



Effects of Air Pollution on Some Chemical Compounds of Cherry Laurel (*Prunus Laurocerasus* L.) in Kastamonu

Nezahat TURFAN¹, Özlem MEŞE^{2*}

¹ Kastamonu University, Science and Art Faculty, Biology Department, Kastamonu, Turkey

^{2*} Çankırı Karatekin University, Faculty of Forest, Department of Forest Engineering, Çankırı, Turkey

Abstract

Industrialization and overuse of automobiles result in the release of toxic matter in the air which may induce harmful effect on the living system such as plant, animal, soil fauna. All pollutants from traffic and factories suppress plant growth and development by preventing physiological and biochemical reactions. However, trees can play an important role in improvement the air quality by prevention of environmental pollution in the urban environment. The present study was conducted in Kastamonu city. It purpose was to point out the impacts of air pollution caused traffic on chemical compositions in *Prunus laurocerasus* L. For this purpose, we collected leaf sample of trees from areas away from the traffic for control, while polluted sample were selected from regions where the traffic was heavy (Salıpazarı) and less dense (Kısla park) in Kastamonu city center. In each of leaf sample, the amount of chlorophyll pigments, carotenoid, total soluble protein, MDA-malondialdehyde, H₂O₂, enzymatic antioxidants such as APX, CAT, GPOX and SOD activities, and non-enzymatic antioxidants measurements were performed. According to data, the amount of chlorophyll b and total chlorophyll, and CAT and GPOX activities was determined higher in non-polluted plant but chlorophyll a, proline, total soluble carbohydrate and SOD activity enhanced excessively contaminated leaf. APX activity was the highest in lighter contaminated leaf samples but H₂O₂ was the lowest. As a result, it could be concluded that the growth and development of cherry laurel was found to be affected traffic pollution depended on the severity of pollution. And also, on the basis of this study it can be said that cherry laurel is resistant to air pollution, and the results could be used in the future research to understand the role of individual tree species in air pollution.

Keywords: Chemicals, pollution, *Prunus laurocerasus* L., tolerance.

Kastamonu'da Taflanın (*Prunus Laurocerasus* L.) Bazı Kimyasal Bileşikler Üzerine Hava Kirliliğinin Etkisi

Öz

Sanayileşme ve aşırı otomobil kullanımı, bitki, hayvan, toprak faunası gibi yaşam sistemi üzerinde zararlı etkiye neden olabilecek havada toksik maddenin salınımına neden olmaktadır. Trafikten ve fabrikalardan kaynaklanan tüm kirleticiler, fizyolojik ve biyokimyasal reaksiyonları engelleyerek bitki büyümesini ve gelişimini baskı altına alır. Bununla birlikte, ağaçlar, şehir ortamındaki çevre kirliliğini önleyerek hava kalitesinin iyileştirilmesinde önemli bir rol oynayabilir. Bu çalışma, Kastamonu ilinde yapılmıştır. Çalışmanın amacı trafiğe bağlı hava kirliliğinin *Prunus laurocerasus* L.'deki kimyasal bileşimler üzerindeki etkilerine dikkat çekmektir. Bu amaçla, Kastamonu şehir merkezinde trafikten uzak alanlardan kontrol numunesi toplanırken, kirliliği ise trafiğin yoğun (Salıpazarı) ve az yoğun (Kısla parkı) olduğu alanlardan seçilmiştir. Yaprak numunesinin her birinde klorofil pigmentleri, karotenoid, toplam çözünür protein, MDA-malondialdehit, H₂O₂, APX, CAT, GPOX ve SOD aktiviteleri gibi enzimatik antioksidanlar ve enzimatik olmayan antioksidanlar ölçümleri yapılmıştır. Verilere göre, klorofil b ve toplam klorofilin miktarı, CAT ve GPOX aktiviteleri, kirlenmeyen bitkilerde daha yüksek olarak belirlenmiştir fakat klorofil a, prolin, toplam çözünür karbonhidrat ve SOD aktivitesi çok fazla kirlenmiş yaprakta artmıştır. APX aktivitesi daha az kirlenmiş yaprak örneklerinde en yüksek ancak H₂O₂ ise en düşük seviyede idi. Sonuç olarak, taflanın büyümesinin ve gelişmesinin, kirliliğin şiddetine bağlı olarak trafik kirliliğinin etkilediği sonucuna varılabilir. Ayrıca, bu çalışmanın temelinde, taflanın hava kirliliğine karşı dirençli olduğu söylenebilir ve sonuçlar, hava kirliliğinde bireysel ağaç türlerinin rolünü anlamak için daha sonraki araştırmalarda kullanılabilir.

Anahtar Kelimeler: Kimyasal, kirlilik, *Prunus laurocerasus* L., tolerans.

