International Journal of Plant & Soil Science
14(1): 1-13, 2017; Article no.IJPSS.29660
ISSN: 2320-7035

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Investigating the Effects of Atmospheric Gaseous Pollutants on the Vegetation of Nezahat Gökyiğit Botanical Garden in Istanbul, Turkey: A Case Study and Survey

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RT and GG conceived the original idea and contributed to designed the study. Author EDG managed the analyses of the study. Author EDG and GG performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. The final manuscript was written by authors EDG and GG. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2017/29660
Editor(s):
(1) Davide Neri, Polytechnic University of Marche - Via Brecce White, Ancona, Italy.
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(4) Dal Singh Kharat, Central Pollution Control Board, India.
(5) Anonymous, University of São Paulo, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/17563

Case Study

Received 22nd September 2016
Accepted 5th January 2017
Published 19th January 2017

ABSTRACT

We assessed the effects of gaseous air pollutants on the vegetation of the Nezahat Gökyiğit Botanic Garden in Istanbul, Turkey, based on the physiological responses of common specie *Fraxinus angustifolia* as it is the most common type of tree located all around the Garden. In order to assess the level of air quality, sulfur dioxide (SO_2), nitrogen dioxide (NO_2) and ozone (O_3) concentrations were measured. The results clearly show that the region, in general, has been

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identified as under the significant impact of NO_2. The leaf samples of *Fraxinus angustifolia* were collected during the period of plant growth months (May, June, August, September), and heavy metal content, water content and photosynthetic pigment analysis were performed in leaves tissues, such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoid amount (µg g^{-1}). Heavy metals accumulation on the leaves were observed at the locations close to the main roads.

Average total chlorophyll content observed in generally 87 ± 10 mg g^{-1} fresh weight (mean ± SD) level. A statistically significant relationship between NO_2 level and photosynthetic activity, total chlorophyll content and carotenoid has been identified. As the NO_2 level increases, increase of photosynthetic activity, chlorophyll a and b and total chlorophyll content and decrease of carotenoid levels were observed. Although the level of air pollution variations over the sampling points have been determined during the growth phase of the plant, *Fraxinus angustifolia* plant species located all around the garden showed resistance to air pollution.

Keywords: Metal; highway ambient air; higher plant; ecological monitoring; Fraxinus angustifolia; urban air pollution; photosynthetic pigments.

1. INTRODUCTION

Air pollution restricts plant growth and size and this effect varies depending on plant species, concentration and distribution of pollutant and some environmental factors [1,2]. The pollution is a complex phenomenon which presents with other factors such as light density, air temperature and water stress [3]. As temperature and drought increase stipulated by global climate change [4], it is envisaged this situation will increase other stress factors on urban plants such as air pollution, soil pollution, lack of proper growth conditions [5].

Leaves, which are the synthesis of organs of plants, are the first to suffer from long-term air pollution. The gas exchange phenomenon regulated by stomatal function in the leaves, exposes the leaves to the air pollutants [3,6]. When exposed to airborne pollutants, most plants experienced physiological changes on leaves, such as stomatal conductivity [7], chlorophyll content [8] and photosynthetic activity [5] which are used to determine the effects of pollution. Particle accumulation over the leaf depends on parameters such as the mass and size of the particle, wind speed, the direction of leaf, water content and leaf surface characteristics [9,10]. Studies especially are focused on usually one year, such as agricultural crops, few studies have been done on trees [11-14]. However, perennial plants are exposed for years to pollution and stress when compared to one-year plants and may show a significant decrease in yield without significant leaf damage [3]. Higher plants absorb not only the metals in the atmosphere but also uptake the heavy metals from the soil and compromises the biomass production. The heavy metals residues in the soils can be uptake by plants' roots and transported to other organs [15,16].

It is known that air pollution in urban and industrial environments can compromise plant growth [2]. SO_2 pollution can affect many morphological features such as a number of leaves, leaf area, shoot and root length, the number of flowers and fruits [6,17], because of increased amount of sulfur in the leaves by atmospheric SO_2 absorption [18]. Therefore, studies show that the various available plant species can be used as indicators of SO_2 pollution (e.g. *Pinus pinea, Pyracantha coccinea and Nerium oleander*) [19]. NO_2 gas directly passing from plant stomata and cuticle [20] assimilated then it is converted to nitrate and nitrite [21]. In a study with *Ricinus communis* plant in areas close to traffic pollution has been observed a higher water content in roots, stems and leaves [22]. Similarly, NO_2 treated bean plants showed higher water content in their leaves most probably due to the osmotic adjustment of the plant to the increasing levels of nitrate in the leaves [23]. O_3 as a result of photochemical reactions occurring is considered to be the most toxic air pollutants for all plants in the world. O_3 as the most important regional air pollutants in the northeastern United States and southern Europe, threaten forest health and productivity [24-26]. Leaf damages caused by ozone reported that also in the Mediterranean region which is a warm and sunny climate [27-29].

