



Analysis of the physiological responses of the plane and willow trees against air pollution in Tehran

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Abstract

Trees can improve air quality, but at the same time, pollution might put them at risk. Plane (*Platanus orientalis* L) and willow trees (*Salix babylonica* L) are important trees in the green space of Tehran. Plane trees in Tehran have experienced early autumn in recent years due to severe air pollution. The present study, investigated some physiological traits of these trees under study to determine the cause of early autumn phenomenon. Three regions in Tehran were chosen for the study: Sadra Park as a clean region, Al-Mahdi Park as the polluted region 1, and Avesta Park as the polluted region 2. The results showed that the highest levels of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and anthocyanins were observed in the leaves of the plane trees of Sadra Park and the leaves of the willow trees of the Avesta Park and Sadra Park. The average concentration of lead (Pb) in the plane leaves was observed in the Avesta Park and the highest cadmium concentration was recorded for the Sadra Park while the concentration of these two metals in the leaves of willow trees in the three regions were not significantly different. This may suggest that the willow trees, by an increase in the amount of anthocyanins of their leaves, prevent the reduction of the amount of photosynthetic pigments under the influence of air pollution. By late spring, the two plants could relatively preserve similar amounts of lead and cadmium in their leaves.

Keywords: air pollution; willow (*Salix babylonica*); plane (*Platanus Orientalis* L); physiological traits

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Introduction

Air pollution is one of the most acute problems in the world. Numerous injuries from various pollutants on human health, living habitats, and a variety of living organisms have attracted scientists' and researchers' attention from all around the world. Air pollution is the presence of any contaminants such as solid, liquid,

and gas in the air in specific amounts and at specific times which put the quality of life for humans and other animals at risk or jeopardize archeological sites and properties (Joodi, 2005, Seyyednsjad et al., 2011).

Tehran, Iran's capital and the largest city, is located 90 kilometers south of the Caspian Sea in northern Iran and in the southern foothills of the Alborz mountain range. The northern part of the city has a cold weather, while the southern part has a dry and warm weather. The population

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growth in the city has turned the city into one of the most polluted cities in the world.

Urban forests and the existing trees in the urban environment can improve the air quality through filtering and absorption of gases and particles in the air, but at the same time, these trees might be at the risk of pollution (Pourkhabbaz et al., 2010; Beckett et al., 2000). Air pollution has adverse effect on plant metabolism and, besides weakening the plants, infects them with pests and diseases. The important role of plants in cleaning up the air has led to serious steps taken to preserve and enhance green spaces. However, air pollution is a major threat to plants and green spaces (Joodi, 2005, Seyyednsjad et al., 2011). Pollutants can reduce photosynthesis, efficiency of water, and plant flowers and fruits. Air pollution can furthermore lead to short stature trees (Moraghebi et al., 2012). One of the most common impacts of air pollution is the gradual disappearance of chlorophyll and concomitant yellowing of leaves, which may be associated with a consequent decrease in capacity for photosynthesis (Joshi and Swami, 2007; Seyyednsjad et al., 2011). Trees, due to their long life as living beings can be the best and most precise measurements for studying environmental stress (Ali Ahmad Koroori et al., 1999).

The best and most compatible tree in old part of Tehran is the plane trees, but the current situation in Tehran and the severely polluted air caused the plane trees to eliminate gradually. The environment for these beautiful trees, once symbol of Tehran, has become unfavorable. Plane trees belong to *Platanus* and are native to the northern hemisphere. Plane trees can have long life and suitable growth in appropriate environment. The leaves are claw-shaped and have 5-7 lobes and its achene fruit is shaped in aggregate. The wood of this tree is used in furniture and woodwork equipment (Sabeti, 1994; Mozaffarian, 1994, 2004).

One of the many species that are planted in Tehran is the weeping willow (*Salix babylonica*). Growth in this species is of short life-span between 40 and 75 years. Weeping willows are of wide usage in green spaces and parks due to their scenery and shading (Mozaffarian, 2004). In

recent years, this tree has shown less apparent changes in response to air pollution.

Cd resources are melting of metals and agricultural uses of the fertilizers and sewage sludge. Most cadmium in urban areas is left from the tire rubber of cars on the road and parking lots (Yakhkeshi, 2002). The smoke from burning oil and gas wells also contain concentrations of cadmium (Prasad et al., 2001). Cadmium also reduces the rate of photosynthesis and thus leads to transpiration and respiration. This element is absorbed through the roots and shoots of the plant.