*Sorumlu Yazar (Corresponding Author):

Özlem MEŞE; Çankırı Karatekin Üniversitesi, Orman Fakültesi, Orman Mühendisliği Bölümü, 18200, Çankırı-Türkiye. Tel: +90 376 212 2757 Faks: +90 376 213 6983, E-mail: ozlemeken@karatekin.edu.tr ORCID No: 0000-0001-9060-0816

Geliş (Received) : 05.03.2019

Kabul (Accepted) : 07.05.2019

Basım (Published) : 15.08.2019

1. Introduction

The quality of air is the most important issue for all living things all over the world, especially in crowded cities. The air pollution originating from the rapidly increasing number of population, urbanization, *vehicle*, and industrial activities have damaged the natural structure intact environment of urban ecosystems, by directly or indirectly impairing plant growth and development (Aguiar-Silva et al. 2016, Yılmaz, 2018). The pollutants arising from pollution can be deposited on leaf surfaces and form a dirt layer, especially as the pollutant accumulation exceeds the threshold value. They can infiltrate into cells, causing changes in physiological and chemical reactions (Tiwari, 2013). In addition, the layer on the leaf surface can inhibit light penetration and stomatal regulation. Many researchers have revealed that pollutants inside cells and tissues induce oxidative stress and suppress photosynthesis, carbon and nitrogen metabolism, and osmotic balance (Pimple 2017; Aggarwal et al., 2012). However, plants respond to pollution in different ways, depending on their tolerance levels, developmental levels, duration of exposure to pollution, as well as seasonal changes. Many authors stated that tolerant species have higher antioxidative defense mechanisms such as antioxidant enzymes, soluble chemicals like proline, protein, sucrose, carbohydrates, pigments, secondary metabolites like flavons, and phenolics (Pukacka and Pukacki, 2000; Cetin et al., 2018). Several studies have shown that those chemicals enhance trees' tolerance to the harmful effect of pollution and protect them. Aguiar-Silva et al. (2016), Chandra and Kang (2016) found out that the amounts of photosynthetic active pigments as chlorophyll and carotenoids in the leaf grown in a contaminated area were significantly higher than the amounts in plants from non-polluted areas, but were lower than the amounts in the tolerant species. Sanaeirad et al. (2017), Rezanejad (2009) studied the effects of pollutants on nitrogenous compounds in plants. They determined that the proline, protein, amino acid and nitrate reductase enzyme levels increased with pollution. Foyer and Shigeoka (2011), Long et al (2003) showed that the air pollution induced oxidative stress and declined the activity of antioxidant enzymes like APX, POD, CAT as well as the SOD activity in plants growing in polluted and less polluted areas, while they were higher in resistant plants. Trees planted in parks, gardens, and places near roadsides are very important for the regulation and improvement of air quality. Hence, monitoring air pollution is becoming increasingly important with screening and using tolerant plant species as biological indicators. In more recent times, scientific studies have quantified the amount of air pollutants removed by urban trees (Kuang et al., 2007; Domingos et al., 2015).

Kastamonu province was classified as a second degree polluted province. However, with the developing industry, the increased population, vehicular traffic and the use of natural gas for heating led to an air pollution problem in Kastamonu, especially in 2016. The rate of natural gas used for heating was found to be 32% in 2016, while it was 11% in 2011. In the organized industrial zones, the rate of natural gas use is 44% in the food sector, 19% in the metal sector, and 13% in the textile sector, in order of highest to lowest. According to the General Directorate of Highways, there is no highway in Kastamonu; however, the automobile density is over 71% and the heavy vehicle density is between 18% and 22% in areas close to the city center and on dense state roads (GDH, 2016). In addition, diesel and gasoline consumptions were reported to be 90% and 75, respectively. The total amount of 122,509 included 59,290 automobiles; 18,752 light commercial vehicles; 6,348 heavy commercial vehicles; and 3,119 other types of vehicles such as tractors, motorcycles and small vehicles (Kastamonu Provincial Directorate of Environment and Urbanization, 2016). Briefly; the rate of automobile use in Kastamonu province was observed to be high on the state roads near the city center, and the high rate of diesel fuel consumption was found to be triggering the air pollution.

Cherry laurel (*Prunus laurocerasus* L.) is an evergreen broadleaf shrub that has long been used for landscaping as a hedge, especially in areas near forest populations. However, it can also be grown in parks and roadsides in city centers. It can grow quickly, adapt extreme environments, tolerate strong winds, shading, and atmospheric pollution, and is also more resistant than most of the other *Prunus* species (Ivanov and Panchev, 2016). It is locally known as “*Taflan*”, “*Karayemiş*”, “*Laz kirazi*”, “*Laz üzümü*” (Karadeniz and Kalkisim, 1996; Alasalvar et al., 2005). Cherry laurel, cypress and sycamore trees are used extensively on the roads used in the center of Kastamonu. Therefore, with this study, the effects of traffic pollution on the chemical compounds of cherry laurel leaves, and its tolerance investigated.

2. Material and Method

2.1. Description of sample areas

The study was carry out in Kastamonu province surrounded by the Black Sea and the provinces in the North Central Anatolia Clean Air Center. Kastamonu is a city located in the Western Black Sea Region, between 41 and 21 degrees north latitude and between 33 and 46 degrees East longitude. The height of the province above sea level is 456 meters. Its pollution level is monitored from the Air Quality Assessment and Management

