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Effects of Iron-Steel Factory and Dense Traffic on Leaf Chemical Compounds of Six Tree Species in Turkey

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Abstract

Aim of study: Tree planting can play an important role in improving the quality of the urban environment. On the other hand, air pollutants can affect growth and health of trees. We studied the impacts of air pollution caused by the iron- steel factory and dense traffic on leaf chemical composition and also tree tolerance to the air pollution using six tree species (Maple, Ash tree, Cypress, Black pine, Sycamore and Aspen).

Material and Methods: The fresh leaf and needle sampling was carried out in April and July, and analyzed for photosynthetic pigments, peroxidation level, hydrogen peroxide, enzymatic antioxidants and non-enzymatic antioxidants.

Main results: Although there were statistically significant variations in the studied chemical compositions of the fresh leaf and needle samples between the six tree species, the pollution types and time, each chemical factor acted different ways with the pollution types and time according to tree species. In general, the pigment values, MDA, H_2O_2 and APX, concentrations in July were lower than those in April, whereas CAT, SOD, proline and protein concentrations in July were higher than those in April. Maple tree was more resistant to the traffic pollution, while Ash tree was more tolerant to both the pollution types. Among all trees, Aspen was found more susceptible to the traffic pollution

Research highlights: The results could be used in the future research directions to improve our understanding of the role of individual tree species in air pollution reduction and also set up a healthy, well managed urban forest which can provide many ecological benefits to urban environment.

Keywords: Air pollution, Chemical composition, Dense traffic, Iron-steel factory, Forest tree.

Türkiye'de Yayılış Gösteren Altı Ağaç Türünün Yapraklarındaki Kimyasal Bileşenlere Demir-Çelik Fabrikası ve Yoğun Trafiğin Etkileri

Öz

Çalışmanın amacı: Ağaçlandırma, kentsel çevrenin kalitesinin artırılmasında önemli bir rol oynamaktadır. Ancak, hava kirliliği ağaçların gelişimini ve sağlığını etkilemektedir. Bu çalışmada; demir-çelik fabrikası ve trafik kirliliğinin olduğu yerde bulunan altı ağaç türünün (Akçaağaç, Dişbudak, Servi, Karaçam, Çınar ve Kavak) yapraklarında kimyasal bileşenleri üzerindeki etkileri ve ayrıca bu ağaçların hava kirliliğine toleransı ortaya konulmuştur.

Materyal ve Yöntem: Yaprak ve iğne yaprak örnekleri Nisan ve Temmuz aylarında toplanılmıştır ve örneklerde fotosentetik pigmentler, lipit peroksidasyon ve hidrojen peroksit seviyesi, enzimatik ve non-enzimatik antioksidant bileşikler ölçülmüştür.

Sonuçlar: Ağaçlara ait yaprak ve iğne yaprak örneklerinde kimyasal bileşenler miktarında istatistiksel olarak önemli değişimler olmakla birlikte, her bir kimyasal değer, kirletici tipi, zaman ve ağaç çeşidine göre farklı etkilenmiştir. Çalışmada, pigment, MDA, H_2O_2 ve APX değerleri Temmuz ayında daha düşüktür ancak CAT, SOD, prolin ve protein miktarı ise daha yüksektir. Akçaağaç trafik kirliliğine yüksek dayanım gösterirken, dişbudak ağacı her iki kirliliğe yüksek tolerans göstermiştir. Ağaçlar içerisinde kavak ağacı ise trafik kirliliğine duyarlı bulunmuştur.

Araştırma vurguları: Bu sonuçlar gelecekte yapılacak olan çalışmalarda ağaç türlerinin hava kirliliğinin indirgenmesindeki rollerinin anlaşılması ve sağlıklı bir şekilde düzenlenmesinde kullanılabildiği gibi kentsel çevreye yararlı ormanların yönetiminde de kullanılabilinir.

Anahtar kelimeler: Hava kirliliği, Kimyasal bileşikler, Yoğun trafik, Demir-Çelik fabrikası, Orman Ağacı

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Introduction

Increase in industrialization and motor vehicle usage has caused air pollution in all around the world, especially in crowded cities. Both pollution types result in accumulation of pollutants such as oxidized and reduced forms of carbon, sulphur and nitrogen, ozone, lead, volatile phenols, suspended particulate matter and also radioactive elements in the atmosphere (Seinfeld, 1986; Tobiszewski et al., 2018). Also they react with each other and produce the new toxic compounds. All pollutants can directly or indirectly repress plant growth and development by changing soil pH followed by solubilization of toxic salts of metals and accumulation of heavy metal in soil (Miller and McBride, 1999). Sarıyıldız, Savacı, & Aljazwei (2017) said that soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues. Trees planted in parks, gardens, near factories and roadsides are very important in the improvement of air quality in a city (Yang, McBride, Zhou & Sun, 2005). Absorbing of pollutants by foliage and accumulation of them inside their organs can help reducing the pollution level in the air. Therefore, the use of trees as bio-indicators of air quality and to maintain and determine the ecological balances are becoming more and more widespread (Oleksyn, 1988; Tripathi and Gautam, 2007). However, tree leaves are highly susceptible to the pollutants. The pollutants can form dirt layer on leaf surface and as the pollutants reach to extreme levels on leaf surface, they can reduce light penetration, prevent stomatal regulation. They can enter inside of cellular structures, and change physiological reactions of them (Halbwacts, 1984; Keller and Lamprecht, 1995). Recently, Bayhan (2016) has shown that the cement dust emissions have a negative effect on photosynthesis by covering the leaves of trees and reducing the amount of chlorophyll, carbohydrates and dry matter of the leaves. Çavuşoğlu, Kılıç, & Kılıç (2009) found that lead pollution increased the cuticle thickness for cedar and pine while it reduced the leaf diameter, vascular bundle size, stomata number, width and index. Also they noted that the stomata length decreased in cedar leaves, while it had no effect on this parameter in pine needles. Inside of tree tissues, the pollutants stimulate oxidative stress, lipid peroxidation, pigment degradation, lacking of relative water content, changing pH and inhibition of enzymes activities (Pukacka and Pukacki, 2000; Michalak, 2006). A study by Deniz (2010) showed that the air pollution by thermal power plant caused an increase in proline, total chlorophyll content, carotenoids, lipid peroxidation, SH-compounds, ascorbate and superoxide dismutase (SOD) but it reduced stomatal density. Yılmaz (2015) found that Pb, heavy metal degraded chlorophyll-a content but the amount of chlorophyll-b, chlorophyll a/b, total chlorophyll and carotenoid were destructed by Cu, heavy metal in *Bryum schleicheri*. Iqbal, Shafig, Zaidi & Athar (2015) determined that the vehicular activities induced an air pollution problem and reduced the level of chlorophyll pigments in trees which were exposed to road side pollution.

