NEEDLE MORPHO-ANATOMY AND POLLEN MORPHO-PHYSIOLOGY OF SELECTED CONIFERS IN URBAN CONDITIONS

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Abstract. Comparison of twelve conifer species (*Abies alba, A. concolor, A. nordmanniana, A. pinsapo, Cedrus atlantica, C. deodara, Picea abies, P. omorika, P. pungens, Pseudotsuga menziesii, Taxus baccata,* and *Pinus nigra*) in the sense of needle morpho-anatomy and pollen morpho-physiology, in correlation to air pollution, was performed for the first time. Analyzed properties of species were also compared with literature sources. Listed conifers were investigated in five Belgrade parks, characterised by different degrees of air pollution, especially CO_2. Their rank, I-V, was performed from non-polluted to heavily-polluted parks. Ranking in the sense of needle morpho-anatomy and pollen morpho-physiology did not match expected ones, but park V remained the worst for many analyzed species. Trees with shorter needles had greater stomatal density, which was particularly prominent in *A. alba, A. nordmanniana, P. abies, P. omorika, P. nigra* and *T. baccata*. The pollen grains of *C. atlantica* and *T. baccata* were the most sensitive to air pollution. In some analyzed species distance of particular trees close to the heavy traffic also was in correlation with needle dimensions (*P. omorika, A. concolor, A. nordmanniana, P. nigra*), stomatal density (*A. alba, P. abies, P. omorika, P. pungens) and pollen vitality (A. pinsapo, C. atlantica, P. menziessi, P. nigra*, and *T. baccata*).
Keywords: *cedar, fir, pine, spruce, yew*

Introduction

The increasing content of air pollutants, such as CO_2, SO_2, nitrogen oxides, soot, dust, etc. is always present in urban habitats and produces an adverse impact on growth and health of the living world. Since plants absorb chemicals and deposit solid particles, green areas, such as parks, are essential for the quality of life in cities. In addition, plants suffer changes which lead to their slow development, to diminished resistance to disease and pest attacks and, ultimately, to their death. These changes could be visible

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(macro-changes) or less visible (micro-changes). Both of them are reliable indicators of the level of vulnerability of urban habitats.

Many scienticists tried to imitate polluted habitats and trees response to them. Fumigation of *Picea omorika'*s pollen with SO_2 resulted in reduced seed production (Krug, 1990). Fumigation of the needles of seedlings of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) with SO_2, O_3 and combination of these two gases greatly reduced soluble carbohydrates in the needles taken from *P. sylvestris*, but to a lesser content in case of needles taken from *P. abies* (Peace et al., 1995). Furthermore, when field with needles of 3-year-old seedlings of Scots pine was fumigated with relatively low concentration of SO_2, their needles emitted H_2S. This emission depended of light and SO_2 concentration (Hällgren and Fredrikson, 1982). On the other hand, some investigations showed that conifer species (Austrian pine, cypress and larch) influenced the lowest level of NO_2, O_3 and NH_3 in the air (Donovan et al., 2005). Conifers also delay ultra-fine dust particles more effectively than broadleaved trees (Freer-Smith et al., 2005). Furthermore, it should be noted that only evergreen and conifer species delay leaves and transpire throughout the whole year. Their transpiration is important especially in winter, when the level of air pollutants is extremely high, or following heavy air pollution (in the period of defoliation of deciduous trees). There are four main ways that urban trees affect air quality: (1) temperature reduction and other microclimatic effects, (2) removal of air pollutants, (3) emission of volatile organic compounds and tree maintenance emissions, and (4) energy effects on buildings (Nowak, 2002).

The purpose of the study is to evaluate, for the first time, the degree of vulnerability of twelve conifer species from five parks to air pollution by means of investigation of both morpho-anatomic leaf properties and morpho-physiological pollen traits. Furthermore, the influence of distance of trees from traffic was also taken into account. Increased content of CO_2 leads to a decrease of stomatal density (Lin et al., 2001) and number of stomata (Woodward and Bazzaz, 1988). In *Pinus crassifolia* (Qiang et al., 2003) their positive correlation (r = 0.63) were found. In one-year old needles of *P. douglasii*, Apple et al. (2000) reported that CO_2 and temperature did not influence stomatal density. They stated that effects could be more visible later (after three years) and genetic influence is very strong, so stomatal density depends on needle elongation.

Materials and methods

Plant materials, selected parks and trees

Five Belgrade parks in Serbia (Europe), exposed to different levels of air pollution, were selected in 2015 (*Fig. 1*). Listed parks were ranked from non-polluted to very heavily-polluted Popović et al. (2016).

For needle and pollen studies twelve and nine tree species were used (*Tables 1* and *2*, respectively).