Heavy metals can cause significant decrease in physiological and biochemical activities in plant [30-32]. Heavy metals can be absorbed from both leaves and through the roots of the plants. The metals in form of gas (eg. Hg) can be absorbed by stoma [33]. Alfani [34] remark that significantly higher metal accumulation in plants grown on the roadside. Ni stress has been reported to affect photosynthetic pigments, lessen yield and cause accumulation of Na^+, K^+ and Ca^{2+} in mung bean [35]. Cu stress leads to reduced germination rate [36,37] and induces biomass mobilization a release of glucose and fructose thereby inhibiting the breakdown of starch and sucrose in reserve tissue by inhibition of the activities of alpha-amylase and invertase isoenzymes. The Cd was the most toxic metal followed by Co, Hg, Mn, Pb, and Cr. Protein content in *Zea mays* L. plants decreased from 68% to 16% in metal exposed plants [31]. Pb and Cd to inhibit root growth, the sunflower plants was determined that reduce the dry weight leaf area [38].

This study is carried out in Nezahat Gökyiğit Botanic Garden (NGBG) which is located at the intersection of heavy traffic loaded Trans European Motorway (TEM) and E5 roads in Istanbul in order to understand the effects of air pollutants over the terrestrial ecosystem. Annually on average, more than 200 000 light vehicles day^{-1}, around 30 000 heavy vehicles day^{-1} has been recorded in the closer highways [39]. It is aimed to understand the effects of gaseous air pollutants originated from the traffic emissions on the vegetation of the NGBG during the growing season (May to September). Four different sampling locations and 2 *Fraxinus angustifolia* trees located at each point within the garden were chosen for air quality monitoring and leaf sampling. In order to observe the effects of air pollution on plant physiology, photosynthesis rate, the fresh and dry weight, photosynthetic pigment (chlorophyll and carotenoid) amounts, the amounts of heavy metals in leaves were determined.

2. MATERIALS AND METHODS

2.1 Description of Study Area and Sampling Sites

Nezahat Gökyiğit Botanical Garden (NGBG) is a memorial park which has been founded in Anatolian side of İstanbul in Kücükbakkalköy on 46 hectare area with more than 50 000 different plant species and provides service since 2002.

NGBG is located at the intersection of the main highways in the region of Istanbul's heaviest traffic area which is the E-5 and TEM highways intersect (Fig. 1). These highways are main arterial roads where heavy traffic occurs each day, additionally, there is no seasonal difference in respect to vehicle numbers. Around the study area, besides of densely populated areas, there are many small and medium-sized industrial organizations. The coordinates of NGBG and the sampling stations are presented in Table 1.

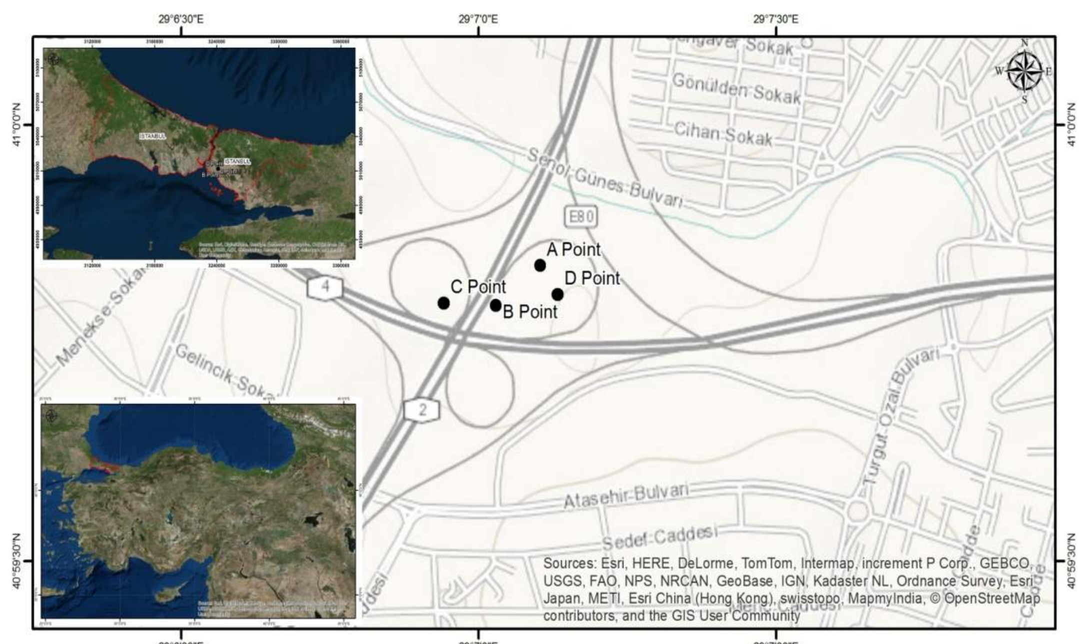

Fig. 1. The sampling locations at NGBG

Table 1. The coordinates of NGBG and the sampling stations

Station	Latitude (m)	Longitude (m)	Height (m)	Distance from traffic (m)
NGBG	40°99'61.41"	29°11'88.19"	-	-
A point	40°59'50.34"	29°7'6.18"	72 m	40 m
B point	40°59'47.59"	29°7'1.71"	74 m	19 m
C point	40°59'47.75"	29°6'56.47"	71 m	45.5 m
D point	40°59'48.35"	29°7'7.95"	81 m	80 m

