www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2022.1086839
15/1 (2022) 114-123

Effect of distance from highway on ''Red Globe'' (*Vitis vinifera* L.) leaf anatomy and morphology

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Abstract

One of the most important pollution in the world is air pollution. One of the factors that cause this pollution is vehicles. Heavy metals have a great role in the component of pollution caused by vehicles and they have great negative effects on plant and human health. In this study, it was aimed to determine the effect of distance from the highway on the leaf morphology and anatomy of the economically important Red Globe grape variety. Leaf samples were collected from four different distances (road side, 250 m, 500 m, 4000 m) in three different periods (spring, summer and autumn). As a result of the morphological and anatomical examinations, it was determined that as the negative effect of heavy metal decreased as the distance to the highway increased. As we approached the highway, important features such as leaf length, width, petiole length, midvein length, midvein thickness, leaf area decreased, while petiole thickness increased. Anatomically, as the distance to the highway decreases, it has been revealed as a result of the measurements that there is an increase in the thickness of the lower epidermis and upper epidermis, and a decrease in the thickness of upper collenchyma, lower collenchyma, upper parenchyma, lower parenchyma, length of vascular bundles and vascular bundles number. This study highlights that the highway causes stress on leaf morphology and anatomy and causes changes in some characters.

Keywords: anatomy, heavy metal, highway, morphology, Red Globe

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Karayoluna uzaklığın ''Red Globe'' (*Vitis vinifera* L.) yaprak anatomisi ve morfolojisi üzerine etkisi

Özet

Dünyadaki en önemli kirliliklerden biri hava kirliliğidir. Bu kirliliğe neden olan faktörlerden biri de araçlardır. Ağır metallerin taşıtlardan kaynaklanan kirliliğin bileşeninde büyük rolü olup, bitki ve insan sağlığı üzerinde büyük olumsuz etkileri vardır. Bu çalışmada, ekonomik önemi olan Red Globe üzüm çeşidinin karayoluna uzaklığının yaprak morfolojisi ve anatomisine etkisinin belirlenmesi amaçlanmıştır. Yaprak örnekleri dört farklı mesafeden (yol kenarı, 250 m, 500 m, 4000 m) üç farklı dönemde (ilkbahar, yaz ve sonbahar) toplanmıştır. Morfolojik ve anatomik incelemeler sonucunda, karayoluna olan mesafe arttıkça ağır metalin olumsuz etkisinin azaldığı tespit edilmiştir. Karayoluna yaklaştıkça yaprak uzunluğu, genişliği, yaprak sapı uzunluğu, orta damar uzunluğu, orta damar kalınlığı, yaprak alanı gibi önemli özellikler azalırken yaprak sapı kalınlığı artmıştır. Anatomik olarak karayoluna olan uzaklık azaldıkça alt epidermis ve üst epidermis kalınlığında artış, üst kollenkima, alt kollenkima, alt parankima, üst parankima kalınlığında, iletim demeti uzunluğunda ve iletim demeti sayısında ise azalma olduğu yapılan ölçümler sonucunda ortaya çıkmıştır. Bu çalışma, karayolunun yaprak morfolojisi ve anatomisi üzerinde baskı oluşturduğunu ve bazı karakterlerde değişikliklere neden olduğunu vurgulamaktadır.

Anahtar kelimeler: anatomi, ağır metal, karayolu, morfoloji, Red Globe

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 Received: 13.03.2022; Published: 15.04.2022 BioDiCon. 1026-130322

1. Introduction

Environmental pollution is one of the most important problems of today. Rapid population growth and industrialization lead to the formation of various pollutants and deterioration of atmospheric quality, and rapid urbanization creates various pressures on the atmosphere. In Europe, more than eighty-five percent of people are exposed to air pollution that exceeds pollution standards. One of the causes of this pollution in the atmosphere is anthropogenic sources [1,2,3]. The main anthropogenic sources of atmospheric pollution are heavy metal containing industrial activities, domestic heating and vehicle traffic [4]. Heavy metals have a significant proportion among the components that cause air pollution. Heavy metal pollution caused by vehicles is also caused by cadmium used in motor oils and automobile tires, and copper and nickel that appear in the wear of motor alloys, in addition to lead from traffic density and exhaust [1,5].