Processing morpho-anatomic properties of needles

Well-developed one-year old leaves (needles) of twelve conifers were collected for laboratory testing: morpho-anatomic analyses of needles, where length, width, shape, dry mass and stomatal density were measured. For the purpose of morpho-anatomic

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analyses, needles all around a tree crown were collected (ca. 20 needles). Stomata rows from abaxial needle side were obtained by the ‘Kolodium method’ (Wolf, 1950).

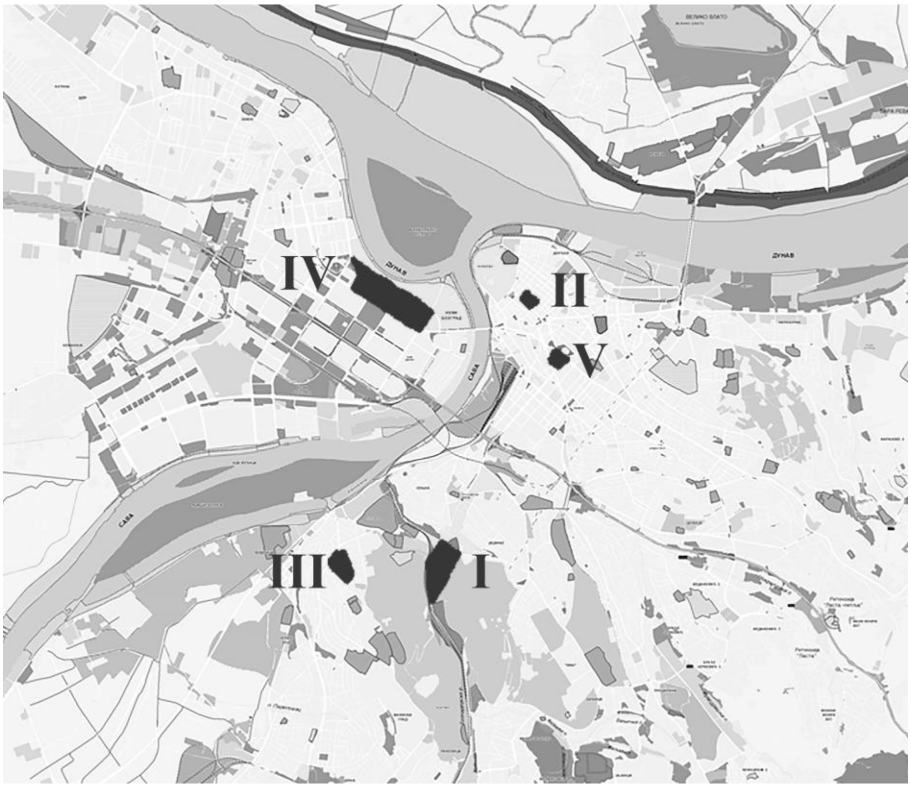

Figure 1. *Shematic view of analysed Belgrade parks performed from non-polluted to heavily-polluted parks: I – Topčider; II – Academic; III – Banovo Brdo; IV – palace ‘Serbia’ and V- Pioneer’s park*

Table 1. *Number of trees per species sampled in five parks for needle studies*

No.	Tree species	Park No.				
		I	**II**	**III**	**IV**	**V**
1.	*Abies alba* Mill.			1		2
2.	*Abies concolor* (Gordon) Lind. ex Hildebr.			3		2
3.	*Abies nordmanniana* (Steven) Spach, 1841	3				
4.	*Abies pinsapo* Boiss.				1	
5.	*Cedrus atlantica* (Endl.) Mann. ex Carrière		3	3	4	4
6.	*Cedrus deodara* (Roxb.) G. Don)		1			
7.	*Picea abies* (L.) Karst.			1		2
8.	*Picea omorika* (Panč.) Pürkyné	1		2	2	
9.	*Picea pungens* Engelm.				2	2
10.	*Pinus nigra* J. F. Arnold	3		3	2	2
11.	*Pseudotsuga menziesii* (Mirb.) Franco			3	4	2
12.	*Taxus baccata* L.				3	2
	Sum	**7**	**4**	**16**	**18**	**18**
	Sum					**63**

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Table 2. *Number of trees per species sampled in five parks for pollen studies*

No.	Tree species	Park No.				
		I	**II**	**III**	**IV**	**V**
1.	*Abies alba* Mill.			1		
2.	*Abies nordmanniana* (Steven) Spach, 1841	2				
3.	*Abies pinsapo* Boiss.				3	
4.	*Cedrus atlantica* (Endl.) Mann. ex Carrière		1	3	3	2
5.	*Picea abies* (L.) Karst.			1		
6.	*Picea omorika* (Panč.) Pürkyné			2	3	
7.	*Pinus nigra* J. F. Arnold			3	3	3
8.	*Pseudotsuga menziesii* (Mirb.) Franco			3		1
9.	*Taxus baccata* L.			2	3	2
	Sum	**2**	**1**	**15**	**15**	**8**
	Sum					**41**

Processing morpho-physiological properties of pollen grains

Pollen was extracted from ripe strobili from nine trees in parks or extracted in laboratory after immersing twigs with half-ripe strobili in water. Observations of needle stomatal density and morpho-physiological analysis of pollen were performed using a light microscope (*Leica Galen III*) and included measurements of length and width of pollen grains, as well as their germinability and length of pollen tubes at 10% of sucrose. Measurements were carried out on 50 pollen grains per tree. The needle and pollen shape coefficient were calculated by the formula: 100*width/length.