2.2 Analytical Procedure

For assessing the effects of air quality on vegetation, *Fraxinus angustifolia*, was selected as the representative plant species, which exist in all four stations. Sampling and analyses were carried out during the growth season, four months (May, June, August, September) period. Two *Fraxinus angustifolia* tree in each region were selected. Three branches in these trees is labeled and leaves collected from these branches for analysis. 3 - 4 leaves from each branch collected, stored in a locked plastic bag and brought to the laboratory and keep in the refrigerator. Thus, six repeated analysis were made for each sampling location by three leaves from each tree. The trees sampled in each sampling area were selected from the same slope and green young leaves were selected from the lower branches of the tree. Thus, possible environmental factors which can cause differences in the samples physiological conditions have been eliminated.

Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll, carotenoid amount (µg mL^{-1}) and water content in leaf tissue have been determined. The standard colorimetric methods were employed for the estimation of pigment analysis. Chl_a, Chl_b and carotenoids were extracted with acetone and, pigment contents were read with the absorbance of the extract at 470, 644.8 and 661.6 nm wavelength of a UV spectrophotometer, respectively. The pigment amounts were calculated using the following equations given by Lichtenthaler and Buschmann [40].

$$Chl_a = (11.23 \times A_{661.6}) - (2.04 \times A_{644.8}) \quad (1)$$

$$Chl_b = (20.13 \times A_{644.8}) - (4.19 \times A_{661.6}) \quad (2)$$

$$\text{Total Chlorophyll} = (7.05 \times A_{661.6}) + (18.09 \times A_{644.8}) \quad (3)$$

$$\text{Carotenoid} = [(1000 \times A_{470}) - (1.9 \times Chl_a) - (63.14 \times Chl_b)] / 214 \quad (4)$$

$$\%\ \text{Water content in leaf} = [(\text{Fresh Weight} - \text{Dry Weight}) / \text{Fresh Weight}] \times 100 \quad (5)$$

Collected leaf samples from the stations were weighted in precision scales shortly after brought to the laboratory and data were recorded for fresh and dry weights. Fresh leaf samples brought to the laboratory were dried in 105 °C approximately for about 3 days in oven (WTB-Binder) and their weights before and after drying have been determined. Specific leaf weights were calculated by proportioning of dry leaf weight to leaf area (dry material amount for 1cm^2 leaf square) [18]. For leaf area measurements, the leaves were scanned and area were calculated with a computer software [39].

For elemental analysis of leaves, dry ashing technique described in EPA Method 3050B was used. Basically, in this method, a representative 1 gram dry weight sample is digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). The elements Pb, Cu, Zn, Cd, Mn, Ni, Fe, Mg and Ca were determined by FAAS method using Atomic Absorption Spectrometer PERKIN-ELMER AA800. Calibration standards Multi VI (MERCK) from 0.5 up to 40 ppm were used for different elements and accuracy of the results was demonstrated using a Certified Reference Material (beech leaves CRM 100).

Gaseous air pollutants, SO_2, NO_2 and O_3 were monitored for one week period each month by passive samplers, imported from Gradko Ltd at four locations in the NGBG during growing season. The passive sampling method used in measurements which is based on sorption of gaseous pollutants onto a the sorbet at a rate controlled by the molecular diffusion of the pollutant in the air, without requiring any pump or electrical power [41]. Each month, the samplers exposed to air for a week period. Passive sampling tubes were placed in the trees at an above of 1.5-2 m off the ground. After sampling period, the open ends of the collected passive sampling tubes were closed and sent for analyses to GRADKO firm using 0°C storage

Table 2. Meteorological character for NGBG

Months	Avg. temperature (°C)	Avg. humidity (%)	Rainy days	Avg. Wind speed (m sn^{-1})	Wind direction	Morning mixture height	Afternoon mixture height
May	18.82	53.85	2	1.6	NE	169.00	1701.63
June	24.75	60.04	-	1.9	NE	460.13	1567.38
August	25.14	61.09	-	2.7	NE	819.78	1305.78
September	18.52	73.26	-	2.04	NE	971.38	1358.88

containers. The monthly mean air quality values at four sampling locations were analyzed. All of the resulting data was performed to investigate the correlation analysis in relation to each other.

2.3 Meteorological Character

Istanbul has a humid subtropical climate that is mild with no dry season, constantly moist (year-round rainfall). Summers are hot and muggy with thunderstorms. Winters are mild with precipitation from mid-latitude cyclones. Seasonality is moderate, there is not big temperature difference between day and night or summer and winter. The average annually humidity is 70%. The humidity decreases down to 55% during the summer. The annual average temperature is 15.5℃. Generally, the hottest months are July and August, the coldest months are December and January. Provided data from General Directorate of Meteorology for Istanbul Kartal Cevizli meteorological station has shown in Table 2 above.

