Carpathian Journal of Earth and Environmental Sciences, November 2014, Vol. 9, No. 4, p. 149 - 156

MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHANGES INDUCED BY ATMOSPHERIC POLLUTANTS ON *POPULUS X CANADENSIS* MOENCH FROM IAŞI CITY AREA

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Abstract: Urban air pollution has become a serious environmental problem to trees and crops. This study was conducted in order to assess the impact of air pollutants on some physiological, morphological and physiological parameters of the *Populus x canadensis* Moench cultivated for ornamental purposes across the five air quality monitoring stations in Iaşi city area. This stations are equipped with analyzers for determination of gaseous pollutants (SO_2, NO_2, ozone), and solid pollutants (PM10). Measurements were made *in vivo*, as well on fresh material measured in different phenological stages, in the months May, July and September, 2012. The most significant response reactions to pollutants was showed by the individuals of *Populus x canadensis* grown at the site of the traffic station Podul de Piatră where SO_2 and particulate solids in suspension are the predominating pollutants with higher values. The SO_2 level was 4.09 µg/m^3 in May, 3.48 µg/m^3 in July, 4.95 µg/m^3 in September. The PM10 gravimetric level was 48.91 µg/m^3 in May, 39.98 µg/m^3 in July, 44.57µg/m^3 in September. The air pollution around Podul de Piatră traffic station lead to a significant leaf area reduction of 72.04% in May, 48.46% in July and 48.46% in September. A photosynthetic rate reduction was observed by 43.95% in May, 43.91% in July and 43.92% in September, a transpiration reduction by 84.23% in May, 77.51% in July and 55.15% in September and stomatal conductance reduction by 29.82% in in May, 26.47% in July and 16.66% in September. Also the amount of chlorophyll a was reduced by 37.04% in in May, 37.34% in July and 62.18% in September and the chlorophyll b was reduced by 40.81% in in May, 36.79% in July and 18.55% in September. The study highlights that the emitted pollutants by the traffic affect the individuals of *Populus x canadensis* Moench.

Keywords: *Populus x canadensis* Moench, atmospheric pollutants, morphological changes, physiological changes

1. INTRODUCTION

Often, talking about human impact on the environment, there are references to pollution. Human assault on the environment consists in various forms of pollution. Environmental pollution with different mineral substances and heavy metals is a global problem (Tomaškin, 2007; Secu et al., 2008; Lăcătuşu & Lăcătuşu, 2008).

Air pollution in urban areas is stronger and here in some industrial areas or in areas with intense traffic. Urban air pollution has become a serious environmental problem to trees and crops (Chauhan & Joshi, 2008). Pollutants are factors that produce phytotoxic effects in all plant organs. Another specific problem occurs in cities where various industrial plants ceased operations. The result was mosaic pollution on the one hand and the existence of complex polluted brownfields. This is the situation of cities such as Iaşi (Secu et al., 2008, Iancu & Buzgar, 2008).

The literature is replete with papers on the assessment of damage caused by air pollutants through the biochemical and physiological changes that occur in stressed plants. Affected plants show some common effects such as chlorosis, necrosis and inhibition in photosynthesis and decreasing plant growth (Davison & Blakemore, 1976).

When exposed to airborne pollutants, most plants experienced physiological changes before exhibiting visible damage to leaves (Liu & Ding, 2008). Pollutants can cause leaf injury, stomata damage, premature senescence, decreased photosynthetic activity, disturb membrane permeability and reduce growth and yield in sensitive plant species (Tiwari et al., 2006). Reducing leaf area and number of leaves may be due to the rate of productivity foliar and early senescence induced by pollutants. (Tiwari et al., 2006). Stress caused by air pollution leads to stomata closure, which reduces the availability of CO_2 in leaves and inhibits carbon fixation. Net photosynthetic rate is a measure commonly used in the study of the impact of air pollutants on woody species (Woo & Je, 2007). Numerous studies have revealed the effect of the gradual disappearance of chlorophyll and, along with it, yellowing leaves and reduce photosynthesis (Joshi & Swami, 2007).

Several studies have shown the impact of automobile exhaust on roadside vegetation throughout their visible and non visible effects (Joshi & Swami, 2007). Shading effects due to deposition of particulate matter on the leaf surface could be responsible for lowering the concentration of chlorophyll in the polluted area. At the same time this leads to blocking of stomata, thus interfering with gas exchange, leading to increase in temperature that can delay leaf chlorophyll synthesis. Solid powder which coates the leaves lead to reduced photosynthesis and causes the reduction of chlorophyll pigments quantity, (Joshi & Swami, 2009).