Statistical analyses

Mean values and differences between parks (at 95% level), as well as analysis of variance were calculated in *Statgraphics Centurion* XVI, Version 16.1.11. Coefficient of correlation (r) by linear regression analysis for all measured properties was performed.

Results

Morpho-anatomic properties of needles

Ranking of parks in *Tables 3* and *4* were performed from heavily-polluted (V, IV) to moderate polluted (III, II) and non-polluted areas (I). The needles of *A. alba*, *A. concolor*, *P. abies* and *P. pungens* were significantly shorter in the park V (*Table 3*). Consequently, trees with shorter needles had greater stomatal density, which is particularly prominent in *A. alba, A. nordmanniana, P. abies*, *P. omorika*, *P. nigra* and *T. baccata* (*Table 3; Fig. 2*). Needles of *P. omorika* and *P. nigra* were significantly larger in the park IV (*Table 3; Figs. 3* and *4*). The needles of *Taxus baccata*, predominantly collected in the proximity of traffic crossroads in the park IV were shorter than in other parks (*Fig. 4*). Interestingly, the needles of *Pseudotsuga menziesii* were larger in the park V than in the park III (*Table 3*). Larger needles very often had a larger leaf-area, perimeter and dry mass. As presented in *Table 3,* especially with respect to morphology of needles, the parks are ranked as follows (from the best to the

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worst results): IV, II V, III and I. This ranking does not correlate to the ranking in terms of park pollution (I-V, from the best to the worst results). As a consequence, we could not conclude that morphological parameters of conifers are reliable indicators of air pollution. However, some species, such as *P. omorika*, *P. pungens*, *P. nigra and T. baccata* had larger needles when situated deep inside the park (far from the heavy traffic). Furthermore, *A. alba* and *P. abies* also produced better results in parks with moderately polluted air than in heavily-polluted parks. With respect to stomatal density, the greatest differences between parks were observed in case of *A. alba*, *A. concolor*, *C. atlantica* and *T. baccata*. Many species had the greatest stomatal density in the most polluted parks (V and IV), but we could not generally conclude that stomatal density of conifers is a dependable indicator of air pollution.