3. RESULTS AND DISCUSSION

In Table 3 the air quality monitoring results of SO_2, NO_2 and O_3 and measured plants physiological parameters results are given. The monthly mean concentrations of SO_2, NO_2 and O_3 measured at NGBG. These analyses values covers one week passive sampling period and took place every month once are shown in Fig. 2. The summer time SO_2 concentrations in A, B, C and D stations are lower than 11 µg m^{-3}. Observed SO_2 level is around the urban background level which shows no threat to vegetation in any sampling station.

Ground level ozone is a harmful air pollutant causing visible leaf injury on sensitive species, commonly in the form of small necrotic flecks or stipples on the upper leaf surface. Although the O_3 concentrations vary depending on the months, it was observed that they exceeded the WHO limit value of 40 µg m^{-3} at June and August months at the NGBG [42]. Ozone has not directly emitted from any source such as other air pollutants. It results from the photochemical reactions of nitrogen oxides and volatile organic compounds. As its production is highly dependent on the intensity of solar radiation, it's concentration is higher during summer months. Measured mean O_3 concentrations at NGBG were 60.54 µg m^{-3}, 43.98 µg m^{-3}, 39.41 µg m^{-3}, 55.89 µg m^{-3} at A, B, C and D locations (Table 3). A and D stations are quite higher than limit values whereas, B and C stations are around the limit value. The highest values of O_3 concentration were measured in the distant locations to highways (A and D stations). This situation is the result known as "NO_x titration" ($NO+O_3 \rightarrow NO_2+ O_2$) and such distribution was seen in many places which close to large emissions sources of NO_x such as dense traffic [43]. An inverse relationship between measured NO_2 and O_3 concentrations at all sampling points are shown in Fig. 3.

The correlation between the plant physiological parameters and air quality levels was tested (Table 4). The ozone levels measured at NGBG has a statistically significant relationship between the chlorophyll and Mg contents of the leaves. Ozone can decrease photosynthesis even at relatively low concentration [44] and this decrease is often associate with decrease of leaf chlorophyll content. It has been observed a reduction of chlorophyll content on leaf samples with increasing ozone concentration at the study area.

A statistically significant negative relation between ambient ozone levels and leaves Mg^{2+} content has been found ($r=-0.53$, $p<0.005$). It has been known that ozone affects plant physiology and consequently the cycle of nutrients [45].

Mean NO_2 concentrations measured in A, B, C and D stations vary from 33 µg m^{-3}, to 75 µg m^{-3} (Table 3). While the highest NO_2 concentration was monitored in closest heavy traffic location, B, the lowest level measured at the most distance location to traffic, D station. The measured NO_2