It has been noted for the pollution tolerant species that amount of enzymatic and non-enzymatic compounds such as phenolic, flavonoids, proline, ascorbate peroxidase, catalase and superoxide dismutase are increased (Michalak, 2006; Mutlu, Atıcı & Kaya, 2009). It seems that those chemicals increase the trees resistance to the pollution damages. Patykowski and Kołodziejek (2016) investigated the seasonal pattern of activity of SOD, CAT, GuPX, POD and the total protein concentration in the European mistletoe (*Viscum album* L. subsp. *album*) which were exposed to pollution with nitrogen dioxide, and they noted that the greater variations of SOD activity and total protein concentration were in Autumn, but only CAT activity was higher in late Spring. Also they have stated that the activity of SOD can mainly be used in the future as markers of the environment purity.

On the other hand, many researchers have expressed that tree reactions to the

pollution can vary with season (Pandey and Agrawal, 1994, Sharma and Tripathi, 2009). Tree species tolerance to the air pollutants can be chosen by determining biochemical compounds by considering the seasonal variations.

Turkey has an impressive richness in terms of flora, earning it a prestigious place among the three continents. Such that, with nearly 30% of its total land area covered with forests, Turkey has 11000 plant species, almost totaling the number of plant species the whole continent of Europe has. On the other hand, Turkey is a developing country. Like other developing countries, Turkey also has many environmental problems caused by rapid development. Air pollution is the most outstanding of these. Increase in industrialization and motor vehicle usage cause serious health problems and loss of life, especially in crowded cities. People have known that trees can help to reduce air pollutants for a long time. In more recent times scientific studies have quantified the amount of air pollutants removed by trees in cities. Trees planted in parks, gardens, near factories and alongside roads can be also seen in Turkey. However, the effects of air pollution on those trees or tree tolerance to the air pollution haven't studied in details in Turkey. Therefore, in this present study, we aimed at (1) studying the impacts of air pollution caused by the iron / steel factory and dense traffics on tree chemical composition with seasonal and (2) finding out the better tree species to tolerance to air pollution using six common forest tree species as Maple (*Acer negundo*), Ash tree (*Fraxinus excelcior*), Cypress (*Cupressus arizonica*), Black pine (*Pinus nigra*), Sycamore (*Platanus orientalis*) and Aspen (*Populus tremula*). Fresh leaf and needle samples were collected around iron steel factories and roadside trees in April and in July, and analyzed for their photosynthetic pigments, non-enzymatic and enzymatic compounds and level of lipid peroxidation and hydrogen peroxide.

Materials and Methods

Study area and tree leaf sampling

This study was carried out in Karabük province, northwest of Turkey (40º05'-40^{0}15'N, 32^{0}15'-32^{0}20'E) (Fig. 1). Turkey's first heavy industry factory Kardemir Karabük Iron Steel Industry and Trade Inc. is among the top 50 industrial organizations in Turkey. The Factory hours are standard, even on weekends. Surface area of Karabük province is about 4.145 km^2 and mean altitude of the city center is 280 m. Although this area generally lies within the Black Sea climate zone, but the distance from the coast prevents it from benefiting from the humid weather of the Black Sea region. Annual average temperature is 13.2 °C. Average monthly temperatures range from 23.1 ºC in July to 2.6 ºC in January and annual average temperature variation is 20.5 °C. In the province, moist mild climate with mild winter is influential with an annual rainfall of 600 mm and annual mean moisture is measured as 76%. In Karabük, 93.020 hectares of total area are used as agricultural land and 271.403 hectares are covered by forest lands, while the rest are pastures, settlements and others. However, plant vegetation is dense throughout the mountains but scarce in the residential areas and surroundings.

Figure 1. Location of the study area

In order to study the effects of air pollution arise from the iron-steel factory and dense traffic in Karabük province, fully maturated fresh leaf and needle samples were collected from 6 different tree species (*Acer negundo*, *Fraxinus excelsior*, *Cupressus arizonica*, *Pinus nigra, Platanus orientalis* and *Populus tremula*) growing near the factory (0-10 m), and from the same tree species growing along the main roads, which were internationally used by all types of vehicles day and nights. Final fresh leaf and needle samples were taken from the same tree species as control samples growing further away (about 2000-3000 m away) from the polluted area (Figure 2 and Figure 3).

Figure 2. Visual differences between ash tree leaves and Arizona cypress needles after one year.

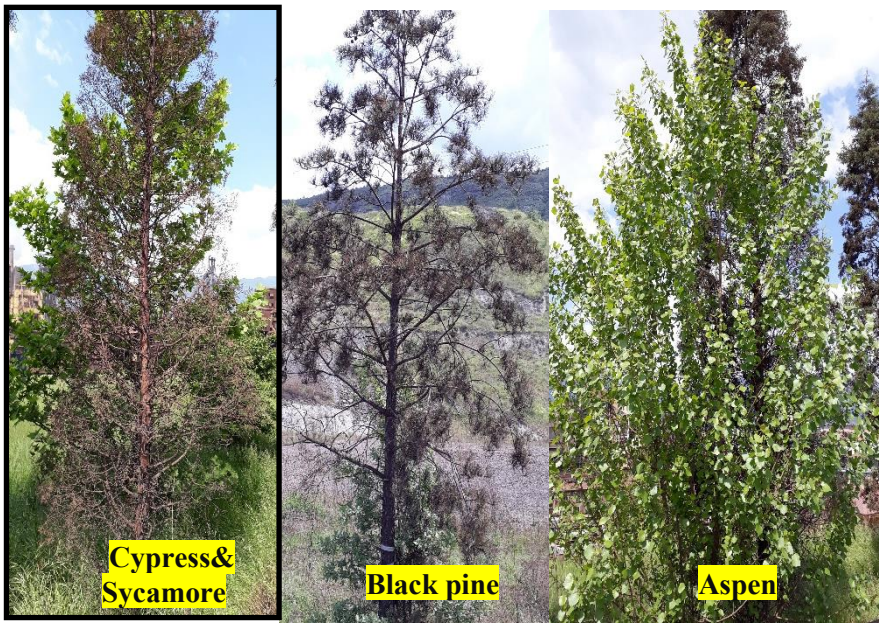

Figure 3. Some tree species (black pine, cypress and sycamore) are more heavily influenced by the air pollution than the other (aspen).

The age of the trees in the both study areas was determined by using an increment borer. Diameter measurements were made by calibrating the diameter of the standing at breast height ($d_{1.30}$). The height of the trees is measured by the The TruPulse 360 Rangefinder equipment in the areas (m). While the minimum and maximum average tree height measurements for maple samples varied between 6 m and 8 m, mean tree diameters are between 10 cm and 12 cm, and average tree ages are between 5 and 8. While the minimum and maximum average tree height measurements for cypress samples varied between 8 m and 13 m, mean tree diameters are between 12 cm and 18 cm, and

average tree ages are between 9 and 11. While the minimum and maximum average tree height measurements for ash samples varied between 8 m and 11 m, mean tree diameters are between 11 cm and 13 cm, and average tree ages are between 5 and 7. While the minimum and maximum average tree height measurements for black pine samples varied between 8 m and 10 m, mean tree diameters are between 20 cm and 25 cm, and average tree ages are between 16 and 17. While the minimum and maximum average tree height measurements for sycamore samples varied between 4 m and 5 m, mean tree diameters are between 4 cm and 6 cm, and average tree ages are between 6 and 8. While the minimum and maximum average tree height measurements for aspen samples varied between 5 m and 8 m, mean tree diameters are between 6 cm and 18 cm, and average tree ages are between 5 and 8 (Table 1).