Station (AQAMS). While the amount of sulphurdioxide (SO₂) mainly arisen from traffic, coal and mines, construction areas and quarries is measured by a Horiba brand device, PM10 (particulate matter less than 10 µm) level is measured by a BAM 1020 Brand device. The contribution of heating to dust emission (PM10) was recorded to be 32% by AQAMS, while the contribution of SO₂ was 52% in 2016. The contribution of the vehicular traffic to PM10 is 44%, while the contribution to SO₂ is 45%. Lastly, the contribution of industry to PM10 was 19%, while the contribution to SO₂ was estimated to be 16% in 2016. In the dispersed SO₂, 76% was scattered as 0-5 µg/m³, 22.8% as 5-10, and 4-5% as 10-55 µg/m³. 7.5 % of the dust concentrations (PM10) ranged from 1 to 10 µg/m³, 26.6% from 10 and 20 µg/m³, and 27.5% from 20 to 30 5 µg/m³. The highest level of PM10 was 190 µg/m³. As seen in Table 1, both pollutants were found to have a high oscillation in seasonal periods in winter, temporarily a high oscillation between 06: 00-12: 00, 18: 00-21: 00, and the highest oscillation on Friday. Whereas annual sulfur content varied between 7 and 15 annual PM10 levels ranged from 48 to 71 µg/m³. As a consequence, SO₂ increased in winter months, while PM10 did not change significantly over the year 2016. Exceeding National Boundary Values were observed to be at the maximum level in February.

Table 1. The monthly mean values of air quality, and the numbers of days when the threshold level of was exceeded in Kastamonu in 2016 (PM10, SO₂ m³).

	PM10	SO ₂	Rate of PM10/SO ₂	AGS
January	32	21	7	0
February	60	7	11	9
March	54	3	10	4
April	57	2	16	7
June	40	2	15	2
July	53	2	20	2
August	34	2	17	0
September	39	2	15	0
October	36	1	14	0
November	46	1	12	1
December	77	2	8	16
Jan.	61	2		6
Wint	55	5		
Summ	43	2		
07:00-10:00	60	5		
12:00-14:00	52	5		
18:00-22:00	52	4		
23:00-06:00	38	3		
Monday	49	4		
Tuesday	50	4		
Wednesday	52	3		
Thursday	53	4		
Friday	53	5		
Saturday	45	4		
Sunday	42	4		

*: (<http://www.weathermonitoring.gov.tr>);

**AGS: Number of days the limit value was exceeded

Site A: Control area is a living area, where plenty of tree species exist in an area away from vehicular traffic.

Site B: It is crossroad located on the Mustafa Kaya street between Kışla park and DSI (The Directorate of State Hydraulic Works), an old settlement with high population and with medium traffic density (lower polluted area).

Site C: It is a busy way with intense traffic on the Rauf Denktas street, known as *Salipazari*. In addition, there is a great number of automotive shops on this road (higher polluted area).

2.2. Sampling of leaf

For leaf sampling, the fully matured fresh leaves were collected from five trees in polluted, less polluted and non-polluted areas near traffic flows in the third week of April in the center of Kastamonu (2017). Leaf samples

were taken from each aspect trees and then the samples were delivered to the laboratory. After the sampling process, the residues on the leaf surfaces were cleaned with tap water. Consequently, they were also cleaned three times with deionized water. The samples taken with the nematic dry paper were used for chemical analysis. All the chemical analyses were conducted in triplicate.

2.3. Chemical analyses

The amount of chlorophyll content was estimated by the method of Arnon (1949). Total carotenoid level was measured using Jaspars Formula according to the method by Witham et al. (1971). Firstly, leaf samples were powdered by liquid nitrogen. After, 500 mg sample were homogenized by 10 ml of 80% (v/v) chilled acetone and centrifuged at 3,000 rpm for 10 min. Then absorbance of the supernatant was noted at 663,645 and 450 nm spectrophotometrically. Proline determination of samples was carried out with approximately 500 mg of the leaf sample by homogenizing with 10 mL of 3% sulfosalicylic acid and measured using the ninhydrin reagent as reported by Bates et al. (1973) and the amount of proline was expressed as $\mu\text{mol proline g}^{-1}\text{FW}$. Total soluble protein level was determined according to Bradford's method (Bradford, 1976) using the Bio-Rad assay as a calibration standard. The amount of lipid peroxidation was evaluated as malondialdehyde (MDA) and determined following Luts et al. (1996) method. 500 mg sample was homogenized in 5 ml of 1% trichloroacetic acid (TCA) on the ice bath. The homogenate was centrifuged at 15,000 g for 10 min. 2 ml of 0.5% thiobarbituric acid (TBA) was added to the supernatant (2 ml). The mixture was heated at 95°C for 30 min, rapidly cooled in an ice bath. After, the absorbance of the supernatant was read at 532–600 nm on a spectrophotometer. The amount of MDA was estimated using the extinction coefficient of $155\text{ mM}^{-1}\text{ cm}^{-1}$ and MDA content expressed as $\mu\text{mol/mg FW}$. Hydrogen peroxide content was determined by the method of Velikova et al. (2000). Antioxidant of the leaf samples was determined by using dry leaf and needle samples (500 mg). The dried leaf and needle samples were grinded in powder using nitrogen liquid. The powder was homogenized in 5 mL phosphate potassium (pH 7.6 with 0.1 mM of EDTA- Ethylenediaminetetraacetic acid). The homogenate was centrifuged to 15.000 x g for 20 min at 4°C. The supernatant was kept, and 0.8 ml phosphate potassium 0.2 M was added. The homogenate was centrifuged again to 15.000 x g during 15 min. The combined supernatants were stored on ice and used in order to determine the activity of detoxifying enzymes. Superoxide dismutase (SOD) activity was assayed as described by Beauchamp and Fridovich (1971). Absorbance was noted at 560 nm and one unit of SOD activity was expressed as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT. The activity of ascorbate peroxidase (APX) was estimated using the method of Nakano and Asada (1981), by observing the decline in absorbance at 290 nm for 1 min caused by ascorbic acid oxidation. Catalase activity (CAT) was estimated following Bergmeyer (1970) method's by the estimation of the destroying of H_2O_2 , measuring the reduce of the absorbance at 240 nm. The guaiacol peroxidase (GPOX) activity was assayed to Chance and Maehley (1995) spectrophotometrically, by the oxidation of guaiacol in the presence of H_2O_2 . All enzyme activity was given per mg protein, and one unit represented 1 μmol of substrate undergoing reaction per mg protein per min. Glucose and sucrose content was estimated by using the "Anthrone Method" of Pearson et al. (1976). Total soluble sugar content was determined following Irigoyen et al., (1992) method.