In this paper we present morphological (foliar surface normal and necrotic), physiological (photosynthetic and transpiration processes intensity and biochemical (water content and dry matter content of photo-assimilating pigments), changes induced at foliar level by some pollutants in samples of *Populus x canadensis* Moench cultivated for ornamental purposes across the five air quality monitoring stations in Iasi city area. The pollutants monitored were gaseous (sulphur dioxide, carbon dioxide, nitrogen dioxide, ozone) and solid (powder prone to sedimentation).

2. MATERIALS AND METHODS

2.1. The study area

The study was done in 2012 in Iaşi city in the locations of the five air quality monitoring stations (Fig.1). The data were recorded in May, July and September performing alongside field observations. The Botanical Garden of "Alexandru Ioan Cuza" University, which is not subject to pollutant emissions, was considered for the reference values. The climate of Iaşi city is temperate continental, influenced by air masses with eastern origin, frosty winters and hot summers (Erhan, 1979). According to Meteorological Science Center the annual average temperature is 15.6ºC. The phenomenon of thermal inversion in Iaşi area is about 20%, during which air pollutants stagnate near the underlying surface, air and soil temperature decreases (Erhan, 1979).

Annual rainfall regime in Iaşi is continental, characterized by the existence of a single maximum in June (90.4 l/m^2) and a single minimum in February (28.2 l/m^2) (Meteorological Science Center). Relative humidity in the air, being inversely associated with temperature, is low (74%) with a mean amplitude of 24% that is specific to the continental climates.

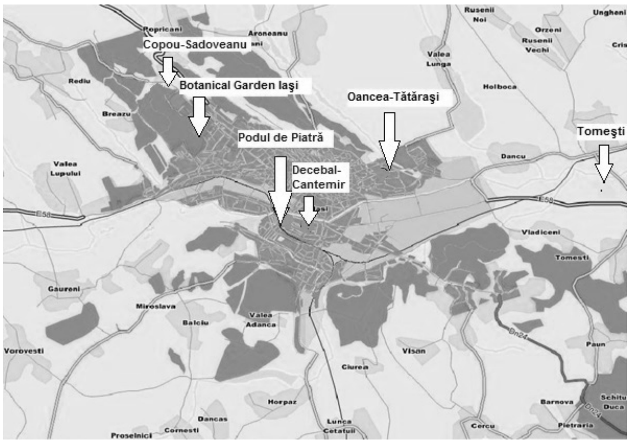

Figure 1. The location of the studied area (modified after Erhan, 1979)

2.2 Plant material

The measurements were conducted on Canadian poplar (*Populus x canadensis* Moench) cultivated for ornamental purposes in Iaşi city area. Collection and measurement "in vivo" were made on leaves situated at the edge of the canopy, of the four cardinal points of each individual, at a distance of 4-5 m above the ground. The height above ground at which were mounted the air quality monitoring station`s analyzers was taken into account because of its relevance.

2.3. Environmental measurements

The climatic factors like temperature, relative humidity and rainfall, influencing physiological processes on plants, can induce the opening of stomata, resulting in the pollutant gases to enter into the plant body. The climatic data of Iaşi city were obtained from the Meteorological Science Center (Table 1). Air quality data were obtained from the automatic monitoring network of air quality in the city of Iaşi, (Table 2).

Table 1. Climatic parameters obtained from Meteorological Science Center at Iaşi City during May, July and September 2012.

Month	Maximum air temperature (° C)	Minimum air temperature (°C)	Average (° C)	Precipitation (Σ, mm)
May	26.7	11.8	19.2	101
July	30.9	14.9	22.9	14.9
September	27.8	12.3	20.1	50.7

Table 2. The averages of the measured air pollutants obtained from the automatic monitoring network of air quality in the city of Iaşi during May, July and September 2012