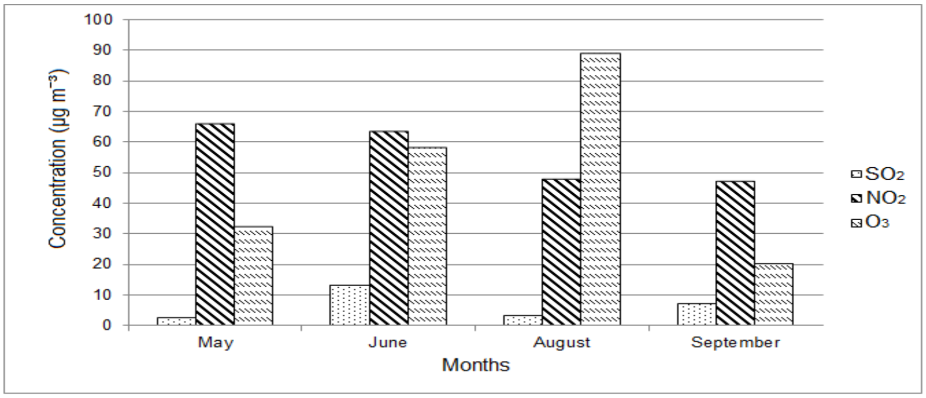

Fig. 2. Monthly mean SO_2, NO_2 and O_3 concentrations at NGBG

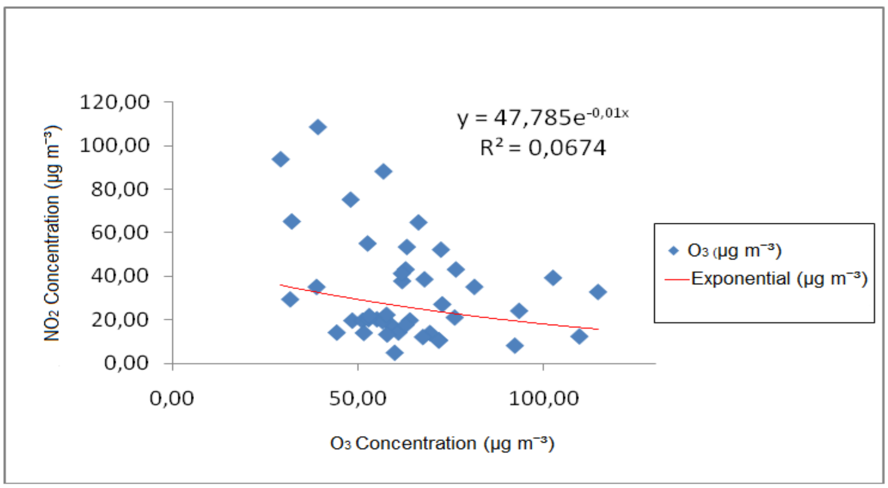

Fig. 3. Variations of O_3 and NO_2 concentrations

concentrations are higher than the annual limit value of 40 µg m^{-3} [42]. In addition to root uptake of NO_3^- atmospheric NO_2 uptake may occur directly via plant stomata [20], dissolved in the Apo plastic solution, and then quickly converted to nitrate and nitrite [21]. High levels of NO_2 can have a negative effect on vegetation depending on a number of factors, which includes: the sensitivity of the species, duation and concentration of exposure and stage of growth. Some of the effects are increase in shoot to root ratio, decreased growth, increased shoot nitrogen which leads to increased susceptibilty to pathogen and insect attack [2,6,18,21-23,46]. Higher concentration of NO_x cause acidification of cytoplasm and that cause ionic balance disorder and prevents ATP (Adenosine Triphosphate) synthesis [21].

A statistically significant correlation between NO_2 levels and the amount of total chlorophyll has been found (Table 4). With increasing ambient NO_2 concentrations, an increase in the amount of total chlorophyll content has been found (Fig. 5). Total chlorophyll amount was observed maximum at the most closest station to heavy traffic road and at the C station which is at the lowest elevation among sampling stations (Fig. 1). Nitrogen precursors located in the chloroplast biogenesis, as a result of increasing emissions of NO_2 can be connected to the accelerating assimilation of nitrogen and could be the cause of high chlorophyll content in B and C stations. The highest carotenoid amount was determined in A station; this shows that station A was resistant place against the air pollution. The decrease of carotenoid level in B station, where the highest NO_2 concentrations were observed, is the result of a struggle with air pollution. The carotenoid level was found quite low in C station, which has several characteristics in common with B station, in respect to air pollution.

Table 3. The mean concentrations of air pollutants and physiological parameters measured at leaf samples at sampling locations A, B, C and D (mean ± SD)

Parameters	A point	B point	C point	D point
SO_2 (µg m^{-3})	11.26±13.67	6.40±2.88	6.64± 3.23	3.00± 2.48
O_3 (µg m^{-3})	60.54±39.53	43.98±31.66	39.41±22.66	55.89± 29.79
NO_2 (µg m^{-3})	50.79±12.10	74.55±21.76	66.36±10.93	32.87±4.12
Chlorophyll-a (µg g^{-1})	34.18±1.55	34.71±1.92	35.51±2.32	34.78±1.32
Chlorophyll-b (µg g^{-1})	45.42±0.47*	57.81±3.32	55.99±6.01	51.91±10.70
T. Chlorophyll (mg g^{-1})	79.05±10.32*	92.56±5.07	91.51±6.59	86.69±10.93
Carotenoid (µg g^{-1})	3.57±2.68*	0.58±0.38**	1.29±1.75**	2.13±2.90*
% Water content	60.88±2.40	67.47±6.16*	63.20±0.95	61.01±2.11
Spec. Leaf weight (mg cm^{-2})	8.30±0.95*	7.89±1.07*	9.08±0.53**	9.38±0.88**
Unit area of leaflet (cm^2 $Leaf^{-1}$)	8.79±0.93*	8.03±0.94**	7.61±1.55**	6.82±0.88***
Number of leaflets	7.96±0.75	6.25±0.32*	8.71±0.75	8.75±1.14
All leaf area (cm^2)	69.14±7.88**	49.98±4.99 ***	67.27±18.67*	58.57±8 .68*
Mg^{2+} (mg g^{-1})	11.99±7.09*	13.04±7.64*	6.95±4.38**	3.22±1.8**
Fe (mg g^{-1})	69.66±89.01	58.93±50.01	48.11±24.44	48.43±38.39
Pb (µg g^{-1})	76.64±23.96	77.21±19.65	71.77±16.04	58.35±8.44*
Cu (µg g^{-1})	10.18±2.62	9.37±2.12	11.21±2.53	10.03±1.58
Cd (µg g^{-1})	2.68±0.82	2.81±1.34	2.67±1.45	3.69±2.48
Zn (µg g^{-1})	17.76±6.79*	13.72±2.94**	16.95±5.79*	22.77±5.71***
Mn (µg g^{-1})	11.49±3.83	13.05±3.27	16.35±4.29	10.91±3.23
Ca^{2+} (mg g^{-1})	4.21±3.41	5.09±3.96	6.16±3.8	7.80±5.54
Na^+ (mg g^{-1})	0.58±0.37	0.42±0.06	0.53±0.13	0.72±0.15
K^+ (mg g^{-1})	0.29±0.21	0.15±0.43*	0.34±0.26	0.47±0.43

p<0.05, level represents groups that are different from other sampling points. Similar groups are indicated by *, **, **

Table 4. Correlation with air quality data in variables with photosynthetic activity in leaf samples

