Xereorthent	Haplic Regosol	6
Brown Forest Soil	Entisol	Orthents	Xereorthent	Typic Xereorthent	Haplic Regosol	7

4. Conclusion

When the catena relations of the soils are examined in the study area, which starts at 2 km of Kırklareli/Demirköy Balaban-Gökyaka villages and extends to the 3rd km of Sarpdere village - Dupnisa cave, it observed that different soil properties occur over a short distance. Although they occur under the same climatic conditions, they differ in slope, parent material, drainage network system, topography, and water movement in the soil profile. The change in the profile properties of the research area soils is due to the Balaban stream and the drainage network system around it, which is effective in the region.

The contribution of slope change to water movement, organic matter accumulation and nutrient distribution can be more specifically addressed to explain the detailed effects of catenation on soil properties. It should be discussed how the drainage network system, in particular the Balaban stream, shapes differences in soil ordo groups and the possible implications of these processes for agricultural productivity, water management and erosion resistance. In addition, comparison of the research results with regions with similar climates and topographies may help to improve the generalisability and specificity of the findings. Finally, long-term monitoring studies are recommended to understand the time-dependent dynamics of the profiles, in particular the effects of slope and water movement on soil physical and chemical evolution processes.

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Conflict of Interest

The authors declare that they have no conflict of interest in relation to this study.

References

Akgül, M. (1992). *Daphan ovası topraklarının sınıflandırılması ve haritalanması* (Doctoral dissertation, Atatürk University). (In Turkish)

Alaboz, P., Demir, S., Şenol, H., Dengiz, O., Yılmaz, K., & Başkan, O. (2021). Yarı-kurak ekolojik koşullar altında farklı kayaç türleri üzerinde oluşmuş toprakların fiziko-kimyasal ve jeo-kimyasal özellikleri. *Harran Tarım ve Gıda Bilimleri Dergisi*, *25*(4), 480-496. https://doi.org/10.29050/harranziraat.903887 (In Turkish)

Anonymous. (1988). *Türkiye gübreler ve gübreleme rehberi*. T.C.T.O.K.B. Köy Hizmetleri Genel Müdürlüğü, Toprak ve Gübre Araştırma Enstitüsü Müdürlüğü. (In Turkish)

Arrouays, D., McBratney, A., Bouma, J., Libohova, Z., Richer-de-Forges, A. C., Morgan, C. L. S., Roudier, P., Poggio, L., & Mulder, V. L. (2020). Impressions of digital soil maps: The good, the not so good, and making them ever better. *Geoderma Regional*, e00255. https://doi.org/10.1016/j.geodrs.2020.e00255

Başarlar, F. F., & Ekinci, H. (2019). Bayramiç-Çan arası farklı jeolojik ve jeomorfolojik araziler üzerinde oluşmuş toprakların profil özellikleri ve sınıflandırılması. *COMU Journal of Agriculture Faculty*, *7*(1), 69-80. https://doi.org/10.33202/comuagri.550835 (In Turkish)

Başayiğit, L., akça, E., Şenol, S., Kapur, S., & Dinç, U. (2004). Konuklar tarım işletmesi yaşlı nehir terasları üzerinde yer alan toprakların fiziksel, kimyasal, mineralojik özellikleri ve oluşumu. *Selcuk Journal of Agriculture and Food Sciences*, *18*(33), 59-67. (In Turkish)

Bouyoucos, G. J. (1951). A recalibration of the hydrometer method for making mechanical analysis of soils. *Agronomy Journal*, *43*(9), 434-438. https://doi.org/10.2134/agronj1951.00021962004300090005x

Çalişkan, H., & Göl, C. (2022). Türkiye’de cumhuriyet döneminde yaşanılan demografik değişimlerin arazi kullanım türü/arazi örtüsü üzerine etkileri. *Anadolu Orman Araştırmaları Dergisi*, *8*(1), 100-112. https://doi.org/10.53516/ajfr.1075531 (In Turkish)

Camacho, M. E., Quesada-Román, A., Mata, R., & Alvarado, A. (2020). Soil-geomorphology relationships of alluvial fans in Costa Rica. *Geoderma Regional*, *21*, e00258. https://doi.org/10.1016/j.geodrs.2020.e00258

Conforti, M., Longobucco, T., Scarciglia, F., Niceforo, G., Matteucci, G., & Buttafuoco, G. (2020). Interplay between soil formation and geomorphic processes along a soil catena in a Mediterranean mountain landscape: An integrated pedological and geophysical approach. *Environmental Earth Sciences*, *79*, 59. https://doi.org/10.1007/s12665-019-8802-2

Dent, D., & Young, A. (1981). *Soil survey and land evaluation*. George Allen and Unwin.