The highest amount of lead, the toxic metal, is absorbed from the soil and is preserved in the root. The primary source of this metal includes mining and smelting of metal ores, burning of leaded gasoline, disposal of sewage and industrial waste (Gisbert et al., 2003). Symptoms of lead poisoning are similar to iron deficiency in the plant (yellowing and chlorosis). The results from the absorption of lead indicate the effect of this element on germination and plant growth, reduced biomass, chlorophyll content, and changes in the efficiency of photosynthesis (Gisbert et al., 2003).

Dos Santos Utmazian et al. (2007) showed that Plant biomass, metal tolerance and metal accumulation pattern in roots and leaves varied greatly between clones. The largest metal concentrations in leaves were detected in *Salix dasyclados* and a *Salix smithiana* clone but these species showed low metal tolerance.

Investigating the biochemical changes in the five species of trees affected by air pollution, Govindaraju et al. (2010) reported that air pollution reduces the photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids). Pourkhabbaz et al. (2010), comparing the traits of plane trees in polluted regions of the city of Mashhad with the clean area of Torghabe, showed that air and soil in urban areas have high concentration of several heavy elements and, among them, there is a huge amount of lead in the leaves of the trees in these areas.

Doganlar and Atmaca (2011) studied the effects of air pollution on urban and industrial areas, considering the amount of heavy metals and pigments in the leaves of many trees and shrubs in Antalya, Turkey. The results showed that

the amount of lead, cadmium, and zinc in the leaves was more rampant in industrial areas. Reduction was observed in the amount of pigments in both polluted urban areas and industrial zones, as compared to the clean areas.

Studying the effects of air pollution on the plant of *Holoptelea integrifolia* during the years 2008-2009 in urban, industrial and forest regions in south India, Kapoor et al. (2012) showed that the average amount of total chlorophyll, chlorophyll a and b, total carotenoids, total carbohydrates, and total protein were highest and lowest in forest and industrial areas, respectively.

In the present study by examining the changes in the content of pigments and the heavy metals of lead and cadmium in the leaves of the willow and plane trees, the two common species in Tehran, the reasons for early autumn of plane trees, together with the possible reasons for resistance of the willow trees were investigated.

Materials and Methods

Sample collection

Plane and willow leaves were collected from three parks in three different regions of Tehran On May 26, 2013, before summer drought. Using data from Tehran Air Quality Control Office Management, polluted and clean areas of Tehran were determined considering the pollutants such as SO₂, O₃, NO₂, CO₂, and PM₁₀. In order to have relatively the same geographical conditions, climate and altitude, the chosen areas were in western Tehran. Sadra Park in Sadra Town, up the Azadi Stadium, was chosen as a clean or low-polluted region, Al-Mahdi Park as the polluted region 1, located on the east Azadi Square, and Avesta Park located on Enghelab St., between Tohid and Jamalzadeh St. were chosen as polluted region 2. In each region, the plane and willow leaves of 5 bases were collected to the most possible extent of similarity from the height of 1.5 meters.

Measuring the pigments

Chlorophyll and carotenoids were measured by the method of Porra (2002). 0.25 gram of leaf tissue was homogenized in 10 ml of 80% acetone. The extract was then centrifuged for 10 min at 1500 g and the resulting absorption was

read at wavelengths of 663.6, 646.6, and 440.5 nm using a spectrophotometer (Cary 100 Bio). The content of pigments was calculated on the basis of micrograms per gram using the Porra (2002) equation.