The accumulation of heavy metals in water and soil is a great risk for the environment, and the presence of high levels of heavy metals in soil causes serious problems [6]. Agricultural and urban soils are exposed to anthropogenic heavy metal pollution and traffic-related factors cause soil pollution. Most of the particles that cause pollution are taken into the body of the plant through the roots and accumulate in the leaves [2,7,8]. It has been stated that the physiological activities of these plants grown in the soil, such as germination, photosynthesis, transpiration, and water intake, are adversely affected by this pollution, and the development and yield of the plants are reduced. In addition, it has been revealed that heavy metal accumulation in the soil is high in areas with heavy vehicle traffic and this rate decreases depending on the distance from the traffic [8,9].

The aim of the study was to examine the anatomical and morphological changes in the Red Globe grape variety, which has a market value, depending on the time and distance from the road.

2. Materials and methods

2.1. Study area and material

The research was carried out in Red Globe vineyards on the Salihli-Alaşehir route (İzmir-Denizli highway), between the latitude 38.4660327^0N and longitude 28.3326680^0E, an elevation of about 113 m above sea level in Alaşehir district of Manisa province in April-September 2019. The annual average temperature of the district is 17.19^0C (min: -4 ^{0}C, max: 40.4^0C), total precipitation is 369.8 mm, average relative humidity (%) is 63.36 [10].

2.2. Sampling and analysis

Plant samples were taken from 4 different distances (0-250-500 and 4000 m) according to the highway in 3 different times (spring - summer - autumn) and the research was conducted with 3 replicates. On the other hand, a total of 150 leaf samples were collected from all sides of the grapevines, from the leaves opposite the bunches on the annual shoots that had completed their development, in 5 selected one's at the sampling stations in each period.

Leaves collected from each sampling station for morphological and anatomical examinations were brought to the laboratory and made ready for analysis. Morphologically, leaf length (LL), leaf width (LW), leaf aspect ratio (LAR), petiole length (PL), midvein length (ML), the ratio of midvein length to petiole length (RMP), petiole thickness (PT), leaf midvein thickness (LMT), leaf area (LA), leaf thickness (LT), the angle between N1 and N2 (A1), N2 and N3 (A2), N3 and N4 (A3) [11] were measured and the data was recorded.

The leaves collected for the anatomy study were washed with tap water and then fixed in 70% ethanol until the necessary examinations were made. Cross sections were taken from petiole by using razor blade. Sections were examined with BAB Image Analyzing Systems Microscope (BAB-95) and anatomical characteristics were determined such as thickness of upper epidermis (UET), lower epidermis (LET), upper collenchyma (UCT), lower collenchyma (LCT), upper parenchyma tissue (UPT), lower parenchyma tissue (LPT), length of vascular bundles (LV) and vascular bundles number (VN). Also, anatomical structures were measured using BAB measurement programme.

2.3. Statistical analysis

The study was designed according to randomized block experimental design with 3 replicates. Statistical analyses were conducted using IBM SPSS Statistics 25 software. Two-way ANOVA was used to analyses of all morphological and anatomical data and the means were compared with Duncan's test at 5% level of significance.

3. Results

3.1. Effect of distance from highway on morphology

As a result of the analyzes, it was determined that the distance to the highway was effective on leaf length, leaf aspect ratio, petiole length, midvein length, petiole thickness, leaf midvein thickness and the angle between N3 and N4 at $p< 0.01$ significance level. On the other hand, it was observed that the distance was effective on leaf width, leaf area, the angle between N1 and N2 at $p<0.05$ significance level. In addition, the effect of distance on the ratio of midvein length to petiole length, leaf thickness, the angle between N2 and N3 parameters was found insignificant (Table 1).

When the data obtained were examined, the highest average values were determined at 250 meters distance in leaf length, leaf width, petiole length, midvein length, leaf midvein thickness, leaf area, leaf thickness, the angle between N3 and N4. On the other hand, the highest mean values in the petiole thickness, the angle between N1 and N2 features were observed at the roadside, at 500 meters in the leaf aspect ratio feature, and at 4000 meters in the ratio of midvein length to petiole length and the angle between N2 and N3 (Table 1).

On the contrary, when we analyzed the data in terms of the lowest mean values, the lowest mean was determined at 500 meters distance in all features except the leaf aspect ratio, the ratio of midvein length to petiole length, the angle between N2 and N3 parameters. In addition to these, the lowest average was found at 4000 meters for the leaf aspect ratio character, at 250 meters for the ratio of midvein length to petiole length, and at the roadside for the angle between N2 and N3 (Table 1).