FAO. (1976). *A framework for land evaluation*. https://www.fao.org/4/x5310e/x5310e00.htm

Follet, R., H. (1969). *Zn. Fe. Mn and Cu in Colorado soils* (Doctoral dissertation, Colorado State University).

İkiz, A. S. (2020). *Doğal kaynaklar ve enerji ekonomisi*. Astana Yayınları. (In Turkish)

Jackson, M. (1958). *Soil chemical analysis*. Prentice-Hall Inc.

Kibaroğlu, Y., & Garipağaoğlu, N. (2022). Aşağı Meriç Havzası'nda arazi kullanımı. *Doğu Coğrafya Dergisi*, *27*(48), 65-78. https://doi.org/10.5152/EGJ.2022.22812 (In Turkish)

Lindsay, W., L. &, Norvell, W. A. (1969). Development of a DTPA Micronutrient Soil Test. *Soil Science Society of America, Proceedings, 35*, 600-602.

Martin, H. (2016). Comparison of indigenous and scientific knowledge on soil classification among Imugan, Nueva Vizcaya, Philippines farmers. *Journal of Environmental Science and Management*, *2*, 71-83.

Nalan, K., & Ekberli, İ. (2020). Çarşamba Ovası'nda soya yetiştirilen tarım alanlarının verimlilik durumlarının belirlenmesi. *Toprak Bilimi ve Bitki Besleme Dergisi*, *8*(1), 14-25. https://doi.org/10.33409/tbbbd.756822 (In Turkish)

Nemcsics, A. (1994). Spacing in the Munsell color system relative to the coloroid color systems. *Colour Research & Application*, *19*(2), 122-125. https://doi.org/10.1111/j.1520-6378.1994.tb00071.x

Olsen, S. R., Cole, C. V., Watanabe, F. S., & Dean, L. A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *U.S. Department of Agriculture Circular No. 939*.

Ormancı, İ. F., Dengiz, O., & Aydın, A. (2023). Aşağı Engiz Havzası topraklarının toprak etüdü, haritalanması ve sınıflandırılması. *Türkiye Tarımsal Araştırmalar Dergisi*, *10*(3), 275-287. https://doi.org/10.19159/tutad.1299891 (In Turkish)

Özden, N., Sökmen, Ö., Uslu, İ., & Aras, S. (2022). Manisa ili tarım topraklarının verimlilik durumları ile mikro element kapsamlarının belirlenerek haritalanması. *ANADOLU Ege Tarımsal Araştırma Enstitüsü Dergisi*, *32*(2), 228-241. https://doi.org/10.18615/anadolu.1225168 (In Turkish)

Ozsahin, E., & Ozdes, M. (2022). Agricultural land suitability assessment for agricultural productivity based on GIS modelling and multi-criteria decision analysis: The case of Tekirdağ province. *Environmental Monitoring and Assessment*, *194*(1), 41. https://doi.org/10.1007/s10661-021-09663-1

Pacci, S., Kaya, N. S., Dengiz, O., & Demirağ Turan, İ. (2021). Van Havzası içerisinde yer alan mera arazilerinde SMAF modeli kullanılarak toprak kalitesinin değerlendirilmesi. *Anadolu Tarım Bilimleri Dergisi*, *36*(2), 301-316. https://doi.org/10.7161/omuanajas.883999 (In Turkish)

Pritchett, W. L. (1980). *Properties and management of forest soils*. Wiley.

Richards, L. A. (1954). *Diagnosis and improvement of saline and alkali soils*. US Department of Agriculture. https://doi.org/10.1097/00010694-195408000-00012

Sağlam, M. (2008). *Toprak ve suyun kimyasal analiz yöntemleri*. Namık Kemal Üniversitesi Yayınları. (In Turkish)

Sokolov, D. A., Androkhanov, V. A., & Abakumov, E. V. (2021). Soil formation in technogenic landscapes: Trends, results, and representation in the current classifications. *Bulletin of Tomsk State University Biology*, *56*, 6-32. https://doi.org/10.17223/19988591/56/1

Soluk, Ö., & Özcan, H. (2020). Hidrotermal akışkanlarca etkilenen toprakların ağır metal içeriği ve jeokimyasal ayrışma düzeyleri (Sarayköy-Denizli örneği). *Lapseki Meslek Yüksekokulu Uygulamalı Araştırmalar Dergisi*, *1*(1), 13-23. (In Turkish)

Soil Survey Staff. (2022). *Keys to soil taxonomy*. United States Department of Agriculture.