The content of anthocyanins was calculated by the method of Mancinelli et al. (1988). 0.25 g of powdered leaves was extracted with 20 ml of cold methanol acid (HCl 1% V / V) for one day. The extract was centrifuged for 15 min at 6000 rpm. The absorption of the filtrated extracts at 530 nm and 657 nm was measured using spectrophotometer (Cary 100 Bio). In order to calculate the anthocyanin content of the extracts, the following formula was used:

$$A = A_{530} - (0.25) A_{657}$$

A = absorbance of anthocyanin

A₅₃₀ = extract absorption in 530nm

A₆₇₅ = extract absorption in 657nm

Measuring the heavy metals

Ten grams of fresh leaves, from willow and plane trees were kept at 75 °C in an oven for 72 hours Dried leaves and also soil samples were collected from three regions using the Digesdahl machine and Pichtel and Bradway (2008) method with the help of sulfuric acid 98% and hydrogen peroxide 30%. Finally, using the spectrophotometry method through the ICP (Atomic Absorption/AAS/England/PG-990) the concentrations of lead and cadmium were determined in leaf extracts and soil samples. Also, by transporting soil to soil laboratory, the physicochemical properties and the tissue of the obtained soil were recorded from 10 cm above the soil surface of the three regions.

Statistical analysis

The experiment was performed in a completely randomized design with 5 replicates and statistical analysis of the data was done using SAS 9.1. Variance analysis was conducted through the Two Way ANOVA method and the mean comparison was performed based on Duncan's multiple range test.

Results

Pigments and heavy metals in plane leaves

The study of the effect of air pollution on the plane leaves pigments (Table 2) showed that the pigments including chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and anthocyanins, in different regions were significant ($P \leq 0.01$). The difference for lead and cadmium concentrations was also significant ($P \leq 0.05$). According to Table 3, the highest amount of all pigments in plane leaves was observed in Sadra Park. The highest amount of chlorophyll (366±20.3 mg/gfw) was related to Sadra Park and the lowest amount was observed in the plane leaves in Avesta Park (182±20.7 mg/gfw). The highest amount of chlorophyll b (1149±58 mg/gfw) was observed in the plane leaves of Sadra Park which is significantly different with the lowest amount (408±84.5 mg/gfw) in Avesta Park (Table 3). Moreover, the highest total chlorophyll (2508±148 mg/gfw) was recorded for the plane leaves in Sadra Park and the lowest amount was related to the plane leaves in Avesta Park (899±146 mg/gfw).

For the amounts of carotenoids, the highest level was recorded in the clean area of Sadra Park (Table 3). The highest absorption of anthocyanins (0.0215±0/10) was also related to Sadra Park.

The maximum mean lead concentration (1.07±0.124) ppm was observed for the plane leaves in Avesta Park, which is significantly higher than that of the plane leaves in Al-Mahdi Park with the lowest lead levels of 0.972±0.117 ppm. The highest amount of cadmium (0.170±0.124) ppm was observed in Sadra Park that was significantly different ($P \leq 0.05$) with that of Al-Mahdi Park (0.157±0.128) ppm (Table 3).

Pigments and heavy metals willow leaves

According to Table 4, in the leaves of the willow trees the difference in the amount of pigments including chlorophyll b, carotenoids, and anthocyanins in different regions were significant ($P \leq 0.01$). The chlorophyll a and total chlorophyll contents were significantly different ($P \leq 0.05$). The difference in the amounts of lead (Pb) and Cadmium (Cd) was not significant.

The comparison of the mean values of pigments in the leaves of willow trees (Table 5) shows that the maximum chlorophyll a content

(206±20.51 mg/gfw) was observed in Sadra Park which is significantly different with its lowest content (144±7/15 mg/gfw) in the willow leaves of Al-Mahdi Park. The highest amount of chlorophyll b (614.2±41.55 mg/gfw) was recorded for the willow leaves of Avesta Park and this is not significantly different from the amount of chlorophyll b in the leaves of willow trees in Sadra Park. However, it is significantly different from its minimum content in Al-Mahdi Park (406.2±47.51 mg/gfw). The highest amount of total chlorophyll (1406/8±118 mg/gfw) was recorded in the willow leaves of Avesta Park that is not significantly different from the amount of total chlorophyll in the willow leaves of Sadra Park (1236±110 mg/gfw). The lowest amount of total chlorophyll was observed in the willow leaves in Al-Mahdi Park (898.3±51.40 mg/gfw).

The highest level of carotenoids was also observed in Avesta Park (2021.3±154.5 mg/gfw) that shows no significant difference with that of the willow leaves in Sadra Park (1842/5±138 mg/gfw). The lowest amount of carotenoids on the other hand was found in the willow leaves of Al-Mahdi Park (1303.5±29.95 mg/gfw) (Table 5).