	Chl_a	Chl_b	Carotenoid	T. Cl	Chl_a /Chl_b	Specific leaf weight	NO_2	SO_2	O_3
T. Chlorophyll	0.45**	0.98	-0.78				0.40		-0.23
Carotenoid		-0.83		-0.78					
Chl_a/Chl_b		-0.92	0.82	-0.82					
Water cont.%						-0.71	0.52		
Leaf Mg^{2+}	0.43**					-0.64	0.70	0.50	-0.53
Leaf Cd					0.50				
Leaf Cu	0.50								
Leaf Ca^{2+}	0.77								
Leaf K^+:Na^+			0.67						
Leaf Mn					-0.44**				0.48**
Leaf Na^+					0.44**				
Leaf Zn							-0.54		
Leaf Fe							-0.54		0.8
NO_2	0.42**	0.42**		0.47**		-0.44**			

p<0.05, level represents groups that are different from other sampling points. Similar groups are indicated by *

Photosynthetic pigments are the most affected biochemical component of the plant's due to urban especially traffic pollution [8,47]. Total chlorophyll content varied between 79 mg g^{-1} and 96 mg g^{-1} within the study areas, whereas, chlorophyll a content don't vary that much (Table 3). Maximum total chlorophyll content has been observed in the sample from B station which is the closest point to heavy traffic. Certain pollutants increase the total chlorophyll content while others decrease it [48]. Nwadinigwe [49] observed that the chlorophyll content of all plants under the air pollution impact was higher than those of the control plants. Chlorophyll content is one of the essential elements that determine the productivity of the plant. While carotenoid is a class of natural fat soluble pigment found principally in plants, where they play a critical role in the photosynthetic process and also protect chlorophyll from phot oxidative destruction. When plants are exposed to the environmental pollution above the normal physiologically acceptable range, photosynthesis gets inactivated and carotenoid content increases. An inverse relation between total chlorophyll and carotenoid content of leaves has been obtained (Fig. 6). The concentration of total carotenoids in the leaf samples from station B, near to heavy traffic and most distant station, A was recorded as 0.58±0.38 mg g^{-1} and 3.57±2.68 mg g^{-1} respectively with a reduction of 84% in leaf samples from polluted sites.

The relative water content of the leaves varied significantly within the NGBG. During the 4 months sampling period water percentage was observed as B > C > D > A (Fig. 8). Higher relative water content of the leaves is observed in B station which is located close to traffic and hence under the high NO_2 impact. It is similar to the report of Gharge and Menon [50] and Rai et al. [51], who found higher relative water content in the polluted plants than in the control plants. A high water content in a plant's body helps to maintain its physiological balance in stress conditions such as exposure to traffic related air pollution [46], when the transpiration rates are usually high. Effect of NO_2 on leafs water content is meaningful for the whole garden. That means *Fraxinus angustifolia* could show tolerant to approximately 60 µg m^{-3} NO_2 concentration.

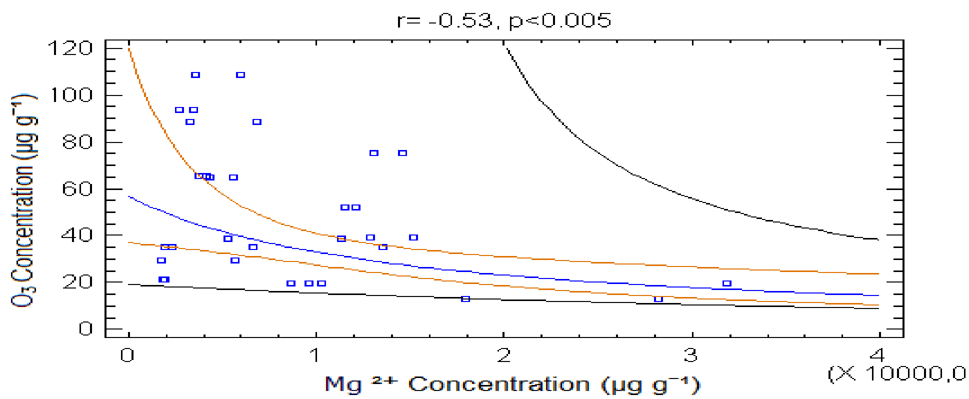

Fig. 4. Variation of Mg^{2+} concentration in the leaves with respect to ambient O_3 concentrations