Table 1. The effect of distance from highway on leaf morphology of Red Globe variety

Parameters	Distance (m)			
	Road side	250	500	4000
LL (cm)**	17,36±2,72ab	18,01±2,63b	16,54±2,61a	17,74±2,21b
LW (cm)*	16,68±2,46a	17,56±2,37b	16,39±2,38a	16,75±1,86a
LAR**	0,96±0,09ab	0,98±0,07bc	0,99±0,07c	0,95±0,06a
PL (cm)**	10,15±2,64a	11,03±3,09b	9,48±2,26a	9,77±2,05a
ML (cm)**	11,85±1,77ab	12,32±1,92b	11,33±1,96a	12,12±1,75b
RMP^{ns}	1,22±0,25	1,17±0,23	1,23±0,23	1,26±0,16
PT (cm)**	4,28±0,77b	4,21±0,86b	3,85±0,76a	3,91±0,71a
LMT (cm)**	2,43±0,52bc	2,47±0,64c	2,19±0,46a	2,28±0,52ab
LA (cm^2)*	198,17±60,61ab	215,61±56,44b	186,13±60,56a	199,63±44,89ab
LT (cm)^{ns}	0,22±0,12	0,25±0,12	0,22±0,12	0,23±0,13
A1(°)*	56,73±5,76b	55,58±6,21ab	53,53±5,92a	56,51±4,91b
A2(°)^{ns}	48,22±5,74	50,80±5,72	48,84±6,80	51,16±6,27
A3(°)**	50,47±5,12a	53,89±5,42b	49,62±5,62a	49,87±7,55a

The data in the table are represented as the mean ± standard deviation and the letters on the same line such as a, b, and c means indicates grouping according to Duncan's test result. * : significant at 0,05 level; ** : significant at 0,01 level, ns: non-significant.

The changes in the morphological characters due to the distance in the leaf samples taken in different periods are shown in Figure 1.