2.4. Statistical analysis

Analysis of variance (ANOVA) was applied for analyzing the differences in the chemical composition of the leaf samples exposed to traffic pollutions using the SPSS program (Version 11 for Windows). Following the results of ANOVAs, Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$) was used for group.

3. Result and Discussion

The mean concentrations of the photosynthetic pigments, total carotenoid, total flavonoid, proline, and soluble protein in the leaf samples exposed to traffic pollution are given in Table 1. The photosynthetic pigments as chlorophyll-a, chlorophyll-b, total chlorophylls and carotenoid are necessary for harnessing light energy (Hörtensteiner, 2006). Pollutants may change their structures and influence the photosynthetic capacity of plant varieties. A reduction in the level of chlorophyll has often been suggested to be an indicator of air pollution damage as SO_2 , O_3 and others (McLaughlin et al., 1982; Shiragave et al., 2015). As seen in Table 1, the higher polluted location showed the highest level of Chl a (0.157 mg/g), while the lowest amount was found in the control plant (0.148 mg/g). Chl b content in the leaf samples was noted as 0.105 and 0.191 mg/g in the lower and higher polluted locations, while it was 0.241 mg in the control location (Table 2). The amount of total chlorophyll was at the lowest level in the lower location plant (0.59 mg/g), while it was at the highest level in the control leaf sample (0.389 mg) and higher polluted leaf tissue (0.348 mg), respectively (Table 2). In the ratio of Chl a: Chl b, there was a n importance lowering as 0.6 in the nonpolluted plant, compared to the polluted plants.

It was a higher level in the lower polluted and higher polluted areas as 1.46 and 0.82 (Table 1). There was no important change in the total carotenoid content but it was the highest in the lower polluted leaf sample (Table 2). The result of Chl-a, ratio of Chl-a:Chl-b, and total carotenoids indicated that Chery laurel is resistant to air pollution. Many researcher stated that photosynthetic pigments play a significant role in the tolerance of the plants to stress conditions, and that higher levels of them enhance tolerance to pollution in plant (Tripathi and Gautam, 2007; Yılmaz 2018).

Table 2. Variation of the mean pigment as chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total Chl), and carotenoid level in the leaf samples of cherry laurel.

	Chl a mg/g	Chl b mg/g	Total Chl mg/g	Chl a:Chl b	Total Carotenoid mg/g	Glucose mg/g	Sucrose mg/g	Total Soluble Carbohy drate (mg/g)
Control	0.148 ± 0.0001	0.241 ± 0.0002	0.389 ± 0.0002	0.61	10.75 ± 0.008	62.32 ± 0.24	27.56 ± 0.77	311.57 ± 0.23
Less polluted	0.154 ± 0.0001	0.105 ± 0.0001	0.259 ± 0.0001	1.46	10.90 ± 0.004	60.86 ± 0.42	24.77 ± 0.45	304.27 ± 0.42
Heavy polluted	0.157 ± 0.0001	0.191 ± 0.0002	0.348 ± 0.0001	0.82	10.59 ± 0.005	65.06 ± 0.34	31.76 ± 0.65	325.30 ± 0.32
F	3072.41	324420.56	743148. 69	679.35	130810.35	39.58	28.87	4761.42
Sig	0.000	0.001	0.000	0.000	0.000	0.001	0.000	0.001

Pigment results are similar to the studies of Geeta and Namrata (2014), Wali et al. (2004), who have expressed that Chl a was more sensitive to gaseous pollutant than Chl b. They also expressed that the chlorophyll level was higher in the tolerant species. As seen in Table 1, the Chl-content and Chl a:Chl b ratios were found to be higher in the polluted leaf. In addition, the carotenoid content was not affected much by pollution (Table 1). It was shown that the chlorophyll level varied depending on the duration of exposure to pollution, leaf characteristics, and seasonal factors; however, the amount of pigment was generally higher in the resistant plants. Strand (1993) and Rüdiger (2003) reported that the Chl a content decreases easily due to breaking down into phaeophytin, but Chl b level may decline by separation of the phytol group of Chl b due to SO₂ accumulation in leaf structures. However, it has been proven that demolition of chlorophyll molecules can also be caused by ROS induced pollutants in chloroplasts during the oxidation of some chemicals (Shimazaki et al., 1980; Kumari et al., 2005). Carotenoids are highly stable compounds that accumulate in the photosynthetically active or inactive in many tissues. They are not sensitive to types of stress like chlorophyll molecules, and protect photosynthetic organisms against oxidative stress that leads to adverse environmental conditions (Adams III et al., 2013; Ramel et al., 2012).