The highest amount of anthocyanins absorption (0.0272±0/004) also was observed in the willow leaves of Avesta Park while the lowest level (0.0071±0) was obtained from the willow leaves of Sadra Park and this is significantly different from the samples from Avesta Park. This is not significantly different from what was recorded in the samples of Al-Mahdi Park (Table 5).

There was no significant difference between the mean concentrations of lead and cadmium in the three regions. Since willow trees are considered as the super absorbents of heavy metals, they have absorbed the highest levels of lead in all three regions as compared to the plane trees, but no difference was observed among the willow trees of the three regions.

Table 1
Physical traits of soil and the amount of lead and cadmium based on ppm in the soils of the three regions

Region	Clay	Sand	Silt	pH	EC	Pb ppm	Cd ppm
Sadra Park	38%	28%	24%	7.4	0.73	1.204	0.197
Al-Mahdi Park	24%	40%	36%	7.7	0.80	1.33	0.0205
Avesta Park	16%	64%	20%	7.6	0.76	0.649	0.076

Table 2
Variance analysis of the pigments and heavy metals in the leaves of plane trees

Resources of change	degree of freedom	chlorophyll a	chlorophyll b	total chlorophyll	carotenoids	anthocyanins	lead	cadmium
Treatment	2	36479 **	551817 **	2697233 **	4123832 **	0.00021 **	0.00985 *	0.000175*
Error	9	1607	20753	75976	152095	0.0000128	0.001841	0.000052
Coefficient of Variation	-	13.84	18.84	17.13	15.99	24.09	4.22	4.47

* and ** = Mean Squares are respectively significant at 5 and 1 percent.

Table 3
Comparison of pigments in the leaves of plane trees in the three regions

Factors	chlorophyll a mg/gFW	chlorophyll b mg/gFW	total chlorophyll mg/gFW	Carotenoids mg/gFW	Anthocyanins absorption	lead ppm	Cadmium ppm
Sadra Park	366±20.3 a	1149±58 a	2508±148 a	3568±124 a	0.021±0.102 a	1.005±0.256 ab	0.170±0.124 a
Al-Mahdi Park	319±19 a	736±70.5 b	1417±116 b	2145±191.5 b	0.016±0.001 a	0.972±0.117 b	0.157±0.128 b
Avesta Park	182±20.76 b	408±84.5 c	899±146 c	1602±248.5 b	0.0069±0.0005 b	1.07±0.124 a	0.160±0.065 ab

The mean treatments that have similar letters, according to Duncan test, are not significantly different at 5% level of probability.

Table 4

Table of variance analysis of the pigments and heavy metals in the leaves of the willow trees in three regions

Resources of change	degree of freedom	chlorophyll a	chlorophyll b	total chlorophyll	carotenoids	anthocyanins	lead	cadmium
Treatment	2	4489*	5552 **	267924 *	556750 **	0.00042 **	0.00985 ns	0.00017 ns
Error	9	1214	6892	38687	69789	0.000023	0.00184	0.00005
Coefficient of Variation	-	19.03	15.31	16.66	15.33	28.88	4.22	4.47

* and ** = Mean Squares are respectively significant at 5 and 1 percent.

Table 5

The comparison of the pigments and heavy metals in the willow trees of the three regions

Factors	chlorophyll a mg/gFW	chlorophyll b mg/gFW	total chlorophyll mg/gFW	Carotenoids mg/gFW	Anthocyanins absorption	lead ppm	Cadmium ppm
Sadra Park	206±20.51 a	606±34.42 a	1236±110 a	1842.5±138 a	0.007125±0.00 b	1.12±0.007 a	0.170±0 a
Al-Mahdi Park	144 ±7.157 b	406.2±47.51 b	898.3±51.40 b	1304.5±95.29 b	0.013063±0.00 b	1.17±0.053 a	0.167±0.004 a
Avesta Park	197.75±20.3 ab	614.2±41.55 a	1406.8±118 a	2021.3±154.5 a	0.027250±0.004 a	1.15±0 a	0.167±0.002 a

Discussion

Plane trees have had significant changes in the amount of pigments in a way that the impact of air pollution in Avesta Park has led to the reduction of total chlorophyll, carotenoids, and anthocyanins pigments. This is in line with the results of Govindaraju et al. (2010). Salama et al. (2011) showed that air pollution reduces the concentration of chlorophyll in *Datura innoxia* Mill plant. These results are consistent with the results of the present research.