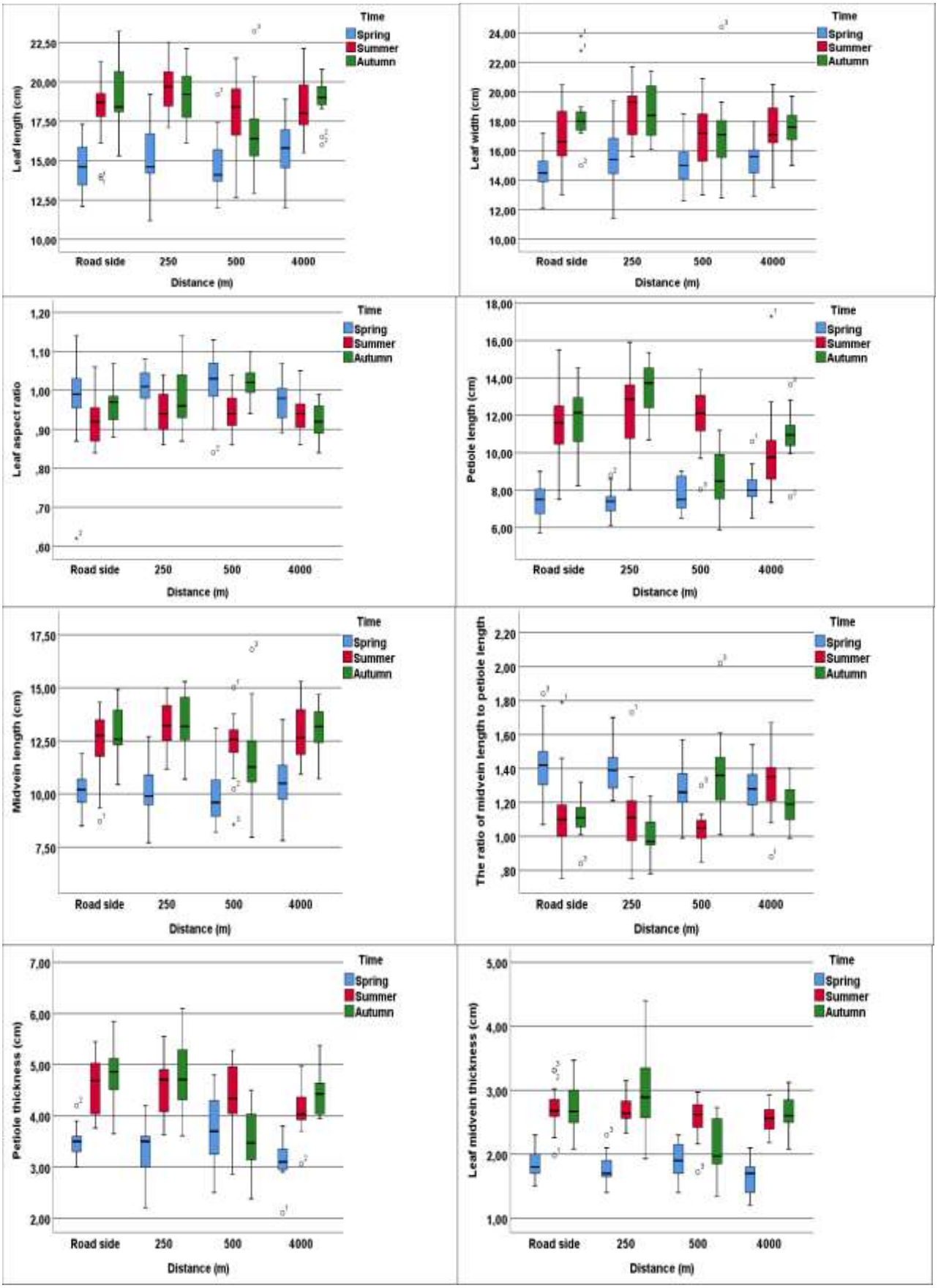

Figure 1. The effect of distance from the highway and time on the leaf morphology of Red Globe