United States Division of Soil Survey. (1993). *Soil survey manual (No. 18)*. US Department of Agriculture. (Yukarıdakini APAya göre böyle düzelttim)

Tağil, Ş., & Danacioğlu, Ş. (2021). Halk toprak bilgisinin planlamadaki önemi ve haritalanması. *Journal of International Social Research*, *14*(76), 881-888. https://doi.org/10.17719/jisr.11443 (In Turkish)

Thorp, J., & Smith, G. D. (1949). Higher categories of soil classification: Order, suborder, and great soil groups. *Soil Science*, *67*(2), 117-126. https://doi.org/10.1097/00010694-194902000-00005

Tufan, Y., Kurt, A. N., & Özkurt, M. (2023). *Sürdürülebilir tarım açısından yem bitkilerinin önemi*. Uluslararası Güncel Akademik Çalışmalar Konferansı. Konya. (In Turkish)

Tüzüner, A (1990). *Toprak ve su analizleri laboratuarları el kitabı*. Köy Hizmetleri Genel Müdürlüğü Yayınları. (In Turkish)

Walkley, A. (1947). A critical examination of a rapid method for determining organic carbon in soils—effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science*, *63*(4), 251-264. https://doi.org/10.1097/00010694-194704000-00001

WRB. (2022). *International soil classification system for naming soils and creating legends for soil maps (4th edition)*. International Union of Soil Sciences (IUSS).

Yüksel, A., & Ekinci, H. (2021). Çanakkale koşullarında andezitlerde toprak oluşumu. *Lapseki Meslek Yüksekokulu Uygulamalı Araştırmalar Dergisi*, *2*(3), 103-115. (In Turkish)

Zheng, Z., Parent, L., & MacLeod, J. (2003). Influence of soil texture on fertilizer and soil phosphorus transformations in Gleysolic soils. *Canadian Journal of Soil Science*, *83*(4), 395-403. https://doi.org/10.4141/S02-073

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Özçelik, H., Taştan, Y., Terzi, E., & Sönmez, A. Y. (2020). Use of onion (*Allium cepa*) and garlic (*Allium sativum*) wastes for the prevention of fungal disease (*Saprolegnia parasitica*) on eggs of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases*, *43*(10), 1325-1330. https://doi.org/10.1111/jfd.13229

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Salem, M. O. A., Salem, T. A., Yürüten Özdemir, K., Sönmez, A. Y., Bilen, S., & Güney, K. (2021). Antioxidant enzyme activities and immune responses in rainbow trout (*Onchorhynchus mykiss*) juveniles fed diets supplemented with dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) extracts. *Fish Physiology and Biochemistry*. https://doi.org/10.1007/s10695-021-00962-5

Book:

Lastname, N., Lastname, M., & Lastname, O. (Year). *Title of the work*. Publisher.

Oidtmann, K., Xao, Q., & Lloyd, A. S. (2018). *The food need by the year 2050*. Elsevier.

Book Chapter:

Lastname, N., Lastname, M., & Lastname, O. (Year). Title of the chapter. In N. N. Lastname, A. Lastname & B. Lastname (Eds.), *Title of the book* (pp. Page numbers). Publisher.

Pickering, A. D. (1993). Growth and stress in fish production. In G. A. E. Gall & H. Chen (Eds.), *Genetics in Aquaculture* (pp. 51-63). Elsevier. https://doi.org/10.1016/b978-0-444-81527-9.50010-5

Dissertation or Thesis:

Lastname, N. (Year). *Title of dissertation/thesis* (Doctoral dissertation/Master's thesis, Name of Institution).

Sönmez, A. Y. (2011). *Karasu ırmağında ağır metal kirliliğinin belirlenmesi ve bulanık mantıkla değerlendirilmesi* (Doctoral dissertation, Atatürk University).

Taştan, Y. (2018). *Tatlısu kerevitindeki (Astacus leptodactylus) siyah solungaç hastalığı etkeni mantar Fusarium oxysporum'un PCR yöntemi ile teşhisi* (Master's thesis, Akdeniz University).

Conference Proceedings:

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Ken, A., & Kumar, S. (2020). *A new statistical model for fuzzy logic evaluation*. 3rd International Congress on Statistics. İstanbul.

Institution Publication:

Institution name. (Year). *Title of the work*. URL

FAO. (2020). *Fishery and aquaculture statistics 2018*. http://www.fao.org/3/cb1213t/CB1213T.pdf

Internet Source:

Lastname, N. (Year). *Title of the work*. Retrieved May 15, 2020, from URL

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