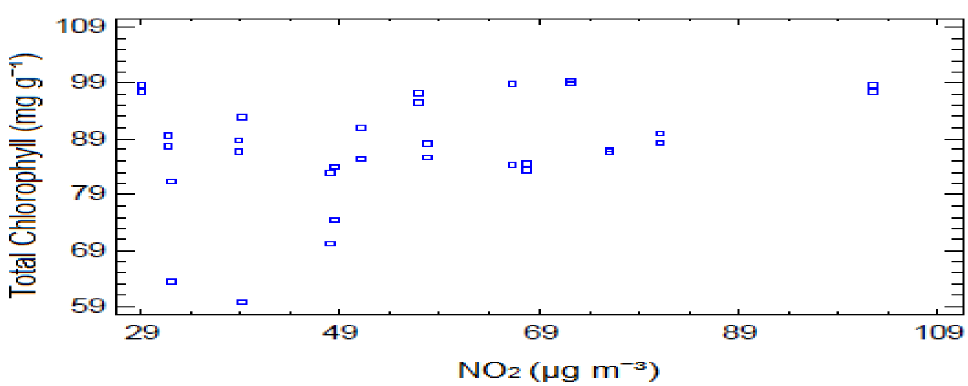

Fig. 5. Variation of Total Chlorophyll and NO_2 concentrations

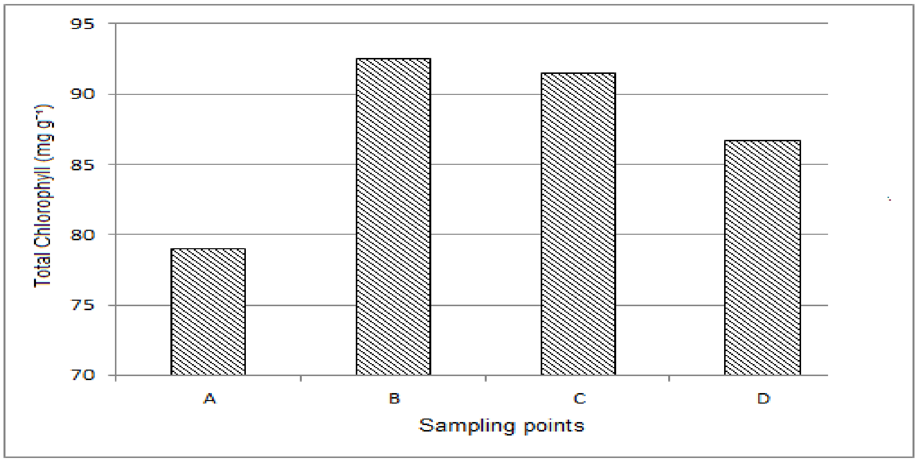

Fig. 6. Total Chlorophyll variations at sampling points

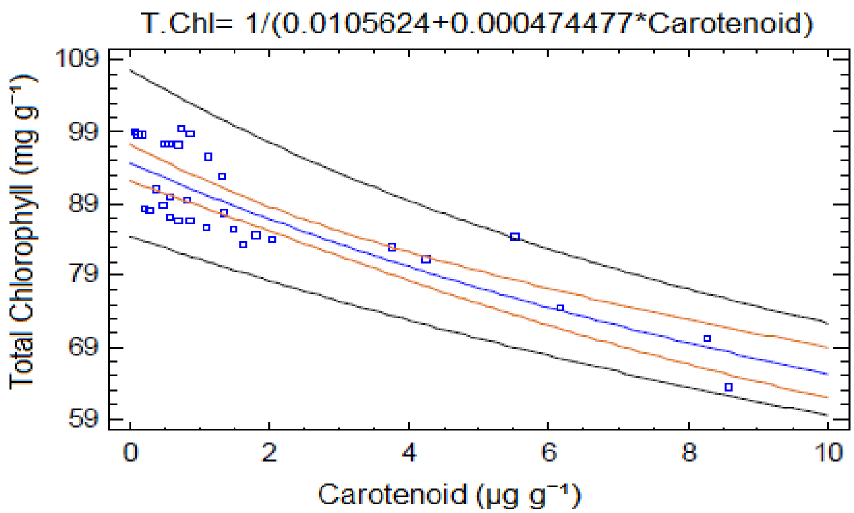

Fig. 7. Relationship between the levels of Total Chlorophyll with Carotenoid concentrations

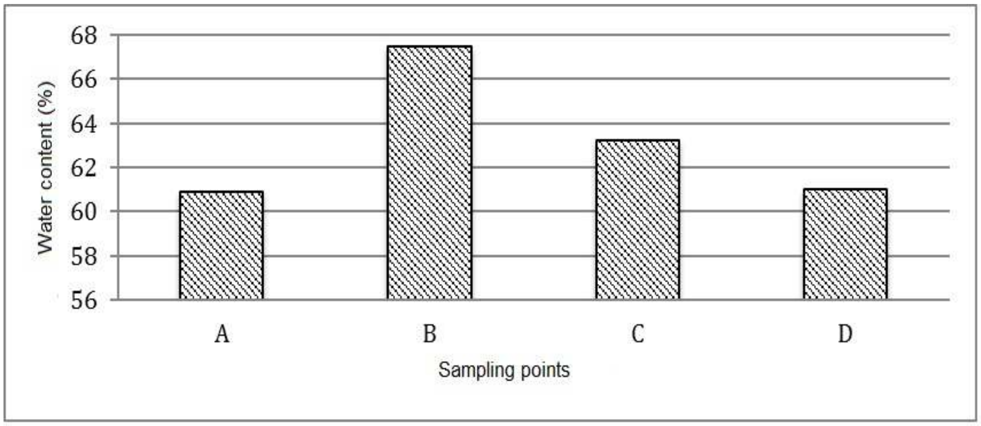

Fig. 8. The percent water content averages of leaves at sampling locations (%)

Mean leaf area (cm^2) has been calculated for 4-month periods at all stations in NGBG. The average leaf area values were found in A, B, C, D stations respectively 69 cm^2, 50 cm^2, 67 cm^2 and 58 cm^2. B station which is the closest station to heavy traffic has lower average leaf area which found meaningful as statistically compared to other stations ($p<0.001$). The decrease in leaf area of plants might be an indication of limited growth due to air pollution, probably indicate that an adaptation against pollution. The average leaflet number per leaflet has been determined respectively D (8.75), C (8.71), A (7.96) and B (6.3) stations. A low number of leaflets found statistically significant compared to other regions at station B because of high NO_2 concentrations in this region ($r=-0.55$, $p<0.001$) (Table 4).