Table 1. Silvicultural characteristics of tree species affected by air pollution.

Species	Age (year)	Height (m)	Tree diameter (cm)
Maple	5-6	6	10-11
	7-8	8	12-13
	6-7	7	10-12
Cypress	10	12-13	17-18
	11	8	13
	9-10	8-9	12-14
Ash	6	8	12-13
	5-6	12	13
	6-7	11	11-12
Black pine	17	9-10	22-25
	16	9-10	20-23
	17	8-9	22-24
Sycamore	6-7	4	4-5
2	7-8	4.5	5-6
3	6-7	4-5	5-6
Aspen	5	5	6-7
2	8	8	17-18
3	6	7	17-16

For each tree species, the fully maturated fresh leaves and needles were collected from 3 trees and combined to form a mixed sample. The fresh leaf and needle samples were carried out in April and July, with the exception of Sycamore leaf in April was not sampled due to poor leaf development. The sample trees around the factory were chosen on the basis of the wind direction, where the effect of the pollution is thought to be the highest. Dust accumulation of capacity and pollutant damage to trees depends on leaf characteristics such as surface geometry, phyllotaxy, leaf external properties and seasonal leaf development status. And also it was not possible to take a sample from the same tree in the second year due to the death of leaf, branch and whole. Therefore, the leaf sample were sampled two times as spring and summer (Katiyar and Dubey, 2001; Sharma & Tripathi, 2009). After the sampling, the dust particles on the leaves and needles were cleaned with tap water and then they were cleaned by deionized water three times. The samples were taken with the nematic dry paper and they were used for chemical analysis. All analysis was performed in triplicate.

In addition, the amount of particulate matter arisen from the iron-steel factory, traffic pollution and retained on leaves was examined according to Prusty, Mishra & Azeez, (2015). The particulate matters were collected from 15-20 pieces of leaves. Each leaf was held individually with tweezers and the particulate matter deposited on leaf surfaces was washed using a no hair-loss brush. And then, the leaves were washed using about 5mL distilled water. The entire procedure typically took less than 10 min, minimizing the water-soluble particles.

Chemical Analyses of Leaf and Needle Samples

Chlorophyll content of the leaves and needles was measured by the method of Arnon (1949). Carotenoid amount was estimated by Jaspars Formula according to the method by Witham, Blaydes & Devli (1971). Proline content was determined according to the modified method of Bates, Waldern & Teare (1973). Total soluble protein contents were determined according to the method of Bradford (1976) using the Bio-Rad assay kit with bovine serum albumin as a calibration standard. The level of lipid peroxidation products was determined and expressed as MDA content according to Lutts, Kinet & Bouharmont (1996). Hydrogen peroxide in the plant samples was determined by the method of Velikova, Yordanov & Edreva (2000). The total phenolic content of leaf samples was determined spectrophotometrically using the Folin-Ciocalteu method (Singleton, Orthofer & Lamuela-Raventós, 1999). The total flavonoid content by spectrophotometrically according to Kumaran and Karunakaran (2006).

Antioxidant of the leaf and needle 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. The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of NBT (nitro blue tetrazolium), adopting the method of Cakmak (1994). The activity of CAT was determined by Bergmeyer (1970), APX was assayed following the procedure described by Nakano and Asada (1981). APX and CAT were expressed per mg protein, and one unit represented 1 μmol of substrate undergoing reaction per mg protein per min. The particulate matter was also analyzed for element concentrations using SPECTRO brand XEPOS model XRF instrument at Central Research Laboratory at Kastamonu University.

Statistical Analysis

Analysis of variance (ANOVA) was applied for analyzing the differences in the chemical composition of the leaf and needle samples from six tree species collected in April and July and exposed to air and 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 significance.

Results

Element concentrations in the particulate matter

Mean element concentrations of the particulate matter on the leaf samples exposed to iron-steel factory pollution are shown in Table 2. As it was expected, the element concentrations in the particulate matter were found to be much higher.

Table 2. Mean element concentrations of the particulate matter on the leaf samples exposed to iron-steel factory pollution (AP) and traffic pollution (TP).

AP				TP			
	ppm		ppm		ppm		ppm
Na	55.9 ± 10.070	**Se**	48.3 ± 0.004	**Na**	1123±20	**Br**	181.82±0.26
Mg	47.9 ± 1.200	**Br**	1548.8 ± 0.122	**Mg**	5260.5±40.60	**Sr**	1.23±0.08
Al	357.4 ± 2.191	**Rb**	295.2 ± 0.016	**Al**	1203.85±8.02	**Y**	0.17±0.06
Si	2033.2 ± 5.225	**Sr**	319.9 ± 0.017	**Si**	7532.5±13.2	**Zr**	5.35±0.6
P	81.5 ± 0.089	**Y**	52.9 ± 0.003	**P**	2257.5±4.2	**Mo**	2.48±0.32
S	3029.5 ± 1.780	**Zr**	16.6 ± 0.015	**S**	5670.5±5.60	**Ag**	5.82±0.42
Cl	35324 ± 4.186	**Mo**	0.39 ± 0.001	**Cl**	2539.33±2.2	**Cd**	2.48±0.32
K	644.3 ± 1.078	**Cd**	22.1 ± 0.03	**K**	17943.33±22	**Sn**	5.82±0.42
Ca	4906.3 ± 6.582	**Sn**	49.9 ± 0.035	**Ca**	30791.67±32	**Te**	9.93±0.48
Ti	279 ± 0.215	**Sb**	4.20 ± 0.0005	**Ti**	81.83±1.11	**I**	5.73±0.72
V	71.2 ± 0.038	**Te**	23.1 ± 0.013	**V**	12.82±0.58	**CS**	21.60±2.18
Cr	20.4 ± 0.005	**I**	60.5 ± 0.029	**Cr**	7.9±0.20	**Ba**	36.10±3.36
Mn	2615.9 ± 0.501	**Ba**	329.9 ± 0.550	**Mn**	246.48±0.98	**La**	6.52±0.94
Fe	73377.6 ±25.840	**Pr**	8.41 ± 0.002	**Fe**	3601.43±6.20	**Ta**	21.28±0.72
Co	3.27 ± 0.002	**Nd**	14.5 ± 0.001	**Co**	7.50±1.42	**W**	0.67±0.08
Ni	29.6 ± 0.007	**Yb**	16.3 ± 0.003	**Ni**	14.77±0.32	**Hg**	0.53±0.04
Cu	61.8 ± 0.014	**Ta**	29.9 ± 0.013	**Cu**	12.87±0.32	**Pb**	12.15±0.26
Zn	85577.7 ± 5.070	**Tl**	58.9 ± 0.001	**Zn**	126.52±0.56		
Ga	95.9 ± 0.011	**Pb**	10691.4 ± 2.002	**Ga**	1.25±0.20		
Ge	13.2 ± 0.002	**Bi**	2151.1 ± 0.345	**As**	1.82±0.14		
As	319.4 ± 0.039			**Se**	9.63±0.12		