Because the healthy appearance of species and the capacity of photosynthesis depend on the carbon level, soluble sugars such as glucose, fructose, sucrose and total soluble carbohydrates are very important. They play very important role in regulating the osmotic potential, increasing the tolerance level of genotypes and adapting to the environmental stresses (Rathinasabapathi 2000; Gupta and Kaur, 2005). And also, it was reported that they are very sensitive to stressful conditions and impact their movement from source organs to sink ones. In this study, the amount of soluble sugar in the leaf sample obtained from a heavily contaminated site were found to be increased when compared with those collected from the non-polluted and less contaminated sites (Table 2). In the most polluted leaf, the glucose, sucrose and soluble carbohydrate levels were found to be the highest as 65.06, 31.76 and 325.30 mg, respectively. They were the lowest values as 60.86, 24.77 and 304.27 mg in the less contaminated leaf sample (Table 2). The results were found to be in conformity with the results obtained by Malhotra and Khan (1980), Konecna et al. (1989). Their investigation exhibited that the amount of soluble sugar increased with SO₂, due to the breaking down of polysaccharides rich in reducing sugars. Seyyednejad et al (2009) determined that the concentration of soluble carbohydrate, proline, chlorophyll a, b, and carotenoid were higher in the polluted regions in comparison with trees in unpolluted sites. Strand et al. (1999) reported that stressful conditions increased the soluble sugar concentrations, while light intensity, heavy metals, nutritional deficiency and ozone lowered the sugar concentrations. In this study, the accumulation of sugar in highly contaminated leaves has been associated with the defensive effect of sugars on pollution and with adjusting the metabolism of cherry laurel against pollution. Our result indicated that the responses of species to pollution are different between varieties and levels of pollution.

The environmental pollutants such as SO₂, NO₂, O₃ and others formed by vehicular traffic and industrial pollution trigger oxidative stress and elevate the level of reactive oxygen species (ROS) such as H₂O₂, O₂, OH, which cause oxidation of some compounds including proteins, enzymes, DNA and others cellular components (Dat et al., 2000). In addition, ROS increase MDA level in cells and tissues due to destruction of membrane lipids hydrolyzed enzymatically or non-enzymatically. However, plants have developed various defensive mechanism including enzymatic and non-enzymatic compounds to eliminate ROS and lipid peroxidation effect (Mittler, 2006). Enzymatic antioxidants contain superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), glutathione reductase (GR), guaiacol peroxidase (GPOX) and ascorbate reductase (AR), while non-enzymatic antioxidants include ascorbate, glutathione, carotenoids, phenolics (Aninbon et al., 2016; Caverzan et al., 2012). In addition, osmolytes such as proline and soluble protein accumulate in response to pollution injury, and serve as non-enzymatic antioxidants (Chiou and Bush, 1998; Agbaire and Akporhonor, 2014). The amount of MDA was the highest in the most polluted leaf (134.00 µM), whereas it was the lowest in the non-polluted control plant (78.00 µM) (Table 3). H₂O₂ concentration in the leaf sample was the lowest in the lower-polluted plant (219.25 µM), but it significantly rose in the control plant (244.67 µM) (Table 3). Antioxidant enzyme activities varied depending on pollution levels. However, APX and SOD were higher in the polluted leaf, while CAT and GPOX reduced in both polluted sites (Table 3). APX was the highest in the least polluted plant (0.696 EU), while SOD was the highest in the most polluted leaf sample (24.06 EU). However, CAT and GPOX were higher in the non-polluted leaf samples as 0.372 EU and 0.143 EU, respectively (Table 3).

Table 3. Variation of the mean MDA, H₂O₂, Proline, Soluble Protein level, APX, CAT, GPOX and SOD activity in the cherry laurel leaf.

	MDA µmol/g	H ₂ O ₂ µmol/g	Proline µmol/g	Total soluble protein mg/g	APX EU/ mg in Protein	CAT EU/ mg in Protein	GPOX EU/ mg in Protein	SOD EU/ mg in Protein
Control	78.03 ± 0.10	244.67 ± 0.034	32.57 ± 0.22	44.24 ± 0.07	0.358 ± 0.007	0.372 ± 0.0006	0.143 ± 0.003	10.57 ± 0.21
Less polluted	109.00 ± 0.05	219.25 ± 0.20	25.69 ± 0.23	54.21 ± 0.19	0.696 ± 0.005	0.211 ± 0.0009	0.119 ± 0.004	19.42 ± 0.18
Heavy polluted	134.00 ± 0.55	227.92 ± 0.33	60.56 ± 0.18	60.08 ± 0.15	0.567 ± 0.008	0.172 ± 0.0028	0.114 ± 0.003	24.06 ± 0.11
F	74.20	3424.28	7746.61	7746.62	676.40	19.54	3961.72	16214 4
Sig	0.001	0.000	0.000	0.000	0.000	0.002	0.000	0.000