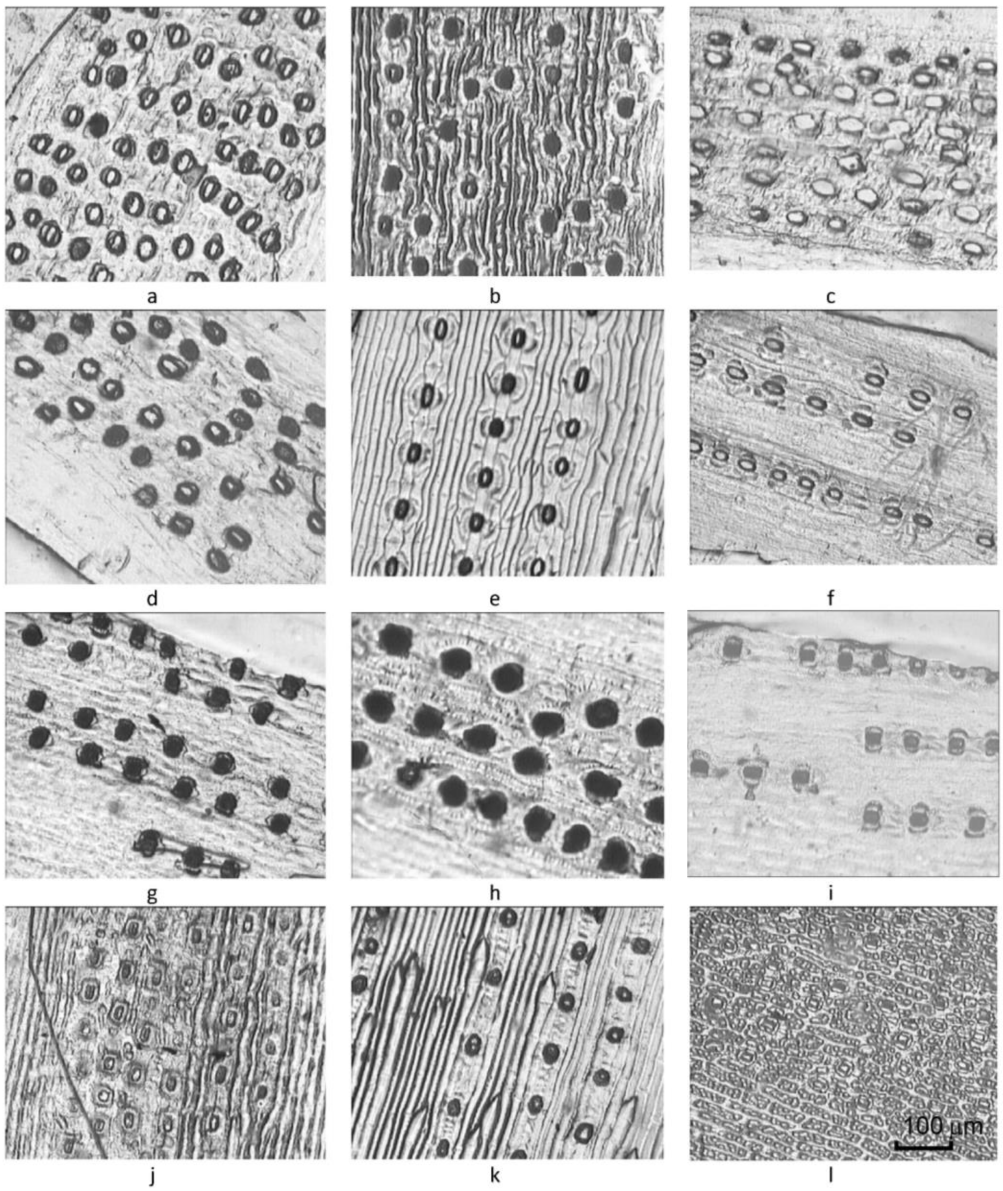

Figure 2. *Rows of stomata on the abaxial needle side (objective 10x): Abies alba - V (a), A. concolor - III (b), A. nordmanniana - I (c), A. pinsapo - IV (d), Cedrus atlantica - III (e), C. deodara - II (f), Picea abies - V (g), P. omorika – IV (h), P. pungens – IV (i), Pseudotsuga douglasii – III (j), Pinus nigra - III (k), Taxus baccata - V (l)*

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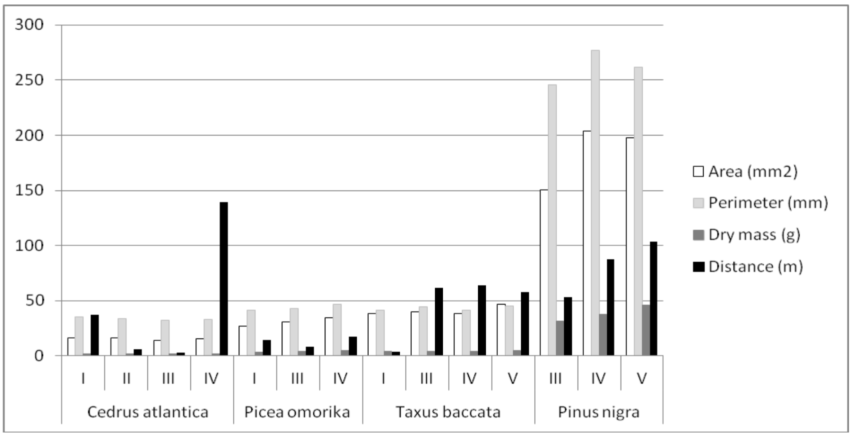

Figure 3. *Graphical illustration of average values of needle area, perimeter, dry mass and distance from traffic in Cedrus atlantica, Picea omorika, Taxus baccata, and Pinus nigra*

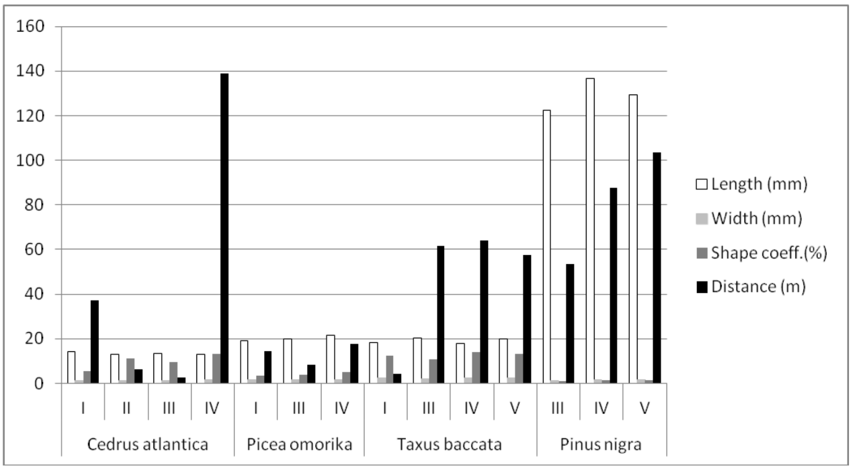

Figure 4. *Graphical illustration of needle length, width, shape and distance from traffic in Cedrus atlantica, Picea omorika, Taxus baccata, and Pinus nigra*