Variation in photosynthetic pigments (chlorophyll-a, and -b, total chlorophyll)

Mean concentrations of photosynthetic pigments (chlorophyll-a, and -b, total chlorophyll) in the leaf and needle samples of six tree species collected in April and July and exposed to air and traffic pollutions are shown in Table 3. The single effects and interactions of tree species, pollution types and sample colleting time are listed in Table 4. The main effects of species, pollution type and time on the photosynthetic pigments were all significant (P<0.001). Tree species x pollution types x time interactions were also significant for the photosynthetic pigments indicating that the photosynthetic pigments behave in different ways with the different pollution type and time according to tree species. These variations in photosynthetic pigments are explained below using mean total chlorophyll concentrations.

Mean total chlorophyll concentration in the control samples in April was in order as Aspen (0.277 mg/g) > Cypress (0.259 mg/g) > Black pine (0.185 mg/g) > Ash tree (0.171 mg/g) > Maple (0.151 mg/g). Compared to April samples, in July samples, mean total chlorophyll concentrations showed a decrease for Maple, Ash tree and Cypress, whereas it showed an increase for Black pine and Aspen. However, it showed similar order among tree species as noted in April; Aspen (0.387 mg/g) > Cypress (0.250 mg/g) > Black pine (0.248 mg/g) > Ash tree (0.105 mg/g) > Maple (0.101 mg/g).

Under the air polluted sites, all three species, except Aspen which had similar values with the control samples, showed an increase in April samples; 0.242 mg/g for Acer, 0.279 mg/g for Ash tree, 0.302 mg/g for Cypress, 0.73 mg/g for Black pine and 0.265 mg/g for Aspen. However, in July samples, compared to the control samples, most tree species showed a decrease in mean total chlorophyll concentration; 0.100 mg/g for Maple, 0.154 mg/g for Ash tree, 0.185 mg/g for Cypress, 0.216 mg/g for Black pine, 0.244 for Sycamore and 0.313 mg/g for Aspen.

Under the traffic polluted sites, in April samples, Maple, Black pine and Aspen tree species showed an increase in mean total chlorophyll concentration; 0.220 mg/g for

Maple, 0.245 mg/g for Black pine and 0.284 mg/g for Aspen, whereas Ash tree and Cypress showed a decrease; 0.156 mg/g for Ash tree and 0.246 mg/g for Cypress. In July samples; Maple, Ash tree and Cypress tree species showed an increase in mean total chlorophyll concentration; 0.112 mg/g for Maple, 0.126 mg/g for Ash tree and 0.282 mg/g for Cypress, whereas Aspen showed a decrease (0.313 mg/g). Black pine and Sycamore, however, showed similar mean total chlorophyll concentrations compared to the control sites; 0.246 mg/g for Black pine and 0.279 mg/g for Sycamore.

Table 3. Mean chlorophyll a, chlorophyll b, total chlorophyll concentrations, and chlorophyll a/b ratios in fresh leaves of 6 different tree species collected from air (AP) and traffic polluted (TP) and control (C) sites at two different times (April and July).

Tree species	Pollution Types	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Total Chlorophyll Ll (mg/g)		Chlorophyll a/b	
		April	July	April	July	April	July	April	July
Maple	C	0.136	0.088	0.016	0.014	0.151	0.101	8.6:1	6.5:1
	AP	0.172	0.073	0.070	0.027	0.242	0.100	2.5:1	2.7:1
	TP	0.161	0.092	0.059	0.020	0.220	0.112	2.8:1	4.6:1
Ash tree	C	0.124	0.086	0.048	0.020	0.171	0.105	2.6:1	4.4:1
	AP	0.165	0.113	0.115	0.042	0.279	0.154	1.4:1	2.7:1
	TP	0.114	0.099	0.042	0.028	0.156	0.126	2.7:1	3.6:1
Cypress	C	0.157	0.163	0.103	0.088	0.259	0.250	1.5:1	1.9:1
	AP	0.152	0.132	0.150	0.054	0.302	0.185	1.0:1	2.5:1
	TP	0.158	0.160	0.089	0.122	0.246	0.282	1.8:1	1.3:1
Black pine	C	0.140	0.177	0.045	0.072	0.185	0.248	3.1:1	2.5:1
	AP	0.170	0.148	0.105	0.068	0.273	0.216	1.6:1	2.2:1
	TP	0.171	0.174	0.074	0.072	0.245	0.246	2.3:1	2.4:1
Sycamore	C	n.a.	0.184	n.a.	0.096	n.a.	0.280	n.a.	1.9:1
	AP	n.a.	0.171	n.a.	0.073	n.a.	0.244	n.a.	2.4:1
	TP	n.a.	0.184	n.a.	0.096	n.a.	0.279	n.a.	1.9:1
Aspen	C	0.168	0.193	0.109	0.195	0.277	0.387	1.6:1	1.0:1
	AP	0.173	0.195	0.093	0.119	0.265	0.313	1.9:1	1.7:1
	TP	0.157	0.199	0.127	0.157	0.284	0.355	1.2:1	1.3:1

C: control, AP: Air polluted sites, TP: Traffic polluted sites, n.a.: not available

Table 4. ANOVA results for mean chlorophyll a and b data.

	Source	Type III Sum of Squares	df	Mean Square	F	Partial Eta Squared
Chlorophyll a	Tree species (TS)	,068	5	,014	4800,743***	,997
	Pollution Type (PT)	,001	2	,000	97,195***	,747
	Time (T)	,005	1	,005	1786,964***	,964
	TS x PT	,007	10	,001	234,379***	,973
	TS x T	,028	4	,007	2486,955***	,993
	PT x T	,004	2	,002	719,385***	,956
	TS x PT x T	,002	8	,000	107,664***	,929
	error					
Chlorophyll b	Tree species (TS)	,115	5	,023	5893,678***	,998
	Pollution Type (PT)	,002	2	,001	294,540***	,899
	Time (T)	,002	1	,002	556,984***	,894
	TS x PT	,018	10	,002	471,705***	,986
	TS x T	,021	4	,005	1336,445***	,988
	PT x T	,014	2	,007	1803,381***	,982
	TS x PT x T	,010	8	,001	303,459***	,974
	error	,000	66	3,92E-006		

Table 4 (continued)