Area	Pollutant	Monthly average µg/m^3		
		May	July	September
PODUL DE PIATRĂ	SO_2	4.09	3.48	4.95
	NO_2			
	PM10 auto	40.18	31.45	50.70
	PM10 gravimetric	48.91	39.98	44.57
	Benzene	3.20	1.81	4.41
DECEBAL CANTEMIR	SO_2	3.26	3.63	3.80
	NO_2	19.01	17.88	23.67
	PM2.5 gravimetric	18.35	15.7	19.14
	Benzene	-	-	-
OANCEA TĂTĂRAŞI	SO_2	3.3	3.53	3.76
	NO_2	15.6	11.62	16.97
	PM10 auto	10.40	15.30	11.15
	Ozone	59.23	78.82	49.20
COPOU SADOVEANU	SO_2	3.05	5.06	3.29
	NO_2	5.12	7.12	8.28
	PM10 gravimetric	22.88	23.71	25.32
	Ozone	78.85	97.71	66.03
TOMEŞTI	SO_2	2.79	5.02	4.87
	NO_2	12.09	15.19	13.41
	PM10 gravimetric	-	-	-
	Ozone	67.09	94.72	60.98

2.4. Research methods

Assessment of the effects of air pollutants on leaf morphology and physiological processes was achieved by tracking several indicators. Determination of leaf area was done with the portable leaf area meter (AM300 apparatus). Optical measurements were made using a simple scanning process.

Water content and dry weight of plant material were determined by gravimetric method (Boldor et al., 1983). For each foliar test we used 3 parallel probes, and the data was selected as the average value of the results.

Leaves assimilating pigments were determined by spectrophotometric method of Arnon (1949). They were determined in 80% acetone extract at the following wavelengths: 663nm, 646nm and 470nm. The results were calculated using formulas developed by MacKinney (1941) and the values were expressed in mg 100g^{-1} plant material.

To analyze the functional parameters - process of photosynthesis, transpiration and stomatal conductance the ADC Bioscientific LCi portable photosynthesis measurement system was used. Recording of the parameters was performed *in vivo* on a number of five leaves from the four cardinal points of each individual analyzed (three individuals of the same species in each determination). Therefore the results are the arithmetic mean of readings taken.

3. RESULTS AND DISCUSSIONS

In the Copou–Sadoveanu, Decebal-Cantemir and Tomeşti areas the individuals of Canadian poplar were vigorous, free from defoliation, with or without chlorosis and necrosis on the leaf surface (Figs 2, 3, 4). It was noted, however, that in July and September the number of leaf with chlorosis and necrosis significantly increased (Fig. 5). In Podul de Piatră and Tătăraşi–Oancea areas the samples of *Canadian poplar* showed severe defoliation in the canopy, in some cases the crown having leaves only at the top.

At the samples from Podul de Piatră the edges of the leaf lamina had reddish- brown color at the end of July (Fig. 6). These marginal burns widened significantly in September. The poplar samples from Podul de Piatră are located at the intersection of Road N. Iorga - Bld. Nicolina in an area with heavy traffic, which is the reason of presence on leaves surface of significant amounts of dust.

Figure 2. Foliar detail of Canadian poplar individual from Podul de Piatră (May 2012)

Figure 3. Foliar detail of Canadian poplar individual from Decebal-Cantemir (May 2012)

Figure 4. Foliar detail of Canadian poplar individual from Tomeşti (May 2012)

Leaf area of all investigated individuals showed lower values compared with the control, in all investigated areas, with minimum values at the individuals from Podu de Piatră area (Table 3). Chlorosis and necrosis appearance since early developmental stages of leaves, physiological stress due to pollutants aggression is consequential harm to the degree of development of the plant's leaves.

Figure 5. Foliar detail of Canadian poplar individual from Tătăraşi-Oancea (July 2012)

Figure 6. Foliar detail of Canadian poplar individual from Podu de Piatră (July 2012

Reduced leaf surface from Podul de Piatră, an intense traffic area, is due to heavy deposits on the leaves or chlorosis and necrosis. Reducing leaf area and number of leaves may be due to the rate of foliar productivity and early senescence induced by pollutants. Dineva (2004) and Tiwari et al., (2006) recorded reduced leaf area and petiole length under stress due to pollution. Some studies showed changes in leaf area and petiole size in polluted air (Jahan & Iqbal, 1992). Significant reduction in the length of the stem and leaf area was also observed in pollution conditions caused artificially. Reduction in leaf area was observed in five species of trees near areas contaminated with solid particulates and SO_2 (Jahan & Iqbal, 1992). Significant effects of automobile exhaust on phenology, morphology and productivity of tree species on the roadside was also reported (Bhatti & Iqbal, 1988).