As seen in Table 3, the variation of MDA and H₂O₂ levels, and the activities of APX, CAT, GPOX and SOD in leaf samples were not homogeneous. The increase in MDA and H₂O₂ concentrations and APX and SOD activities, and the decrease in CAT and GPOX activities in the presence of pollution have led us to consider this result (Table 3). The increase of the quantity of MDA and H₂O₂ has been related with membrane structure deterioration of cherry plant (Chaffai et al., 2009). Morsy et al. (2012) found out that the level of lipid peroxidation rose in the shoot sample grown in the contaminated area. However, the activities of APX and SOD proline, soluble protein, glucose and sucrose levels were enhanced in heavy polluted plants and they may contribute to the reduction of MDA and H₂O₂ effect (Delauney and Verm, 1993; Woo and Je, 2006). It has been reported that compounds such as proline, proteins and sucrose accumulated the most in stress conditions correlated with resistance, and served as the inhibitor of ROS overproduction, stabilizer of membrane structures, balancer of cellular redox potential, metal chelators and osmotic regulator in resistant species under pollution (Deak and Malamy, 2005; Yasseen et al., 2018). Sanaeirad et al. (2017) showed that the air pollution increased proline and soluble proteins in tolerant plants, but Sofo et al. (2004) noted that the pollutants decreased the MDA level in those genotypes. Ogagaoghene (2017), Tingey et al. (2006) found out that the proline and protein levels enhanced in the polluted seedling, and this suggested an induced biosynthesis of amino acids and proteins with pollution. Ghorbanli et al (2007) measured CAT and POD activities higher in the polluted *Nerium oleander* and *Robinia pseudo acacia* leaf samples. APX was at a higher level in *Nerium oleander*, but did not change significantly in *Robinia pseudo acacia*. As a result of the investigation, they expressed that both species can be considered as indicators of air quality in contaminated sites. Achiella et al (2015) examined pollution to detect the capacity of detoxifying peroxide enzymes in *Jatropha integerrima* and *Cassia surattensis*, and found out that the CAT level was higher while the APX activity was lower in both plants compared to the unpolluted plant. Kavitha and Shailaja (2016) conducted a study to reveal the tolerance capacity to pollution by using CAT, POD and polyphenol oxidase activity in 12 plant species. Based on their results, four plant as *Ficus religiosa*, *Bauhinia variegata* L., *Acacia nilotica* and *Peltaforum ferrigoenum* showed higher resistance to air pollution.

Based on based on the amount of MDA and H₂O₂ and enzyme activity, it can be said that Cherry laurel is a tolerant variant of pollution.

The study concluded that the amount of total chlorophyll, chlorophyll b, and CAT and GPOX activities was increased in non-polluted plant but chlorophyll a, proline, total soluble carbohydrate and SOD activity enhanced heavily contaminated leaf. APX activity was the maximum level in lighter contaminated leaf samples but H₂O₂ was the minimum. All the results were considered, it can be said that Cherry laurel is resistant to pollution, and cultivation of it in the park, garden and road side can contribute to the reduction of air pollution. Apart from, it is a every green leafy species and may be more effective in reducing air pollution. Therefore, green However, further studies on the intolerance of Cherry laurel o pollution will lead to more accurate results.

References

1. **Adams III WW., Muller O., Cohu CM., Demmig-Adams B. (2013).** May photoinhibition be a consequence, rather than a cause, of limited plant productivity? *Photosynth. Res.* 117, 31-44.
2. **Agbaire PO, Akporhonor EE (2014).** The Effects of Air Pollution on Plants around the Vicinity of the Delta Steel Company Ovwian-Aladja, Delta State, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology* 8(7), 61-65.
3. **Aguiar-Silva C, Brandão SE, Domingos M, Bulbovas P. (2016).** Antioxidant responses of Atlantic Forest native tree species as indicators of increasing tolerance to oxidative stress when they are exposed to air pollutants and seasonal tropical climate. *Ecological Indicators* 63 (2016) 154–164.
4. **Alasalvar C, Al-Farsi M, Shahidi F. (2005).** Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez, *J. Food Sci* 70, 47-52.
5. **Aninbon C, Jogloy S, Vorasoot N, Patanothai A, Nuchadomrong S, Senawong T (2016).** Effect of end of season water deficit on phenolic compounds in peanut genotypes with different levels of resistance to drought. *Food Chemistry*, 196: 123-129.
6. **Arnon DI (1949).** Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, 24, 1-15.
7. **Bates LS, Waldren RP, Teare, ID (1973).** Rapid determination of free proline for water-stress studies, *Plant and soil*, 39(1), 205-207.
8. **Beauchamp C, Fridovich I (1971).** Superoxide dismutase: improved assays and an assay applicable to acrylamide gels, *Analytical Biochemistry* 44, 276-287.
9. **Bergmeyer HU (1970).** Methoden der Enzymatischen Analyse, Akademie Verlag 1,636-562.
10. **Bradford MM (1976).** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analytical biochemistry*, 72(1-2), 248-254.
11. **Caverzan A, Passaia G, Rosa SB, Ribeiro CW, Lazzarotto F, Margis-Pinheiro M. (2012).** Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. *Genet. Mol. Biol.* 35: 1011-1019.
12. **Cetin IZ, Cesur A, Keskin R, Aarsu H (2018).** Bazı Peyzaj Bitkilerinde Klorofil Miktarının Değişimi: Samsun Örneği, *Kastamonu University Journal of Engineering and Sciences* 4(1),1-10.
13. **Chaffai R, Seybon TN, Marzouk B, Ferjani E (2009).** A comparative analysis of fatty acid composition of root and shoot lipids in *Zea mays* under copper and cadmium stress, *Acta Biologica Hungarica* 60,109-125.
14. **Chance B, Maehly SK. (1995).** Assay of catalase and peroxidase", *Methods Enzymol.* 2:764-775.
15. **Chandra R, Kang H. (2016).** Mixed heavy metal stress on photosynthesis, transpiration rate, and chlorophyll content in poplar hybrids. *Forest Science and Technology*, 12 (2):55-61.
16. **Chiou TJ, Bush DR (1998).** Sucrose is a signal molecule in assimilate partitioning, *Proc Natl Acad Sci USA* 95, 4784-4788.
17. **Dat J, Vandenabeele S, Vranov AE, Van Montagu M, Inz ED, Van Breusegem F. (2000).** Dual action of the active oxygen species during plant stress responses, *Cellular and Molecular Life Sciences* 57, 779-795.
18. **Deak KI, Malamy J (2005).** Osmotic regulation of root system architecture, *Plant J.* 43, 17-28.
19. **Delauney AJ, Verma, DPS (1993).** Proline Biosynthesis and osmoregulation in plants, *Plant J.* 4,215- 223.
20. **Domingos M, Bulbovas P, Camargo CZS, Aguiar-Silva C, Brandão SE, Dafré-Martinelli M, Dias APL, Engela MR, Gagliano J, Moura BB, Alves ES, Rinaldi MCS Gomes EP, Furlan CM, Figueiredo AMG (2015).** Search for native tree species and respective potential biomarkers for future assessment of pollution effects on the highly diverse Atlantic Forests in the SE-Brazil. *Environ.Pollut.* 202, 85–95.
21. **Foyer CH, Shigeoka S. 2011.** Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant. Physiol.* 155, 93-100.
22. **Geeta C, Namrata C (2014).** Effect of Air Pollution on the Photosynthetic Pigments of Selected Plant Species along Roadsides in Jamshedpur, Jharkhand. *Res. Plant Biol* 4(5), 65-68.