Total Chlorophyll	Tree species (TS)	,336	5	,067	6430,067***	,998
	Pollution Type (PT)	,005	2	,003	239,985***	,879
	Time (T)	,014	1	,014	1326,403***	,953
	TS x PT	,036	10	,004	340,544***	,981
	TS x T	,086	4	,021	2053,278***	,992
	PT x T	,033	2	,017	1594,113***	,980
	TS x PT x T	,012	8	,002	148,339***	,947
	error	,001	66	1,04E-005		
Chlorophyll a/b	Tree species (TS)	120,237	5	24,047	925,743***	,986
	Pollution Type (PT)	24,491	2	12,245	471,404***	,935
	Time (T)	2,126	1	2,126	81,825***	,554
	TS x PT	66,578	10	6,658	256,301***	,975
	TS x T	6,835	4	1,709	65,780***	,799
	PT x T	3,276	2	1,638	63,048***	,656
	TS x PT x T	13,399	8	1,675	64,477***	,887
	error	1,714	66	,026		

*Asterisk refers the level of significance: *, P<0.05; **, P<0.01; ***, P<0.001.*

Variation in MDA and H_2O_2

Mean concentrations of MDA and H_2O_2 are shown in Table 5. The single effects and interactions of tree species, pollution types and sample colleting time are listed in Table 6. The main effects of species, pollution type and time on mean MDA and H_2O_2 were all significant (P<0.001). Tree species x pollution types x time interactions were also significant for mean MDA and H_2O_2 were indicating that mean MDA and H_2O_2 were behave in different ways with the different pollution type and time according to tree species.

Mean MDA concentration in April samples for all 6 tree species were also higher than in July samples, whereas mean H_2O_2 concentration showed different ways according to the tree species. For Maple, Cypress and Aspen, mean H_2O_2 concentration in April samples were lower than in July samples, while for Ash tree and Black pine, mean H_2O_2 concentration in April samples were higher than in July samples (Table 5). Variation in mean MDA concentration was depended on tree species and time, but mean H_2O_2 concentration in the air or traffic polluted samples were notably higher than the control samples (Table 5).

Table 5. Mean MDA and H_2O_2 concentrations in fresh leaves of 6 different tree species collected from air (AP) and traffic polluted (TP) and control (C) sites at two different times (April and July).

Tree species	Pollution Type	MDA µmol/g		H_2O_2 µmol/g	
		April	July	April	July
Maple	C	67.6	13.6	64	78
	AP	57.4	18.7	66	134
	TP	39.4	18.4	63	126
Ash tree	C	37.4	18.4	120	38
	AP	42.3	21.7	129	51
	TP	40.6	27.7	123	56
Cypress	C	45.7	15.3	48	82
	AP	64.3	10.8	44	114
	TP	49.3	19.7	56	101
Black pine	C	25.7	16.8	69	63
	AP	29.5	19.4	75	65
	TP	27.3	20.4	80	42
Sycamore	C	n.a.	22.7	n.a.	141
	AP	n.a.	22.4	n.a.	152
	TP	n.a.	25.1	n.a.	142
Aspen	C	60.7	19.6	42	128
	AP	63.7	15.2	48	146
	TP	42.6	19.5	46	122

Table 6. ANOVA results for H_2O_2 and MDA data.

	Source	Type III Sum of Squares	df	Mean Square	F	Partial Eta Squared
H_2O_2	Tree species (TS)	30430,606	5	6086,121	68782,254***	1,000
	Pollution Type (PT)	2557,457	2	1278,728	14451,539***	,998
	Time (T)	7435,877	1	7435,877	84036,515***	,999
	TS x PT	2207,322	10	220,732	2494,603***	,997
	TS x T	74526,118	4	18631,529	210564,096***	1,000
	PT x T	1640,138	2	820,069	9268,006***	,996
	TS x PT x T	3646,363	8	455,795	5151,169***	,998
	error	5,840	66	,088		
MDA	Tree species (TS)	2363,819	5	472,764	9361,798***	,999
	Pollution Type (PT)	232,319	2	116,160	2300,223***	,986
	Time (T)	17508,822	1	17508,822	346714,515***	1,000
	TS x PT	856,739	10	85,674	1696,538***	,996
	TS x T	3471,738	4	867,935	17187,079***	,999
	PT x T	996,637	2	498,318	9867,837***	,997
	TS x PT x T	943,701	8	117,963	2335,928***	,996
	error	3,333	66	,050		

*Asterisk refers the level of significance: *, P<0.05; **, P<0.01; ***, P<0.001.*

Variation in mean enzymatic antioxidant (APX, CAT and SOD)

Mean concentrations of mean enzymatic antioxidant (APX, CAT and SOD) in the leaf and needle samples of six tree species collected in April and July and exposed to air and traffic pollutions are shown in Table 7.

The single effects and interactions of tree species, pollution types and sample colleting time are listed in Table 8. The main effects of species, pollution type and time on mean enzymatic antioxidant were all significant (P<0.001). Tree species x pollution types x time interactions were also significant for mean enzymatic antioxidant were indicating that mean enzymatic antioxidant were behave in different ways with the different pollution type and time according to tree species. Mean APX concentration in April samples for all 6 tree species were higher than in July samples, whereas mean CAT and SOD concentrations in April samples were much lower than in July samples. Although variation in mean enzymatic antioxidant concentration was depended on tree species and time, in general, compared to the control samples, mean enzymatic antioxidant concentrations in the air polluted samples were much higher than in the traffic polluted samples (Table 7).

Table 7. Mean enzymatic antioxidant concentrations in fresh leaves of 6 different tree species collected from air (AP) and traffic polluted (TP) and control (C) sites at two different times (April and July).

Tree species	Pollution Type	APX EU/mg Protein		CAT EU/mg Protein		SOD EU/mg Protein	
		April	July	April	July	April	July
Maple	C	0.641	0.138	0.088	0.759	17	92
	AP	0.909	0.153	0.077	0.491	25	132
	TP	0.468	0.181	0.091	0.538	15	114
Ash tree	C	0.640	0.148	0.057	0.880	17	93
	AP	0.785	0.167	0.053	0.601	24	101
	TP	0.530	0.103	0.055	0.546	16	102
Cypress	C	0.723	0.209	0.099	0.660	17	103
	AP	0.812	0.163	0.114	0.600	26	111
	TP	0.876	0.216	0.107	0.620	22	101
Black pine	C	0.743	0.227	0.081	0.411	24	98
	AP	0.868	0.145	0.061	0.394	38	100
	TP	0.806	0.211	0.054	0.511	30	104

Table 7 (continued)

Sycamore	C	n.a.	0.266	n.a.	0.523	n.a.	71
	AP	n.a.	0.235	n.a.	0.473	n.a.	64
	TP	n.a.	0.248	n.a.	0.513	n.a.	87
Aspen	C	0.641	0.138	0.088	0.759	17	92
	AP	0.909	0.153	0.076	0.491	25	132
	TP	0.468	0.181	0.091	0.538	15	114

Table 8. ANOVA results for APX, CAT, SOD.