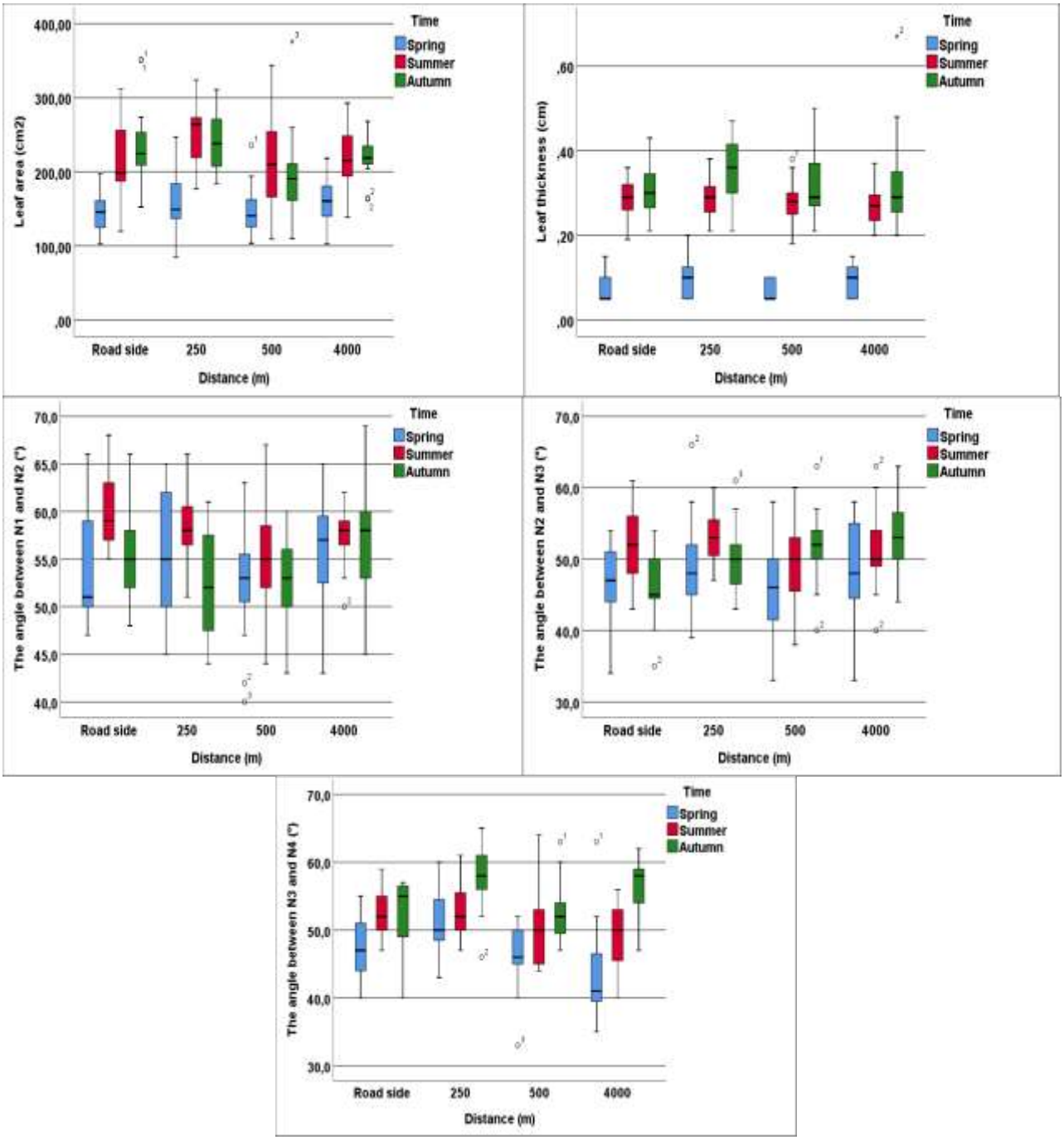

Figure 1. Continues

3.2. Effect of distance from highway on anatomy

According to the results of the analysis, it was determined that the distance from the highway was effective on the thickness of upper epidermis, lower epidermis, upper collenchyma, upper parenchyma tissue, lower parenchyma tissue, length of vascular bundles and vascular bundles number at $p < 0.01$ significance level; but it was observed that the effect of distance on the thickness of lower collenchyma was insignificant (Table 2).

When the anatomical data obtained were examined, the highest average value was determined at 4000 meters in thickness of upper epidermis, lower epidermis and vascular bundles number characteristics, and the highest average values in all parameters except for these features were observed in leaf samples collected from 250 meters.

According to the results of the analysis, the lowest average value was obtained in the thickness of upper epidermis and lower epidermis at 250 meters. The lowest mean value in the characters of upper collenchyma thickness, upper parenchyma tissue thickness and length of vascular bundles was determined in samples collected from 500 meters distance. In addition, the lowest values were observed at the roadside for lower collenchyma thickness and vascular bundles number, and at 4000 meters for lower parenchyma tissue thickness characters (Table 2).

Table 2. The effect of distance from highway on leaf anatomy of Red Globe variety

Parameters	Distance (m)			
	Road side	250	500	4000
UET (µm)**	13,97±0,77b	13,41±0,83a	13,54±0,75a	14,23±0,90c
LET (µm)**	12,82±0,66b	12,43±0,89a	12,58±0,73a	13,11±0,70c
UCT (µm)**	183,51±31,65b	188,26±31,34b	174,76±28,46a	187,37±21,96b
LCT (µm)^{ns}	149,07±21,43	153,72±26,92	152,50±25,26	149,78±23,48
UPT (µm)**	374,02±90,79b	401,22±119,59c	330,93±61,70a	382,45±81,00bc
LPT (µm)**	149,58±29,86bc	154,10±35,03c	144,57±30,21b	132,94±30,48a
LV (µm)**	435,08±37,96bc	438,84±66,99c	411,76±37,54a	420,64±52,41ab
VN**	32,33±3,54a	33,51±3,06b	33,77±2,97b	34,43±2,76b

The data in the table are represented as the mean ± standard deviation and the letters on the same line such as a, b, and c means indicates grouping according to Duncan's test result. ** : significant at 0,01 level, ns: non-significant.

The changes in some anatomical characters due to the distance in leaf samples taken in different periods are given in Figure 2.