Table 3. *Results of the morphological analyses of conifer leaves (needles), dry mass, stomatal density and distance of trees from traffic*

Park No.	**Tree No.**	**Needle morphology**					**Dry mass (g)**	**Stomatal density (number/ mm^2)**	**Distance of trees from traffic (m)**
		Area (mm^2)	**Perimeter (mm)**	**Dimensions**					
				Length (mm)	**Width (mm)**	**Shape coefficient (%)**			
	Abies alba								
V	197	31.66	39.82	17.99	1.92	11.00	3.80	134.38	21.7
	199	33.34	39.35	17.56	2.11	12.52	4.20	132.82	21.6
	Mean ± SD	*32.50 ± 1.19*	*39.58 ± 0.33*	*17.78 ± 0.30*	*2.02* ± 0.13*	*11.76* ± 1.07*	*4.00 ± 0.28*	*133.60 ± 1.10*	*21.7 ± 0.07*
III	96	41.51	50.42	23.13	2.08	9.41	4.55	93.23	100.3
	Mean ± SD	*41.51 ± 0.00*	*50.42 ± 0.00*	*23.13 ± 0.00*	*2.08 ± 0.00*	*9.41 ± 0.00*	*4.55 ± 0.00*	*93.23 ± 0.00*	*100.3 ± 0.00*
Mean ± SD		**35.50* ± 5.27**	**43.20* ± 6.26**	**19.56* ± 3.10**	**2.04 ± 0.10**	**10.98 ± 1.55**	**4.18 ± 0.38**	**120.14* ± 23.32**	**65.0* ± 45.41**