	Source	Type III Sum of Squares	df	Mean Square	F	Partial Eta Squared
APX	Tree species (TS)	,187	5	,037	1136,900***	,989
	Pollution Type (PT)	,028	2	,014	429,931***	,929
	Time (T)	6,026	1	6,026	182701,406***	1,000
	TS x PT	,244	10	,024	738,525***	,991
	TS x T	,217	4	,054	1646,836***	,990
	PT x T	,050	2	,025	753,527***	,958
	TS x PT x T	,267	8	,033	1011,390***	,992
	error	,002	66	3,30E-005		
CAT	Tree species (TS)	,215	5	,043	2640,719***	,995
	Pollution Type (PT)	,055	2	,028	1702,675***	,981
	Time (T)	5,782	1	5,782	355455,220***	1,000
	TS x PT	,115	10	,012	707,019***	,991
	TS x T	,140	4	,035	2152,254***	,992
	PT x T	,045	2	,022	1373,898***	,977
	TS x PT x T	,135	8	,017	1037,290***	,992
	error	,001	66	1,63E-005		
SOD	Tree species (TS)	6227,455	5	1245,491	3940,881***	,997
	Pollution Type (PT)	1125,214	2	562,607	1780,155***	,982
	Time (T)	124058,630	1	124058,630	392536,244***	1,000
	TS x PT	1988,808	10	198,881	629,282***	,990
	TS x T	6238,326	4	1559,581	4934,701***	,997
	PT x T	327,004	2	163,502	517,340***	,940
	TS x PT x T	1212,811	8	151,601	479,685***	,983
	error	20,859	66	,316		

*Asterisk refers the level of significance: *, P<0.05; **, P<0.01; ***, P<0.001.*

Variation in non-enzymatic antioxidant (carotenoid, flavonoid, phenolic, proline and protein)

Mean concentrations of carotenoid, flavonoid, phenolic, proline and protein in the leaf and needle samples of six tree species collected in April and July and exposed to air and traffic pollutions are shown in Table 9. The single effects and interactions of tree species, pollution types and sample colleting time are listed in Table 10. The main effects of species, pollution type and time on carotenoid, flavonoid, phenolic, proline and protein were all significant (P<0.001). Tree species x pollution types x time interactions were also significant for carotenoid, flavonoid, phenolic, proline and protein indicating that carotenoid, flavonoid, phenolic, proline and protein behave in different ways with the different pollution type and time according to tree species. Although there were statistically significant variations in mean carotenoid, flavonoid and phenolic concentrations among the six tree species, the pollution types and time, they acted different ways with the pollution types and time according to tree species. For example, in April samples, mean carotenoid concentration in Maple control leaves (9.63 mg/g) was lower than those in the air polluted leaves (10.5 mg/g), but similar to those in the traffic polluted leaves (9.61 mg/g). In July, mean carotenoid concentration showed a decrease, but this time, mean carotenoid concentration in Maple control leaves (4.59 mg/g) was lower than both the air polluted leave (6.13 mg/g) and the traffic polluted leaves (8.37 mg/g). Similar results

were noted for the other species and the flavonoid and phenolic concentrations (Table 9).

Mean proline and protein concentrations, however, showed more clear indications between the six tree species and time. In general, all tree species had much higher concentrations in July samples than in April samples (Table 9). However, variation in mean proline and protein concentrations among the pollution types did not show clear directions (Table 9).

Table 9. Mean non-enzymatic antioxidant concentrations in fresh leaves of 6 different tree species collected from air (AP) and traffic polluted (TP) and control (C) sites at two different times (April and July).

Tree species	Pollution Type	Carotenoid mg/g		Flavonoid µg/g		Phenolic µg/g		Proline µmol/g		Protein mg/g	
		April	July	April	July	April	July	April	July	April	July
Maple	C	9.63	4.59	2.05	2.23	6.61	9.85	4.53	15.8	79.6	150
	AP	10.5	6.13	2.34	3.86	7.00	10.5	3.18	17.6	86.5	171
	TP	9.61	8.37	2.09	3.27	7.53	9.76	4.15	18.8	71.5	166
Ash tree	C	10.8	7.20	4.32	6.39	12.2	12.6	8.93	20.8	70.5	152
	AP	11.0	9.68	4.01	10.5	12.4	8.19	10.8	18.4	93.1	159
	TP	10.0	11.1	4.31	12.3	9.66	9.30	10.4	14.2	64.4	157
Cypress	C	9.61	9.60	3.43	4.30	8.47	10.9	3.97	43.4	67.4	161
	AP	9.64	15.1	3.64	5.60	14.5	20.3	3.55	17.4	83.8	163
	TP	9.61	10.2	2.87	4.27	15.1	19.1	4.20	18.6	79.8	157
Black pine	C	10.4	11.7	3.59	4.53	16.4	15.2	10.7	28.7	73.8	153
	AP	11.5	9.11	3.98	4.48	15.3	13.0	12.1	31.5	95.8	150
	TP	11.6	12.9	4.28	6.16	17.7	24.5	11.3	17.6	87.5	156
Sycamore	C	n.a.	12.2	n.a.	7.57	n.a.	18.5	n.a.	18.1	n.a.	155
	AP	n.a.	13.6	n.a.	7.98	n.a.	27.8	n.a.	34.3	n.a.	157
	TP	n.a.	10.1	n.a.	3.90	n.a.	13.8	n.a.	25.4	n.a.	151
Aspen	C	11.0	11.2	2.53	3.28	16.5	8.82	5.49	20.4	91.8	154
	AP	9.49	11.0	3.55	2.86	16.6	6.51	4.11	17.9	81.7	165
	TP	9.71	11.0	5.05	4.58	16.5	7.30	4.74	18.0	70.4	160

Table 10. ANOVA results for non-enzymatic antioxidant data.

	Source	Type III Sum of Squares	df	Mean Square	F	Partial Eta Squared
Carotenoid	Tree species (TS)	130,479	5	26,096	1436,181***	,991
	Pollution Type (PT)	9,011	2	4,506	247,963***	,883
	Time (T)	2,873	1	2,873	158,112***	,706
	TS x PT	75,460	10	7,546	415,297***	,984
	TS x T	83,762	4	20,941	1152,463***	,986
	PT x T	15,319	2	7,659	421,540***	,927
	TS x PT x T	55,371	8	6,921	380,917***	,979
	error	1,199	66	,018		
Flavonoid	Tree species (TS)	200,288	5	40,058	1068,542***	,988
	Pollution Type (PT)	9,101	2	4,550	121,382***	,786
	Time (T)	70,721	1	70,721	1886,497***	,966
	TS x PT	72,089	10	7,209	192,297***	,967
	TS x T	85,328	4	21,332	569,035***	,972
	PT x T	8,124	2	4,062	108,358***	,767
	TS x PT x T	26,107	8	3,263	87,051***	,913
	error	2,474	66	,037		

Table 10 (continued)