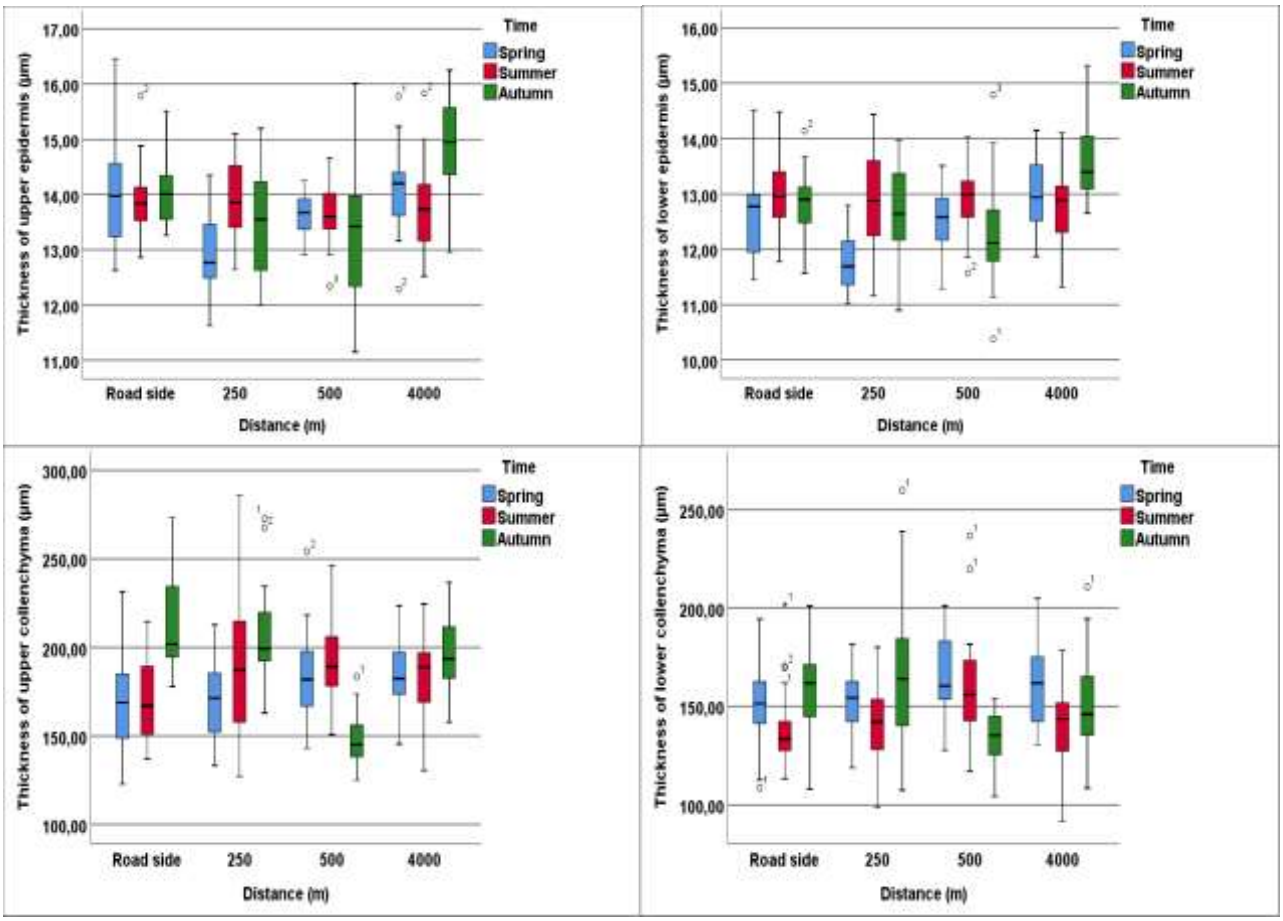

Figure 2. The effect of distance from the highway and time on the leaf anatomy of Red Globe