It should be noted that high dry weight content isn't directly correlated with macroscopically visible necrosis, which means that in some cases disruption of physiological functions is directly followed by defoliation (Ivănescu & Toma, 1999). The decrease of the water amount, combined with the increase of dry substance amount, can be correlated to the close-open stomata mechanism. The photosynthetic rate is a measure commonly used in the study of the impact of air pollutants on woody species (Woo & Je, 2007). Plants can integrate and accumulate the pollutants in their systems.

Table 3. Variation of leaf area at *Populus x Canadensis* Moench individuals derived from the five areas of investigations (May, July and September 2012)

Month	Studied area	Leaf area				
		Polluted leaves without chlorosis and necrosis		Polluted leaves with chlorosis and necrosis		
		Area cm^2	% from control	Area cm^2	Damaged area cm^2	% damaged
May	Control	47.57				
	Podu de Piatră	34.27	72.04	30.18	1.91	6.26
	Decebal - Cantemir	43	90.37			
	Oancea – Tătăraşi	37.43	78.67			
	Copou- Sadoveanu	37.9	79.65			
	Tomeşti	45.73	96.12			
July	Control	65.31				
	Podu de Piatră	31.65	48.46	26.69	13.08	48.97
	Decebal - Cantemir	62.72	96.03			
	Oancea – Tătăraşi	60.88	93.20			
	Copou- Sadoveanu	50.84	77.84			
	Tomeşti	65.52	100.31			
Sept.	Control	65.31				
	Podu de Piatră	31.65	48.46	26.69	13.08	48.97
	Decebal - Cantemir	62.72	96.03			
	Oancea – Tătăraşi	60.88	93.20			
	Copou- Sadoveanu	50.84	77.84			
	Tomeşti	65.52	100.31			

Table 4. Variation of water and dry matter content at *Populus x Canadensis* Moench individuals derived from the five areas of investigations (May, July and September 2012)

Month	Studied area	Water content and dry matter (g%)			
		Water content %	% from control	Dry matter %	% from control
May	Control	69.08		30.91	
	Podu de Piatră	66.26	95.90	33.73	109.14
	Decebal - Cantemir	66.08	95.65	33.91	109.70
	Oancea – Tătăraşi	65.98	95.51	34.01	110.02
	Copou- Sadoveanu	69.52	100.62	30.47	98.60
	Tomeşti	66.42	96.14	33.57	108.61
July	Control	67.74		32.25	
	Podu de Piatră	66.64	98.37	33.35	103.41
	Decebal - Cantemir	64.39	95.05	35.60	110.38
	Oancea – Tătăraşi	65.33	96.44	34.66	107.45
	Copou- Sadoveanu	68.39	100.96	31.60	97.97
	Tomeşti	64.63	95.40	35.36	109.64
Sept.	Control	67.61		32.38	
	Podu de Piatră	65.93	97.51	34.06	105.18
	Decebal – Cantemir	63.78	94.33	36.21	111.82
	Oancea – Tătăraşi	62.40	92.30	37.59	116.05
	Copou- Sadoveanu	61.95	91.63	38.04	117.46
	Tomeşti	60.89	90.06	39.10	120.74

Table 5.Variation of photosynthesis rate, transpiration rate and stomatal conductance at *Populus x canadensis* Moench individuals derived from the five areas of investigations (May. July and September 2012)

Month	Studied area	The physiological process					
		Photosynthesis µmols CO_2 m^{-2} s^{-1}	% from control	Transpiration µmols $H_2O/m^2/s$	% from control	Stomatal conductance mol m^{-2} s^{-1}	% from control
May	Control	3.83		1.62		0.57	
	Podu de Piatră	1.68	43.95	1.37	84.23	0.17	29.82
	Decebal - Cantemir	1.91	49.98	1.52	93.78	0.21	36.84
	Oancea – Tătăraşi	1.95	50.90	1.51	93.14	0.25	43.85
	Copou- Sadoveanu	1.53	39.93	1.79	110.0	0.19	33.33
	Tomeşti	2.32	60.47	1.58	97.24	0.24	42.10
July	Control	3.05		1.33		0.34	
	Podu de Piatră	1.34	43.91	1.03	77.51	0.09	26.47
	Decebal - Cantemir	1.620	53.02	1.17	87.52	0.12	35.29
	Oancea – Tătăraşi	1.50	49.29	1.12	83.96	0.16	47.05
	Copou- Sadoveanu	1.25	41.07	1.44	108.2	0.11	32.35
	Tomeşti	1.94	63.72	1.10	82.56	0.15	44.11
Sept.	Control	2.35		1.41		0.24	
	Podu de Piatră	1.03	43.92	0.78	55.15	0.04	16.66
	Decebal - Cantemir	1.20	51.29	0.92	65.07	0.09	37.5
	Oancea – Tătăraşi	1.18	50.08	0.85	60.21	0.08	33.33
	Copou- Sadoveanu	0.94	39.94	1.09	77.13	0.09	37.5