Phenolic	Tree species (TS)	1237,799	5	247,560	9904,731***	,999
	Pollution Type (PT)	51,983	2	25,991	1039,905***	,969
	Time (T)	4,456	1	4,456	178,294***	,730
	TS x PT	694,215	10	69,421	2777,515***	,998
	TS x T	487,885	4	121,971	4880,003***	,997
	PT x T	17,381	2	8,690	347,693***	,913
	TS x PT x T	90,015	8	11,252	450,181***	,982
	error	1,650	66	,025		
Proline	Tree species (TS)	866,780	5	173,356	1831,161***	,993
	Pollution Type (PT)	103,974	2	51,987	549,138***	,943
	Time (T)	4713,596	1	4713,596	49789,747***	,999
	TS x PT	1227,396	10	122,740	1296,499***	,995
	TS x T	501,197	4	125,299	1323,538***	,988
	PT x T	280,415	2	140,208	1481,013***	,978
	TS x PT x T	573,113	8	71,639	756,725***	,989
	error	6,248	66	,095		
Protein	Tree species (TS)	363,271	5	72,654	4,600***	,258
	Pollution Type (PT)	1585,616	2	792,808	50,198***	,603
	Time (T)	138202,475	1	138202,475	8750,503***	,993
	TS x PT	851,045	10	85,105	5,389***	,449
	TS x T	789,276	4	197,319	12,494***	,431
	PT x T	451,793	2	225,896	14,303***	,302
	TS x PT x T	1887,348	8	235,918	14,938***	,644
	error	,002	66	3,30E-005		

*Asterisk refers the level of significance: *, P<0.05; **, P<0.01; ***, P<0.001.*

Discussion

The impacts of air pollutants on vegetation have been studied since the early part of the 20th century, with effort being focused on sulfur dioxide, oxidized and reduced nitrogen, and ozone. Many authors have also stated that pollutant uptake depends on climatic conditions and the nature and extent of damage to plants depends on the chemistry and physical characteristics of the pollutant, and also on the plant's ability to detoxify it (Kozuharova, Nedyalkova, Gergov & Simeonov, 2017; Szczepanska, Kudlak, Nedyalkova, Simeonov & Namiesnik, 2017).

This present study investigated the variation in the photosynthetic pigments, oxidative stress, enzymatic and non-enzymatic compound using 6 different forest tree species exposed to the air pollution by the iron-steel factory and dense traffic. The results have shown that the chemical composition of tree leaves also significantly vary with air pollution, but the extent of the variations depends on the tree species, pollution types and season.

It has been known that photosynthetic pigments are the photoreceptors in photosynthesis that carry out the bulk of energy fixation by some pigments as chlorophyll-a, chlorophyll-b and carotenoid. Many researchers used chlorophyll as an index of productivity of plant by photosynthesis, and so understanding the amount, composition and variation of it between different plants and by the environmental factors received much attention (Cuming and Bennet 1981). The results in this present study have indicated that the photosynthetic pigments can significantly vary with plant species, pollution types and also seasons. We found that the pigment values in July were generally lower than those in April. However, both sampling periods we found significant differences in the pigment values between the six tree species and between the pollution types. The reactions of the trees to the pollution types in April, it was seen that total chlorophyll amount increased in the samples from Maple, Ash tree Cypress, Black pine and Aspen compared to the control samples. In addition, the ratios of

chlorophyll a/b were higher in Ash tree, Cypress and Aspen (Table 3, Table 4). In July, the amount of chlorophyll-a was noted higher in Maple, Ash tree, Black pine and Aspen, but total chlorophyll increased in Maple, Ash tree and Cypress compared to the control samples. The ratios of chlorophyll a/b in July were higher than those in April. This time, the ratios of chlorophyll a/b were higher in Cypress, Sycamore and Aspen tree species (Table 3, Table 4).

In general, all results in this present study indicate that some certain pollutants increase the photosynthetic pigments as chlorophyll a, b and total chlorophyll contents, but the others decrease them. And also deposition and harmful effects of pollutant on pigment content varied according to the seasons. The results of this present study which studied the effects of pollutants by the vehicular exhausts and factory pollutions on the pigment content show similarities with the results obtained by other studies. Sharma et al. (2017) showed that total chlorophyll content varied significantly in different species, and the maximum chlorophyll was detected in *Quercus* sp. during the monsoon season. Gheorghe and Ion (2011) found that the older needles of conifers were more sensitive to NO_2, and they were more damaged in spring and summer due to destruction of thylakoid membrane. Bhattacharya, Kriplani & Chakraborty (2013) showed for six tree species that the amount of chlorophyll increased during monsoon while it decreased in winter and summer with the pollution. They also noted that the tolerant species had more pigment than the susceptible ones. In this present study, based on total chlorophyll, it was noted that Maple was more resistant to the traffic pollution, while Ash tree was more tolerant to both the pollution types. Among the all six trees species, Aspen was found more susceptible to the traffic pollution.

A number of authors stated that tolerant species maintained higher chlorophyll under polluted conditions. They also reported that deposition and harmful effects of pollutant on leaf samples varied according to the variations in seasonal parameters such as temperature level, wind direction, rainfall as well as sources of pollution, distance to them. A study by Wang, Shi & Wang (2014) showed the highest pollutant accumulated on leaf surface was in August and the lowest in November. The small sized matters were tightly adhered to leaf surface, but larger substances were washed away by rain. Anoob, Santhoshkumar & Roby (2017) showed that chlorophyll pigment amount decreased as pollution was heavier and dirt layer was highest in dry summer by the reason of long exposure time and not enough rainfall to wash the dust off the leaves. Prajapati and Tripathi (2008) showed that level of chlorophyll reduced with the increase of dust deposition and the maximum dust deposition was observed in winter by summer. On the other hand, Geeta and Namrata (2014) showed that chlorophyll-a and chlorophyll-b reduced in Industrial area in Alstonia and in Polyalthia, whereas the industrial area in Thevetia chlorophyll content increased.

As seen above, many studies have stated that air pollutants such as SO_2 and NO_2 (Hamid and Jawaid, 2009; Joshi and Swami, 2009) and the pollutants from the automobile exhaust such as the dust particles, metals and polycyclic hydrocarbons (Chauhan, 2010, Wittenberghe et al., 2013) deplete the photosynthetic pigments in plant leaves. Some authors have argued that the reduction of chlorophyll content in plant species due to pollution can be attributed to the breaking of chlorophyll molecules, blocking of biosynthesis of pigment pathway or functional enzymes activities in pathways or stimulating oxidative stress (Cakmak, 1994; Dubey and Pandey 2011). Other authors have stated that the destruction of chloroplast membrane by oxidant, the closing of stomata, the changing of the leaf sap pH, photosynthetic electron transfers and redox reactions are the other effects of the pollution (Cunyan and Xuefeng, 2014; Geeta & Namrata 2014). All these adverse effects by the pollution prevent leaf development, stimulate premature senescence and even cause death (Katiyar and Dubey 2001; Gheorghe & Ion 2011). Chatterjee, Dube, Sinha & Srivastava (2004) found that excess of heavy metals accumulation in leaf cell such as lead, iron, cadmium, and toxic compounds like SO_2, NO_2, volatiles and suspended substances

prevented the incorporation of Fe in phytoporphyrin ring of chlorophyll molecule. Langmeier, Ginsburg & Matile (1993) observed that high amount of SO_2 destroyed chlorophyll structure by restraining Mg^{2+} insertion into protoporphyrin or Mg^{2+} in the chlorophyll molecule was replaced by two atoms of hydrogen thereby changing the light-spectrum characteristic of the chlorophyll molecules. The number authors have also noted that the pollutants can stimulate mineral insufficiency/toxicity in soil (Cakmak and Horst, 1991).