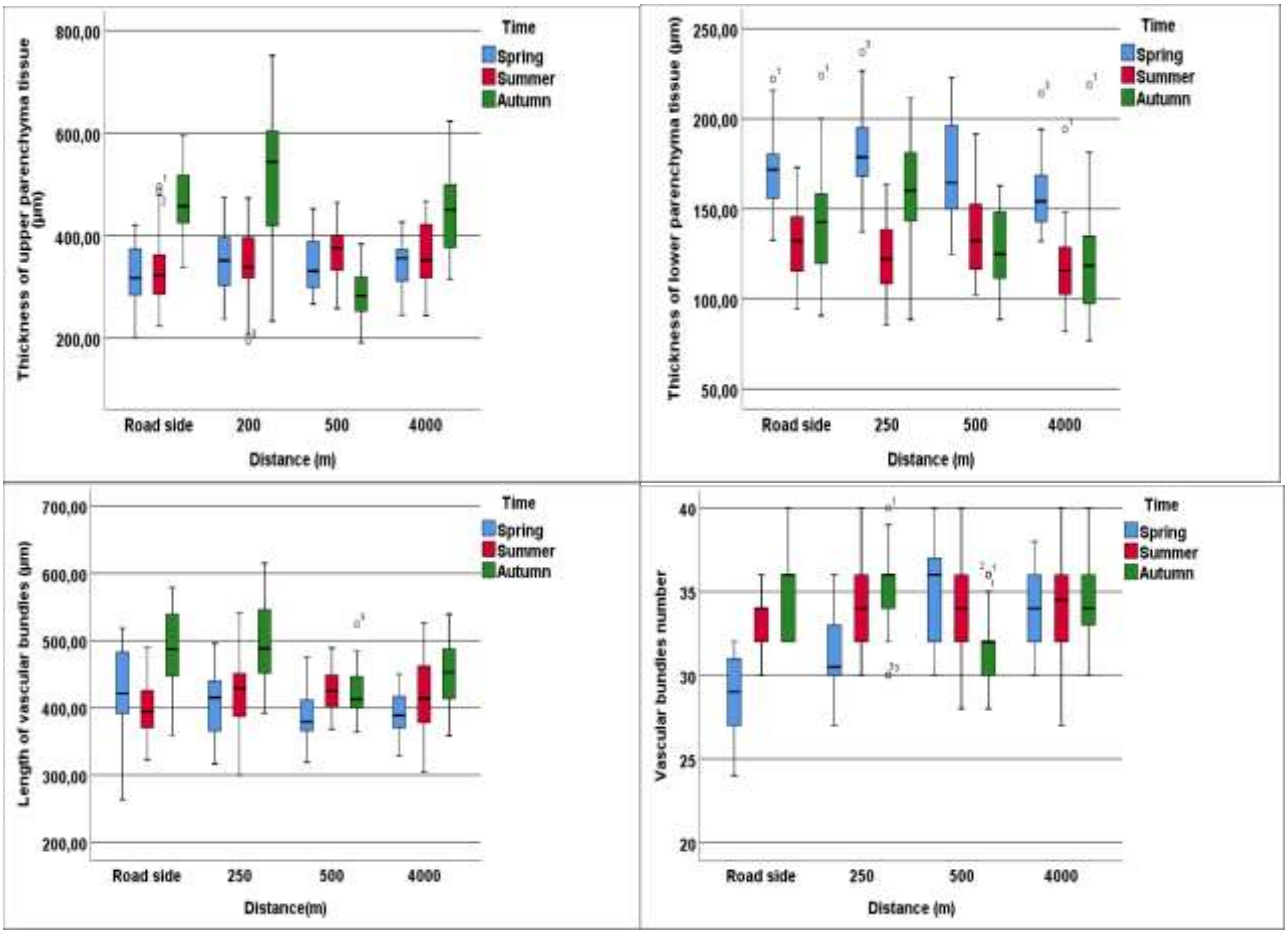

Figure 2. Continues

4. Conclusions and discussion

With the development of technology, the number of motor vehicles on the road increases every year in our country and in the world. The increase in this number brings about an increase in the amount of harmful gases released to nature and environmental problems such as air pollution. Heavy metals cause irreparable damage to health, food chain and nature.

Urban plants are constantly exposed to high levels of pollutants that cause morphological, physiological and anatomical damage to plants [12]. Leaves are more affected by traffic-related pollutants than roots and stems. Exhaust gases have harmful effects on the structure and physiology of plants. For this reason, morphology and anatomy studies are also carried out on the leaves to reveal the negative effect [13].

When the data on the effect of distance on leaf anatomy and morphology were evaluated, it was determined that this effect was significant on some parameters. When all the data obtained were examined, it was concluded that the effect of heavy metal was clearly observed at the roadside and 250 meters away, but a different effect than expected in some parameters, at 500 meters and 4000 meters distances. The change in the soil structure and the difference in the prevailing wind direction may be the reason for the different results at 500 and 4000 meters.

Traffic-induced pollutants accumulate on the leaf surface, adversely affect the anatomical, morphological and physiological activities of plants, as well as cause a decrease in very important physiological events such as transpiration, photosynthesis [14]. In areas with pollution, there is a decrease in leaf width, length and leaf aspect ratio. It is stated that the reason for this may be that pollutants adversely affect the photosynthesis mechanism [15]. It is thought that heavy metals cause the production of reactive oxygen species, which have a negative effect on the enzymes that synthesize chlorophyll, and that the decrease in chlorophyll content directly affects the growth and vitality of plants [16]. On the other hand, it is stated that heavy metals stress the plant and reduce the leaf area of the plant to reduce water loss by transpiration [17]. When we evaluated the morphological data of present study, it was observed that there was a decrease in leaf width, length, leaf aspect ratio, petiole length, midvein length, midvein thickness and leaf area. In the article, which includes some of these parameters and effect of lead on *Glycine max* (L.) Merr., it was concluded that as the concentration of lead increased, the midvein thickness decreased [18]. In another study on heavy metals in *Vachellia campechiana* (Mill.) Seigler & Ebinger, a decrease in petiole length and length of the intermediate vein parameters and an increase in petiole diameter were observed in plants exposed to heavy metals [19]. Similar results