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Abies concolor									
V	220	77.18	84.39	39.90	2.30	6.08	14.30	68.91	24.4
	221	82.11	96.35	46.14	2.03	4.68	12.85	94.61	20.8
	Mean ± SD	*79.65 ± 3.49*	*90.37 ± 8.46*	43.02 ± 4.41	*2.17* ± 0.19*	*5.38 ± 0.99*	*13.58 ± 1.02*	*81.76 ± 18.17*	*22.6 ± 2.54*
III	248	106.70	98.07	46.20	2.83	6.19	21.33	84.58	19.6
	282	65.69	76.59	36.18	2.11	6.06	8.80	88.23	26.2
	283	89.22	95.01	45.26	2.25	5.28	13.70	93.51	22.8
	Mean ± SD	*87.40* ± 20.58*	*90.40* ± 11.62*	*42.82* ± 5.53*	*2.39* ± 0.38*	*5.78 ± 0.49*	*14.61 ± 6.31*	*88.77 ± 4.49*	*22.8 ± 3.30*
Mean ± SD		**83.96* ± 15.23**	**90.39 ± 9.24**	**42.91 ± 4.50**	**2.29* ± 0.31**	**5.61 ± 0.66**	**14.20 ± 4.53**	**85.97 ± 10.36**	**22.8 ± 2.66**
Abies nordmanniana									
I	149	31.96	40.50	18.41	1.84	10.32	3.87	116.25	74.1
	150	28.52	38.34	17.33	1.84	10.66	3.95	184.22	70.3
	153	22.98	31.31	13.83	1.82	13.23	2.40	127.97	59.7
	Mean ± SD	*28.44* ± 4.53*	*37.50* ± 4.80*	*16.91* ± 2.39*	*1.84 ± 0.01*	*11.12* ± 1.59*	*3.41 ± 0.87*	*142.81 ± 36.33*	*68.0 ± 7.46*
Mean ± SD		**28.44 ± 4.53**	**37.50 ± 4.80**	**16.91 ± 2.39**	**1.84 ± 0.01**	**11.12 ± 1.59**	**3.41 ± 0.87**	**142.81 ± 36.33**	**68.0 ± 7.46**
Abies pinsapo									
IV	8	40.07	47.14	21.34	2.23	10.86	5.60	133.99	25.6
	Mean ± SD	*40.07 ± 0.00*	*47.14 ± 0.00*	*21.34 ± 0.00*	*2.23 ± 0.00*	*10.86 ± 0.00*	*5.60 ± 0.00*	*133.99 ± 0.00*	*25.6 ± 0.00*
Mean ± SD		**40.07 ± 0.00**	**47.14 ± 0.00**	**21.34 ± 0.00**	**2.23 ± 0.00**	**10.86 ± 0.00**	**5.60 ± 0.00**	**133.99 ± 0.00**	**25.6 ± 0.00**
Cedrus atlantica									
V	26	13.96	28.70	11.07	1.29	11.88	1.80	-	77.7
	80	19.54	45.76	19.15	1.11	*5.77*	1.80	78.20	44.3
	176	16.62	37.56	15.51	1.13	*9.28*	1.95	83.13	7.7
	181	12.25	23.85	8.73	1.50	17.32	1.40	81.67	7.0
	Mean ± SD	*16.03* ± 3.19*	*35.50* ± 9.70*	*14.42* ± 4.64*	*1.20 ± 0.18*	*11.06* ± 4.86*	*1.74 ± 0.24*	*81.00 ± 2.53*	*34.2 ± 33.84*
IV	59	18.66	43.59	17.67	1.16	7.03	2.13	-	148.2
	68	13.35	25.77	9.72	1.61	19.22	2.07	-	161.9
	83	15.60	29.37	11.48	1.57	15.77	2.25	78.75	133.1
	445	15.43	33.76	13.11	1.31	10.50	1.95	56.15	113.2
	Mean ± SD	*15.73* ± 2.19*	*32.90* ± 7.70*	*12.90* ± 3.41*	*1.42* ± 0.21*	*13.13* ± 5.42*	*2.10 ± 0.12*	*67.45 ± 15.98*	*139.1 ± 20.89*
III	322	18.74	39.52	15.52	1.33	9.05	2.25	56.33	10.8
	329	10.53	27.09	10.61	1.17	11.13	1.00	77.11	32.2
	335	13.71	31.38	12.72	1.13	9.54	1.65	73.28	37.9
	Mean ± SD	*15.08* ± 4.14*	*33.78* ± 6.31*	*13.42* ± 2.46*	*1.22* ± 0.11*	*9.67 ± 1.09*	*1.63 ± 0.62*	*68.91 ± 11.06*	*29.7 ± 14.29*
II	45	18.47	40.16	13.27	1.34	8.88	2.05	51.77	113.6
	47	17.21	32.41	12.47	1.59	13.52	2.45	-	104.2
	57	12.37	28.23	11.41	1.18	10.89	1.30	65.63	5.6
	Mean ± SD	*16.02* ± 3.22*	*33.60* ± 6.05*	*13.05* ± 0.93*	*1.37* ± 0.21*	*11.10* ± 2.33*	*1.93 ± 0.58*	*58.70 ± 9.80*	*73.3 ± 59.82*
Mean ± SD		**15.71 ± 2.84**	**33.72 ± 6.89**	**13.31 ± 2.99**	**1.32* ± 0.18**	**11.30 ± 3.84**	**1.86 ± 0.40**	**70.20 ± 11.75**	**69.0 ± 56.93**