The environmental contaminants may stimulate oxidative stress and elevate the level of oxygen species (ROS) which cause oxidation of proteins, enzymes, DNA and others cellular components (Palma et al. 2002, Dubey & Pandey, 2011). And also ROS increase MDA concentration due to disruption membrane structures by lipid peroxidation reactions (Fermer and Muller, 2013). But tolerant genotypes have inhibitors of lipid peroxidation reactions and detoxification mechanisms of ROS and efficiently as several non-enzymatic as well as enzymatic antioxidants. The non-enzymatic compounds consist of carotenoid, flavonoids, phenolics, proline and total soluble proteins (Takahama and Oniki, 2000, Sanaeirad, Majd, Hossein Abbaspour & Peyvandi, 2017), while enzymatic antioxidants catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), superoxide dismutase are formed which enhance the resistance of plants to pollution (Foyer and Noctor, 2011). We found that the MDA amount in July were generally lower than those in April but H_2O_2 concentration was higher in July in Maple, Cypress and Aspen. The APX activity decreased in July while the CAT and SOD activities increased significantly in July for all six tree species. The carotenoid amount did not show any significant changes, but Maple had the lowest concentrations compared to the other tree species in July. The amount of flavonoid in the leaf samples was higher in April compared to those in July, but it was low in Aspen leaves, except control leaves of Aspen in July. Total phenolic compound was higher for Maple and Cypress in July than in April, whereas for Aspen leaves, it decreased significantly compared to April. The amounts of proline and total soluble protein for the six tree species were significantly higher in July than in April. Much higher increase in proline and protein concentration was seen for Maple and Cypress in July. According to the literature, the higher activity of CAT and SOD was effective in decreasing the MDA level. A part from that, higher proline and soluble protein contents may have caused a reduction in the MDA content in July. And also being at the higher H_2O_2 concentration in April, but the lower CAT activity shows an inverse relation between the H_2O_2 and CAT activity. Those results are in line with the findings of Sanaeirad et al. (2017) who showed that the levels of proline and total protein was higher via to the nitrate reductase activity in *Laurus nobilis* growing in a polluted area, and also stated that broadleaf and evergreen plant can be used for monitoring pollution. Yan et al. (2010) for *Quercus* spp., Tiwari, Agrawal & Marshall (2006) for carrot plants and Verma and Dubey (2003) for rice seedling reported that pollutants resulted in elevating the MDA level, the destructions of pigments molecules, and reductions of cell lipid. On the other hand, many studies indicated that there was an adverse interaction between the pollution and the oxidative stress, activity of enzymes and soluble osmolytes such as proline, soluble protein and reduced sugars. They can act as inhibitors of ROS overproduction and reducing agents' injuries in tolerant genotypes under pollution, drought and heavy metals and also they play important role in maintain of osmotic potential (Sharma, Villamor & Verslues, 2011). Some authors showed that proline and soluble proteins increased in plant which contacted with the air pollutants (Mattioli, Costantino & Trovato, 2009; Sanaeirad et al. 2017) and heavy metals (Wang et al., 2014), whereas the other showed that the pollutants decreased MDA level (Sofoa, Dichioa, Xiloyannisa & Masiab, 2004). Woo, Lee & Lee (2007) noted that as long as exposure to the pollution was prolonged, the MDA and ROS production was increased, but the accumulations of proline and protein led to increasing the resistance to the pollutants. Patykowski and Kołodziejek (2016)

investigated the seasonal pattern of activity of antioxidant enzymes as well as the soluble protein by pollution from at the beginning of May to at the end of November. The effects of pollutant on chemicals varied according to duration of pollution of mistletoe leaves, and the SOD activity was higher in autumn but the CAT activity increased in late spring. Seyyednejad, Koochak & Vaezi (2013) also observed that industrial pollution increased the POD and CAT activity as well as ascorbic acid.

As non-enzymatic antioxidants, total phenolic and carotenoids have various functions in the protection of cellular components in plant species such as preventing of harmful effect of UV, photo inhibition of leaf by high light intensity, pollution, and heavy metal by chelating metals/contaminants (Michalak, 2006). Many authors reported that level of total phenolic, flavonols and also carotenoid increased with leaf maturation especial at the end of summer or autumn (Chandawat, Verma, Solanki, & Patel 2014).

Takahama & Oniki (2000), Prajapati & Tripathi (2008) showed that the amount of total phenolic and carotenoids changed under stress factories in respect to seasons. Increasing of total phenolic and falvonols due to heavy metals, SO_2, NO_2, RSPM, SPM and O_3 was also shown by Michalak (2006) and Cordoba, Marquez-Garcıa & Fernandez-Recamales (2012). Pasqualini, Cannac, Greff Fernandez & Ferrat (2007) found for pine tree that total phenolic increased after a prescribed burning from May to July. They stated that total phenolic compounds could be used as bio-indicators the impact of prescribed burning due to higher concentration in mature leaf. Muszyńska, Kałużny & Hanus-Fajerska (2014) examined heavy metal effect on total phenolic, flavonols and anthocyanins in sea buckthorn which was collected in spring and autumn. They noted that total phenolic, especially levels of phenylpropanoids and flavonols were higher in the samples growing on the waste heaps. However, the amount total phenolic and flavonols vary with the leaf age and the season.

Conclusion

Many urban areas have high concentrations of air pollution sources resulting from human activities; sources such as iron-steel factory, motor vehicle traffic, power generation, residential heating and industry. It is known that tree planting has the ability to remove air pollutants from the atmosphere. On the other hand, the pollutants can enter inside of cellular structures, and also they change morphological, physiological and cellular properties of tree leaves. The results presented in this study have shown that leaf chemical compounds such as photosynthetic pigments, oxidative stress, and antioxidants system as enzymatic and non-enzymatic molecules vary significantly with the air pollution, but the extent of the variations are dependent on the tree species, pollution types and season. In general, the pigment values, MDA, H_2O_2 and APX, concentrations in July were lower than those in April, whereas CAT, SOD, proline and protein concentrations in July were higher than those in April. All the results were considered, it was noted that Maple was more resistant to the traffic pollution, while Ash tree was more tolerant to both the pollution types. Among the all six trees species, Aspen was found more susceptible to the traffic pollution. The results provided in this study could be used in the future research directions to improve our understanding of the role of individual tree species in air pollution reduction and also set up a healthy, well managed urban forest which can provide many ecological benefits to urban environment.

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