were obtained in our study. In a study in which the effect of auto-vehicular pollution on the morphology and anatomy of *Achyranthes aspera* L. and *Chenopodium album* L. was observed [20], it was determined that pollution reduces leaf length, width and petiole length, and it was also stated that pollution reduces leaf area. Likewise, it has been reported that leaf length and width decrease as traffic density increases in *Olea europea* L. [21]. In another study using *Elaeagnus angustifolia* L., *Eucalyptus tereticornis* Sm., *Ficus carica* L., *Fraxinus excelsior* L., *Melia azedarach* L., *Morus alba* L., *Morus nigra* L., *Pistacia vera* L., *Prunus armeniaca* L., *Punica granatum* L., *Robinia pseudoacacia* L., *Rosa indica* L. and *Vitis vinifera* L. species, it was concluded that there was a decrease in leaf length, width, petiole length and leaf area in polluted areas [22]. There are significant differences between grapevine varieties in terms of features such as leaf area, length of veins, angles between veins, and petiole length [23]. Since the angle between the veins is an important character in the grapevine, it was also investigated whether the distance from the highway has an effect on this feature. It was observed that there were significant differences in terms of the angle between N1 and N2 and the angle between N3 and N4. It was concluded that heavy metal might have a negative effect on cell division, and that the speed differences in cell division and cell growth could create different angles in the grapevine. The changes that occur in the leaf are of critical importance; because the photosynthesis mechanism takes place in the leaf and any negative situation in the leaves will affect photosynthesis.

The part of the study related to anatomy was examined in the petiole. It has been reported that petioles are more effective as a particle impactor than twigs or leaf lamina for polluting particles [24]. In the study, it was observed that the upper and lower epidermis were thicker at the roadside than at a distance of 250 meters. An increase in epidermis thickness is the expected result; because the epidermis tissue thickens, it is possible for the plant to show an anatomical change in order to reduce the entry and negative effects of heavy metals in the internal tissues [25]. In addition, the thickening of the epidermis layers may be a strategy of the plant to reduce water loss by transpiration [26]. In the research conducted on this subject, data were presented that cadmium increases the upper and lower epidermis thickness of *Eichhornia crassipes* Mart. [27]. Plants can also develop responses to toxic substances such as reducing the number of vascular bundles, increasing the total area of the vascular bundle, and the thickness of the xylem and phloem [25]. In another study [17], it was stated that lead reduced vascular bundle size and leaf diameter. It is emphasized that the reduction in trachea diameter and vascular bundle size may facilitate the transport of organic and inorganic substances, thus paving the way for adaptation. When we examined the data we obtained, it was observed that there was a decrease in the number of vascular bundles and length of the vascular bundles. In this study, it was also tried to determine whether the highway has an effect on the thickness of the lower collenchyma, upper collenchyma, lower parenchyma tissue and upper parenchyma tissue in the petiole, and as a result of the research, lower values were obtained at the roadside compared to the 250 meters distance in terms of these properties. In the article examining the effect of lead on the leaf anatomy of *Schinus molle* L., it was reported that lead increased the thickness of the epidermis in the leaf and reduced the collenchyma [28]. Likewise, in a study conducted to determine the effect of cadmium stress on *Glycine max* (L.) Merr., it was concluded that cadmium, a heavy metal, decreased palisade parenchyma, sponge parenchyma and mesophyll thickness [29]. In another study, there is information that changes occur in plants exposed to high heavy metals, such as decrease in parenchyma tissues and xylem size, shrinkage of mesophyll tissue, decrease in leaf growth and vascular bundle [30]. In a study examining the effect of chromium on the anatomy of *Triticum aestivum* L. cv. "Ekiz", it was observed that chromium increased the length and width of the upper and lower epidermis in the leaf. In addition, it was determined that chromium decreased the thickness of mesophyll, xylem, phloem and diameter of vessel elements [31].

The reason for the decrease in many morphological and anatomical characteristics used in this study in areas close to the highway may be due to the negative roles of heavy metals in many metabolic reactions such as photosynthesis, enzyme activity, chlorophyll biosynthesis and mitosis [17]. Environmental conditions such as humidity of the air and precipitation significantly affect the entry of heavy metals into the plant [32]. In this study, leaf samples were taken at different periods, and while making the evaluations, the average of the data was taken and inferences were made accordingly. In the spring period, there is a possibility of washing the heavy metal with the effect of precipitation, and the probability of precipitation is less in the summer and autumn periods. In addition, there is a high probability of heavy metal accumulation due to more vehicle traffic and lack of precipitation during the summer period. When we examine the results, we have obtained, periodic changes in plant anatomy and morphology are visible (Figure 1 and 2).

In conclusion, it was observed that the distance from the highway was effective on the leaf morphology and anatomy of the Red Globe grape variety, and there were obvious differences in the vineyards at the roadside. In addition, it was concluded that the cultivate of this economically important variety in areas further away from the highway would be better for health.

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