Table 6. Variation of foliar pigments content (chlorophyll a, chlorophyll b and carotenoidic pigments) at *Populus x canadensis* Moench derived from the five areas of investigations (May, July and September 2012)

Month	Studied area	Chlorophyll a		Chlorophyll b		Carotenoidic pigments	
		mg/100g	% from control	mg/100g	% from control	mg/100g	% from control
May	Control	64.68		23.85		21.65	
	Podu de Piatră	23.95	37.04	9.73	40.81	11.94	55.17
	Decebal - Cantemir	40.49	62.60	19.20	80.53	16.25	75.05
	Oancea – Tătărași	42.02	64.97	22.24	93.26	17.45	80.61
	Copou- Sadoveanu	34.12	52.76	16.17	67.79	14.98	69.20
	Tomeşti	43.04	66.55	16.41	68.82	18.01	83.20
July	Control	82.74		25.39		23.67	
	Podu de Piatră	30.89	37.34	9.34	36.79	13.65	57.69
	Decebal - Cantemir	50.91	61.52	14.91	58.74	18.65	78.78
	Oancea – Tătărași	63.07	76.22	20.81	81.97	19.47	82.24
	Copou- Sadoveanu	43.53	52.61	16.08	63.36	19.59	82.74
	Tomeşti	49.23	59.50	16.20	63.83	19.13	80.82
Sept.	Control	58.82		49.31		19.78	
	Podu de Piatră	36.58	62.18	9.15	18.55	12.40	62.68
	Decebal - Cantemir	50.59	86.00	11.28	22.88	14.77	74.63
	Oancea – Tătărași	38.80	65.96	11.11	22.53	13.30	67.25
	Copou- Sadoveanu	61.18	104.01	13.55	27.49	17.76	89.75
	Tomeşti	47.55	80.83	11.08	22.47	17.67	89.32

It is reported that, depending on their level of sensitivity, the plants show visible changes that would include biochemical and physiological modification (Agbaire & Esiefarienrhe, 2009).

In all investigated areas the average of photosynthetic rate at *Populus x canadensis Moench* individuals was lower than the control in May, July and September (Table 5). The minimum photosynthetic rate was recorded at the individuals from Podu de Piatră area (traffic station). The photosynthesis rate was 43.95% from the control in May, 43.91% in July and 43.92% in September. The stomatal conductance values were lower than the controls (Table 5). The minimum values were recorded at the individuals from Podu de Piatră area. The stomatal conductance values were 29.8 % from the control in May, 26.47% in July and 16.66% in September 2012.

This stomatal conductance values can be explained by the existence of adaptive mechanisms by which the plant responds to the stress caused by the presence of pollutants in the atmosphere, to which the thermal and hydric stress by closing and opening of stomata is also added, (Popescu et al., 2012).

The average of transpiration values was in general, lower in affected individuals than in the controls, the exception was the Copou-Sadoveanu area, in May and in July, where the transpiration rates of affected individuals was higher than those of controls, (Table 5). The minimum values were recorded at the individuals from Podu de Piatră area (traffic station).The transpiration process value was 84.23% from the control in May, 77.51% in July and 55.15% % in September. A relationship between traffic density and photosynthetic activity, total chlorophyll content and leaf senescence degree was studied by Honour et al., in 2009. One of the most common effects of air pollution is the gradual disappearance of chlorophyll and yellowing leaves along with it, which as a consequence reduce photosynthesis (Joshi & Swami, 2007).