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	Cedrus deodara								
II	91	19.46	39.71	15.82	1.34	8.90	2.67	128.13	18.6
	Mean ± SD	*19.46 ± 0.00*	*39.71 ± 0.00*	*15.82 ± 0.00*	*1.34 ± 0.00*	*8.90 ± 0.00*	*2.67 ± 0.00*	*128.13 ± 0.00*	*18.6 ± 0.00*
Mean ± SD		**19.46 ± 0.00**	**39.71 ± 0.00**	**15.82 ± 0.00**	**1.34 ± 0.00**	**8.90 ± 0.00**	**2.67 ± 0.00**	**128.13 ± 0.00**	**18.6 ± 0.00**
	Picea abies								
V	15	34.26	44.45	20.41	1.82	9.02	2.75	74.38	36.8
	18	21.80	37.92	17.67	1.29	7.60	2.45	85.86	44.0
	Mean ± SD	*28.03* ± 8.81*	*41.18* ± 4.62*	*19.04* ± 1.94*	*1.55* ± 0.37*	*8.31* ± 1.00*	*2.60 ± 0.21*	*80.12 ± 4.52*	*40.4 ± 5.09*
III	382	28.00	45.30	17.88	1.60	8.99	3.45	-	62.1
	Mean ± SD	*28.00 ± 0.00*	*45.30 ± 0.00*	*17.88 ± 0.00*	*1.60 ± 0.00*	*8.99 ± 0.00*	*3.45 ± 0.00*	-	*62.1 ± 0.00*
Mean ± SD		**28.02* ± 6.23**	**42.56 ± 4.04**	**18.65* ± 1.52**	**1.57* ± 0.27**	**8.54 ± 0.81**	**2.88 ± 0.51**	**80.12 ± 4.52**	**51.3 ± 13.03**
	Picea omorika								
IV	362	34.74	49.02	22.81	1.70	7.54	4.61	103.54	115.9
	495	34.26	44.45	20.41	1.82	9.02	5.20	105.00	51.2
	Mean ± SD	*34.50 ± 0.34*	*46.73* ± 3.23*	*21.61* ± 1.70*	*1.76* ± 0.08*	*8.28* ± 1.05*	*4.91 ± 0.42*	*104.27 ± 1.03*	*83.5 ± 45.11*
III	1	33.30	47.44	22.14	1.58	7.27	4.15	123.59	9.3
	4	27.59	38.26	17.46	1.67	9.82	3.50	108.28	19.7
	Mean ± SD	*30.45* ± 4.04*	*42.85* ± 6.49*	*19.80* ± 3.31*	*1.63 ± 0.06*	*8.55* ± 1.80*	*3.83 ± 0.46*	*115.94 ± 10.82*	*14.5 ± 7.35*
I	338	26.82	41.38	19.13	1.56	8.35	3.60	-	17.8
	Mean ± SD	*26.82 ± 0.00*	*41.38 ± 0.00*	*19.13 ± 0.00*	*1.56 ± 0.00*	*8.35 ± 0.00*	*3.60 ± 0.00*	-	*17.8 ± 0.00*
Mean ± SD		**31.34* ±3.82**	**44.11* ±4.39**	**20.39* ±2.18**	**1.67* ± 0.10**	**8.40 ± 1.05**	**4.21 ± 0.71**	**110.10 ± 9.21**	**38.6 ± 43.48**
	Picea pungens								
V	23	37.44	52.75	24.60	1.77	7.47	5.80	75.47	65.9
	25	29.44	45.27	21.12	1.52	7.31	5.60	80.39	75.0
	Mean ± SD	*33.44* ± 5.66*	*49.01* ± 5.29*	*22.86* ± 2.46*	*1.65* ± 0.18*	*7.39 ± 0.11*	5.70 ± 0.14	*77.93 ± 3.48*	*70.5 ± 6.43*
IV	272	35.63	53.76	25.36	1.52	6.10	6.55	107.19	148.6
	273	50.09	64.51	30.42	1.83	6.08	10.70	82.03	149.5
	Mean ± SD	*42.86* ± 10.22*	*59.13* ± 7.60*	*27.89* ± 3.58*	*1.68* ± 0.22*	*6.09 ± 0.01*	*8.63 ± 2.93*	*94.61 ± 17.79*	*149.0 ± 0.64*
Mean ± SD		**38.15* ± 8.66**	**54.07* ± 7.92**	**25.37* ± 3.84**	**1.66 ± 0.16**	**6.74* ± 0.75**	**7.16 ± 2.39**	**86.27 ± 14.22**	**105.7 ± 45.53**
	Pinus nigra								
V	44	211.75	276.13	136.39	1.68	1.22	49.80	77.93	96.8
	46	184.11	247.54	122.19	1.59	1.30	42.95	83.79	110.5
	Mean ± SD	*197.93* ± 19.54*	*261.84* ± 20.22*	*129.29* ± 10.04*	*1.63 ± 0.06*	*1.26 ± 0.06*	*46.38 ± 4.84*	*80.86 ± 4.14*	*102.6 ± 9.69*
IV	496	190.58	233.51	114.96	1.80	1.59	37.80	84.96	80.7
	504	192.97	267.13	131.96	1.61	1.22	36.30	-	88.5
	506	208.87	294.01	145.25	1.75	1.21	34.00	-	88.8
	507	234.46	323.62	160.18	1.63	1.02	41.60	-	92.1
	Mean ± SD	*203.97* ± 20.20*	*277.08* ±38.41*	*136.86* ± 19.25*	*1.68 ± 0.09*	*1.25* ± 0.24*	*37.43 ± 3.19*	*84.96 ± 0.00*	*87.5 ± 4.83*