23. **Ghorbanli M, Bakand Z, Bakhshi khaniki G, Bakand S (2007)**. Air Pollution Effects on the Activity of Antioxidant Enzymes in Nerium Oleander And *Robinia pseudacacia* Plants In Tehran, Journal of Environmental Health Science & Engineering 4(3), 157-162.
24. **Gupta AK, Kaur N (2005)**. Sugar signalling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants, J Biosci 30,761-76.
25. **Hörtensteiner S. (2006)**. Chlorophyll degradation during senescence. Annu. Rev. Plant Biol. 57: 55-77.
26. **Irigoyen JJ, Emerich DW, Sanchez-Diaz M (1992)**. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol Plan 84,55-60.
27. **Karadeniz T, Kalkisim O (1996)**. Investigations on selection of cherry laurel (*Prunus laurocerasus* L.) grown in Akcaabat, J. YYU Agr. Fac. 6(1),147-153.
28. **Kavitha S, Shailaja K (2016)**. Effect of Air Pollutants on Enzyme Activity of Plants. International Journal of Innovative Research in Science, Engineering and Technology, 5(8), 15791-15794.
29. **Khan AA, Malhotra SS (1982)**. Peroxidase activity as an indicator of SO₂ injury in jack pine and white birch, Biochem. Physiol. Pflanzen. 177,643-650.
30. **Konecna B, Fricand F, Masarovicova E (1989)**. Ribulose-1,5-biphosphate carboxylase activity and protein content in pollution damaged leaves of three oak species, Photosynthetica, 23(4), 566-574.
31. **Kuang Y, Zhou G, Wen D, Liu S (2007)**. Heavy metals in bark of *Pinus massoniana*(Lamb.) as an indicator of atmospheric deposition near a smeltery at Qujiang, China. Environmental Science and Pollution Research, 14, 270-275.
32. **Kumari SI, Rani PU, Suresh CH (2005)**. Absorption of automobile pollutants by leaf surfaces of various road side plants and their effect on plant biochemical constituents, Poll Res., 24(3),509-512.
33. **Long XX., Yang XE, Ni WZ, Ye ZQ, He ZL, Calvert DV, Stoffella JP (2003)**. Assessing zinc thresholds for phytotoxicity and potential dietary toxicity in selected vegetable crops. Communications in Soil Science and Plant Analysis. 34, 1421-1434.
34. **Malhotra SS, Khan AA (1980)**. Effects of sulphur dioxide and other air pollutants on acid phosphatase activity in pine seedlings, Biochem. Physiol. Pflanzen 175, 228-326.
35. **McLaughlin SB, McConathy RK, Duvick D, Mann LK (1982)**. Effect of chronic air pollution stress on photosynthesis, carbon allocation, and growth of white pine trees, For. Sci., 28, 60-70.
36. **Mittler R (2006)**. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 11, 15-19.
37. **Morsy AA, Salama KHA, Kamel HA, Mansour MMF (2012)**. Effect of heavy metals on plasma membrane lipids and antioxidant enzymes of *Zygophyllum* species, EurAsian Journal of BioSciences Eurasia J Biosci., 6(1),1-10.
38. **Nakano Y, Asada K (1981)**. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast, Plant Cell Physiol., 22(5), 867-880.
39. **North Anadolu Clean Air Center Directorate (2016)**. Kastamonu Province Air Quality Analysis Report (2010-2016). www.kiathm.csb.gov.tr.
40. **Ogagaoghene AJ (2017)**. pH level, Ascorbic Acid, Proline and Soluble Sugar as Bio - indicators for Pollution. Chem Search Journal, 8(2), 41- 49.
41. **Pearson D, Melon H, Ronald S (1976)**. Chemical analysis of Food,8th edition. Churchill Livingstone. Pp 5-63.
42. **Pimple NS (2017)**. Adverse Effect of Air Pollutants on the Chlorophyll Content in Leaves from Pune, Maharashtra (India), International Journal of Pharmaceutical Sciences Review and Research, 44(2),131-135.
43. **Pukacka S, Pukacki PM (2000)**. Seasonal changes in antioxidant level of Scots pine (*Pinus sylvestris* L.) needles exposed to industrial pollution, I. Ascorbate and thiol content, Acta Physiologiae Plantarum, 22(4),451-456.
44. **Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylides C, Havaux M (2012)**. Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc. Natl. Acad. Sci. USA, 109, 5535-5540.
45. **Rathinasabapathi B (2000)**. Metabolic engineering for stress tolerance: installing osmoprotectant synthesis pathways, Ann Bot 86,709-16.
46. **Rezanejad F (2009)**. Air pollution effects on structure, proteins and flavonoids in pollen grains of Thuja orientalis L. (Cupressaceae) Grana, 48: 205-213.
47. **Rüdiger W (2003)**. The last step of chlorophyll synthesis, in: K.M. Kadish, K.M. Smith, R. Guillard (Eds.), The Porphyrin Handbook, Elsevier Science, Amsterdam, 71-108.
48. **Sanaeirad H, Majd A, Abbaspour H, Peyvandi M (2017)**. The Effect of Air Pollution on Proline and Protein Content and Activity of Nitrate Reductase Enzyme in *Laurus nobilis* L. Plants, Journal of Molecular Biology Research, 7(1).