In our study, the average of chlorophyll a amount was in general lower in affected individuals than in controls, with the exception of Copou-Sadoveanu area in September where the quantity of pigments was higher in affected individuals than in controls (Table 6). Minimum values were recorded at the individuals of *Populus x canadensis* Moench species from Podul de Piatră area. The average of chlorophyll a values was 37.04% from the control in May, 37.34% in July and 60.18% in September. The average of chlorophyll b amount was lower in affected individuals than the controls in all studied moments and areas, (Table 6). Minimum values were recorded at the individuals of *Populus x canadensis* Moench species from Podul de Piatră area (traffic station). The average of chlorophyll b values was 40.81% from the control in May, 36.79% in July and 18.55% in September. The average of carotenoidic pigments was was lower in affected individuals than the controls in all studied moments and areas. (Table 6). Minimum values were recorded at the individuals of *Populus x canadensis* Moench species from Podul de Piatră area (traffic station).

The average of carotenoidic pigments values was 55.17% from the control in May, 57.69% in July and 62.68% in September. Under the influence of solid and gas polluting agents the average of chlorophyll a, chlorophyll b and carotenoidic pigments quantity can decrease. If the sulphur dioxide (SO_2), nitrogen oxides (NO_x) and CO_2 emissions and particulate matter are absorbed by the leaves, they can cause a reduction in the concentration of photosynthetic assimilating pigments (chlorophylls and carotenoids) (Joshi & Swami, 2009).

4. CONCLUSIONS

This study focused on morphological (foliar surface normal and necrotic), physiological (photosynthetic and transpiration processes intensity and biochemical (water content, dry matter content and photo-assimilating pigments), changes induced at foliar level by some pollutants in samples of *Populus x canadensis* Moench cultivated for ornamental purposes in Iasi city area. From the correlated interpretation of the obtained data, due to vegetation condition for the year 2012 we can conclude that:

The individuals of *Populus x canadensis* Moench from Podul de Piatră area showed the most significant response reactions to pollutants harmful action. The lowest values of foliar surface and physiological processes compared to the control, were recorded at the individuals of Canadian poplar from Podul de Piatră area. Also the largest number of chlorosis of the leaf unit accompanied by necrosis of the limb edges were found at the individuals investigated from this area. The foliar chlorosis and necrosis resulted from deep physiological alterations that affect the water and dry substance contents. The lowest values of foliar surface and physiological processes compared to the control, were recorded at the individuals of *Populus x canadensis* Moench from Podu de Piatră area**.**

We outline the fact that not always the high dry substance content is related to necrotic leaf surface, which means that in certain cases the disturbance of some physiological functions is directly followed by defoliation.

The response of each species or even individual to pollutant aggression is conditioned by a multitude of genetic factors, pedo-climatic conditions, natural habitat and the nature of pollutant.

Foliar necrosis and chlorosis are a clear proof of profound physiological modifications that affect the average amount of water, dry substance and assimilating pigments. If the sulphur dioxide (SO_2), nitrogen oxides (NO_x), CO_2 emissions and particulate matter are absorbed by the leaves, they can cause a reduction in the concentration of photosynthetic assimilating pigments (chlorophylls and carotenoids), which directly lead to lower photosynthetic process intensities (Joshi & Swami, 2009).

The minimum rate of photosynthesis, stomatal conductance and transpiration values recorded at individuals from the Podu de Piatră area is explained by the presence of both coarse and fine particles resulting from heavy traffic. Both fine and coarse particles were reported to be responsible for increased leaf temperature that influenced transpiration and decreased light absorption, thus affecting photosynthesis (Tomašević & Aničić, 2010).

The lowest quantity of assimilating pigments was found at the individuals of *Populus x canadensis* Moench species from Podu de Piatră area. We can conclude that the amount of chlorophyll *a*, chlorophyll *b* and the intensity of photosynthesis aren't always correlated.

Effects of air pollution on physiological processes are complex and not yet fully understood, so that responses to pollutants action can vary greatly. Plants grown in urban areas, have developed a number of defense mechanisms against the harmful action of air pollutants, such as stomatal closure, but this reaction can be blocked by the action of another pollutant, which is reflected by variations in physiological response from one area to another. At the same time, the synergistic action of pollutants and also the multitude of combinations of pollutants to other environmental factors such as climate, soil, etc. are difficult to assess the action of an individual pollutant. Further studies conducted in a controlled environment to highlight clearly the mechanism of action of each pollutant separately.

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Received at: 02. 06. 2014
Revised at: 06. 09. 2014
Accepted for publication at: 09. 09. 2014
Published online at: 18. 09. 2014