Inorganic chemical compositions of leaves collected throughout the NGBG were investigated. Results of this study shows lower heavy metal concentrations compared to similar heavy traffic emissions impacted areas [52-54]. According to the ranking of the observed concentration of macro elements in *Fraxinus angustifolia* leaf samples is following; Mg^{2+} > Ca^{2+} > Na^+ > K^+ > Fe and the ranking of microelements has been determined; Pb > Zn > Mn > Cu > Cd. It has been found that Mg^{2+} was significantly low and Zn, Cd, Ca^{2+}, K^+ and Na^+ were higher than the other regions in D sampling station, which are expected to be affected least in terms of traffic emissions. The low Mg^{2+} content observed on plant materials of D station should be evaluated with high content K^+ and Ca^{2+} at sampling stations in the garden. As we observed in the case of a plant-available form of Mg^{2+} is absence, the plant is increasing K^+ and Ca^{2+} contents. The plant material collected from D station did not show any physiological deterioration. It is known that as the plants level of Mg^{2+} reduce down of 550 mg g^{-1}, plants will show chronic stress [55], being no physiological deterioration in D station is an expected situation. However the decreasing of Mg^{2+} level in parallel to increase of O_3 level pass under the limiting value (550 mg g^{-1}; [55]), there may be mentioned that the physiological degradation. The Zn level of leaves collected from B station is low where NO_2 concentrations were observed highest. There is a negative correlation between Mg^{2+} and Zn ($r=-0.47$ $p<0.5$) (Table 4) and Mg^{2+} and NO_2, Mg^{2+} and SO_2 and Mg^{2+} and O_3. Mg^{2+} can't be formed due to the transformation of NO_2 from O_3 in the region D. Descending Mg^{2+} is depending on increasing O_3 which increases the K^+, Ca^{2+} and Na^+ amounts of leaves. Tree leaves near the salt garden which is close to station A shows the lowest chlorophyll and highest the carotenoid contents most probably due to the accumulation of Na^+ and Cl^- particle deposition over the soil (Fig. 6).

Pb concentrations in the leaves of plants decrease up to 45 - 48% while moving away from the traffic [56]. Pb levels observed in leaves samples varies according to the sampling station. Traffic proximity relatively similar stations, A, B, and C, showed no difference in the level of Pb but the lowest level observed in D station, which is the farthest station from the traffic (Fig. 1). Cu concentration range observed in leaf samples from 3.43 to 16.53 µg g^{-1}. It is unlikely to observed a toxic effect due to Cu at this concentration level. Zn levels were observed as a mean of 17.8 µg g^{-1}. Cd level observed on the plant leaves of the region varies from 1.08 µg g^{-1} (at B station) to 7.0 µg g^{-1} (at D station) with a mean value of 2.96 µg g^{-1}. This level is considered as a high level that could be observed on plants.

4. CONCLUSION

Physiological responses of *Fraxinus angustifolia* under different levels of polluted air conditions were analyzed in a botanical garden located at the intersection of heavy traffic TEM and E5 highways in İstanbul. The level of air pollution has been determined with passive air monitoring tubes for SO_2, NO_2 and O_3 at four sampling sites which were chosen as representatives of the whole profile of the botanical garden. Along with air pollution monitoring, some physiological parameters of the leaves of *Fraxinus angustifolia*, total chlorophyll and carotenoid contents, Chl_a/Chl_b ratio, specific leaf dry weight, leaf area, percent water content and heavy metal concentrations were analyzed during the growing period. The levels of SO_2 have been in a very low range in the region so the impact of leaves has not been determined depending on SO_2. Elevated O_3 concentration is measured during summer months due to the photochemical ozone formation in this period. Reduction of Mg^{2+} content of leaves is the only interaction due to high level of O_3 measured at the distant location to traffic, Station D. Highest NO_2 concentrations measured at the B station which is located at the nearest traffic emissions. With increasing ambient NO_2 concentrations, an increase in the amount of water and total chlorophyll content and has been found. It is possible to say that the plant has developed a tolerance mechanism against high NO_2 concentrations. This is *Fraxinus angustifolia* approximately 74 mg m^{-3} mean NO_2 concentrations can tolerate. The generally decrease in leaf area, leaflet number, carotenoids, the amount of Mg^{2+} of the leaves although increased chlorophyll content and the water percentage content, Ca^{2+} and Na^{+} of the leaves which are exposed to pollution, not just an indicator of growth has slowed also probably is an adaptation against pollution. We can conclude that to *Fraxinus angustifolia* plant have endurance species in urban pollution caused by heavy traffic.

ACKNOWLEDGEMENTS

This study was supported by Ali Nihat Gökyigit Foundation. We thank Mr. Ali Nihat Gökyiğit, Mr. Prof. Dr. Adil Guner, and garden employees for support this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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