49. **Seyyednejad SM, Niknejad M, Yusefi M (2009)**. The Effect of Air Pollution on Some Morphological and Biochemical Factors of *Callistemon citrinus* in Petrochemical Zone in South of Iran, *Asian Journal of Plant Sciences* 8, 562-565.
50. **Shimazaki K, Sakaki T, Sugahara K (1980)**. Active oxygen participation in chlorophyll destruction and lipid peroxidation in SO₂-fumigated leaves of Spinach. *Studies on the Effects of Air Pollutants on Plants and Mechanisms of Phytotoxicity: Res. Rep. Natl Inst. Environ. Stud., Japan* 11, 91-101.
51. **Shiragave PD, Ramteke AA, Patil SD (2015)**. Plant responses to vehicular pollution: specific effect on photosynthetic pigments of plants at divider of NH-4 highway Nipani Area, Karnataka State, India. *Central European Journal of Experimental Biology* 4 (2): 1-4.
52. **Sofa A, Dichio B, Xiloyannis C, Masia A (2004)**. Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. *Plant Science* 166, 293-302.
53. **Strand Å, Hurry V, Henkes S, Huner N, Gustafsson P, Gardeström P, Stitt M (1999)**. Acclimation of Arabidopsis leaves developing at low temperatures. Increasing cytoplasmic volume accompanies increased activities of enzymes in the Calvin Cycle and in the sucrose-biosynthesis pathway, *Plant Physiol* 119, 1387-97.
54. **Strand M (1993)**. Photosynthetic activity of Scot pine (*Pinus sylvestris* L.) needles during winter is affected by exposure to SO₂ and NO₂ during summer, *New Phytol* 23, 133-141.
55. **Tingey DT, Fites RC, Wickle C (2006)**. Activity Changes in Selected Enzymes from Soybean Leaves Following Ozone Exposure, *Physiologia Plantarum* 33(4), 316-320.
56. **Tiwari SH (2013)**. Air pollution induced changes in foliar morphology of two shrub species at Indore city, India, *Research Journal of Recent Sciences*, 2, 195-199.
57. **Tripathi AK, Gautam M (2007)**. Biochemical parameters of plants as indicators of air pollution, *Journal of Environmental Biology* 28(1): 127-132.
58. **Varshney SRK, Varshney CK (1985)**. Response of peroxidase to low levels of SO₂. *Environ. Exp. Bot.*, 25, 107-114.
59. **Velikova V, Yordanov I, Edreva A (2000)**. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective roles of polyamines, *Plant Sci.*, 151, 59-66.
60. **Wali B, Bushra, Iqbal MM (2004)**. Plant growth, stomatal response, pigments and photosynthesis of *Althea officinalis* as affected by SO₂ stress. *Ind. J. Plant Physiol* 3, 224-233.
61. **Witham FH, Blaydes DF, Devli R (1971)**. *Experiments in plant physiology*. pp 55-56. Van Nostrand Reinhold Company, New York.
62. **Woo SY, Je SM (2006)**. Photosynthetic rates and antioxidant enzyme activity of *Platanus occidentalis* growing under two levels of air pollution along the streets of Seoul, *Journal of Plant Biology* 49(4), 315-319.
63. **Yasseen BT, Al-Thani RF, Alhadi FA, Abbas RAA (2018)**. Soluble Sugars in Plants Under Stress at the Arabian Gulf Region: Possible Roles of Microorganisms, *J Plant Biochem Physiol*, 6, 4.
64. **Yilmaz MT (2018)**. Effects of Cement Dust on Chlorophyll and Metabolism Products. *Kastamonu Univ., Journal of Forestry Faculty* 18 (3), 279-287., *Mehmet Akif Ersoy Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 7(1): 67-74