Willow trees in the polluted region of Al-Mahdi Park had the lowest amount of chlorophyll and carotenoids pigments and the polluted region of Avesta Park and clean region of Sadra Park had the highest amounts. Perhaps the willow trees in Avesta Park by increasing the amount of anthocyanins in their leaves tried to stop the decrease in photosynthetic pigments. It is possible that plane deficiency in raising the amount of anthocyanins against air pollution has caused the reduction of its resistance. However, under the influence of air pollution the photosynthetic pigments are decreased and these results are consistent with the results reported by Kapoor et al. (2012) and Doganlar and Atmaca (2011).

It is probable that the different responses of the two plants in the two polluted regions go back to the different amount and different types of the pollutants in Azadi Square and Enghelab St., or it might be the differences in their genus. The wide surface of the plane leaves together with the trichome on them exposes them to more pollutants. Heavy dust coating in heavily polluted areas may block stomata (Bacic et al., 1999) and abrade leaf surface, and may even bury organisms and photosynthetic organs through physical or chemical reactions (Grantz et al. 2003, Cui et al., 2006).

The highest amount of lead were accumulated in plane leaves of Avesta Park and the highest amount of cadmium were accumulated in the plane leaves in Sadra Park, while the lowest amount of these two elements were observed in Al-Mahdi Park where the given results of cadmium disagrees with the results by Pourkhabbaz et al. (2010) and Doganlar and Atmaca (2011). The study by Doganlar and Atmaca (2011) indicated that higher Cd^{2+} concentration was observed in *Platanus orientalis* L. and *Nerium*

oleander L. in the urban and roadside plant leaves as compared to control which were collected from the city. The most obvious use of cadmium is in vehicles brakes. With regard to this fact that Sadra Park is located alongside the exit zone of Azadegan Highway, it is thus possible the drivers used more severe braking. This can be a justification for the high amount of cadmium in this park. Pirzada et al. (2009) documented that Pb^{2+} concentration in soil decreased with increasing distance from roadside. Considerable increase in Pb^{2+} and Cd^{2+} was reported in urban industrial areas of North Central India (D'Souza et al., 2010).

By comparing the amount of lead in the leaves and in the soil, and considering that lead is of low mobility, it seems the high amount of lead in plane and willow leaves that often exists more than the soil under these plant is because of the existence of this element on the surface of these leaves. The amount of cadmium in the soil of Sadra Park region was more than the two other regions, but the amount of this metal in the leaves of these two plants was less than that in the soil, while in two other regions this was more than that in the soil, which might be due to the mobility of this element.

French et al. (2006) showed that the absorption of lead was relatively low and cadmium was high in the willow leaves due to lack of physical mobility of lead and mobility of cadmium in the soil; Dos Santos Utmazian et al. (2007) showed that the concentration of cadmium in *S.rubens* and *Salix babylonica* were more in the roots than the leaves; therefore, there is little transfer of this metal to the shoots of these two species of willow; it is thus accumulated in the roots. In the present study, lack of transfer of these heavy metals could be a probable reason for no difference in the concentration of the heavy metals in the willow leaves of the three regions under study.

In a study carried out by Yanqun et al. (2004) on a lead and zinc mine in China, the coefficient of bioaccumulation of metals under study was recorded as $Zn > Cd > Cu > Pb$ in plants around the mines and in *Salix cathayana* the coefficient of bioaccumulation was higher than 1, qualifying it as a Hyperaccumulator plant.

By studying the effect of the pollution of heavy metals such as lead and cadmium, resulted

from the vehicles on certain roadside species, up to a distance of 50 meters in Pakistan, it was found that the concentration of these metals in the leaves of plants and the soil is reduced as they get distance from the road;. Moreover, the photosynthetic proportion is reduced under the influence of the lead and cadmium pollution, which is consistent with the results of the present study on plane trees (Nawazish et al. 2012). Pb²⁺ and Cd exhibited decrease in chlorophyll contents and photosynthetic activity in two wheat varieties (Oencil et al., 2000). Sunghyun and Hojeong (2011) observed that increase in CO₂ as compared with an increase in lead content in soil is has more effects on *Pinus densiflora* metabolic activities.

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