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III	9	137.45	242.86	120.45	0.98	0.81	28.65	83.21	50.5
	10	127.12	211.56	105.72	1.08	1.02	28.70	80.86	53.6
	11	186.42	283.54	140.59	1.18	0.84	37.95	110.16	56.5
	Mean ± SD	*150.33* ± 31.68*	*245.98* ± 36.09*	*122.25* ± 17.50*	*1.08* ± 0.10*	*0.89* ± 0.11*	*31.77 ± 5.35*	*91.41 ± 16.28*	*53.5 ± 3.00*
Mean ± SD		**176.29* ± 34.34**	**257.28* ± 34.26**	**127.43* ± 16.81**	**1.38 ± 0.31**	**1.08* ± 0.24**	**37.53 ± 6.78**	**86.82 ± 11.70**	**81.2 ± 21.30**
Pseudotsuga menziesii									
V	205	51.21	72.08	34.42	1.62	4.86	7.60	-	53.6
	224	41.62	60.77	28.83	1.56	5.62	4.35	147.11	26.0
	Mean ± SD	*45.73* ± 6.78*	*65.61* ± 8.00*	*31.22* ± 3.95*	*1.58 ± 0.04*	*5.24 ± 0.54*	*5.98 ± 2.30*	*147.11 ± 0.00*	*39.8 ± 19.52*
III	46	39.30	54.91	25.87	1.58	6.32	6.00	127.55	78.2
	383	48.46	64.30	30.54	1.61	5.40	5.95	142.19	71.6
	384	30.14	44.79	20.71	1.68	8.50	3.87	97.89	62.6
	Mean ± SD	*40.22* ± 9.16*	*55.63* ± 9.76*	*26.19* ± 4.92*	*1.62 ± 0.05*	*6.74* ± 1.59*	*5.27 ± 1.21*	*122.68 ± 22.57*	*70.8 ± 7.83*
Mean ± SD		**42.48* ± 8.28**	**59.74* ± 10.25**	**28.26* ± 5.15**	**1.61 ± 0.04**	**6.06* ± 1.42**	**5.55 ± 1.48**	**128.79 ± 60.66**	**55.3 ± 20.35**
Taxus baccata									
V	58	39.29	42.80	19.00	2.40	12.85	4.45	98.44	65.8
	231	53.64	47.98	21.21	2.78	13.28	5.40	100.08	49.4
	Mean ± SD	*46.47* ± 10.15*	*45.39* ± 3.66*	*20.11* ± 1.56*	*2.59* ± 0.27*	*13.06 ± 0.30*	*4.93 ± 0.67*	*99.26 ± 1.16*	*57.6 ± 11.60*
IV	358	43.21	42.74	18.84	2.53	13.52	5.90	150.21	100.8
	791	34.36	37.63	16.56	2.26	14.17	3.45	143.83	27.1
	Mean ± SD	*38.79* ± 6.19*	*40.18* ± 3.61*	*17.70* ± 1.61*	*2.39* ± 0.19*	*13.84 ± 0.46*	*4.68 ± 1.73*	*147.02 ± 4.51*	*63.9 ± 52.11*
III	379	41.73	49.65	22.84	1.99	8.80	4.30	130.70	59.6
	380	40.85	41.26	18.35	2.28	12.80	3.65	132.71	64.2
	381	38.06	43.32	19.54	2.12	10.97	3.80	-	61.8
	Mean ± SD	*40.21 ± 1.92*	*44.74* ± 4.37*	*20.24* ± 2.33*	*2.13* ± 0.14*	*10.86* ± 2.00*	*3.92 ± 0.34*	*131.71 ± 1.42*	*61.8 ± 2.30*
I	241	38.23	41.82	18.69	2.21	12.14	3.95	137.26	26.7
	265	47.60	46.25	20.68	2.44	12.00	5.30	124.69	28.0
	340	29.64	35.55	15.76	2.02	12.98	2.75	121.95	61.5
	Mean ± SD	*38.49* ± 8.98*	*41.21* ± 5.38*	*18.38* ± 2.47*	*2.22* ± 0.21*	*12.37 ± 0.53*	*4.00 ± 1.27*	*127.97 ± 8.16*	*38.7 ± 19.73*
Mean ± SD		**40.66* ± 6.66**	**42.90* ± 4.33**	**19.15* ± 2.09**	**2.30* ± 0.24**	**12.35* ± 1.53**	**4.30 ± 0.98**	**126.65 ± 17.83**	**54.6 ± 22.96**

*****Significant differences ($p < 0.05$)

Morpho-physiological properties of pollen grains

Morpho-physiological pollen properties are presented in *Table 4* and *Figures 5* and *6*. The impact of air pollution was calculated and graphically presented in four tree species: *Cedrus atlantica*, *Picea omorika*, *Taxus baccata* and *Pinus nigra* (*Fig. 6*). These four species were selected because they belong from different genera and simultaneously were present in two or more parks. The best results were obtained in the park IV (in case of *P. omorika*, *T. baccata* and *P. nigra*). The largest amount of pollen of *C. atlantica* was found in the park II, but its vitality (pollen tube length) was the

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highest in the park III. The parks were ranked as follows (from the best to the worst results with respect to morpho-physiological pollen properties): IV, II, III, I (only for *T. baccata*) and V. This ranking of parks is not similar to the rankings obtained by the analysis of morphometric values of tree needles (statistic results are not presented). However, we could generally conclude that the analysed conifers have the smallest pollen grains and the lowest pollen vitality in the park with the heaviest air pollution (V) (*Fig. 5*). The pollens of *T. baccata* and *C. atlantica* were most sensitive to air pollution (*Table 4; Fig. 5*). Furthermore, *P. omorika* and *P. nigra* showed better results when situated far from the traffic zones. Small, abnormal and non-vital pollen grains lead to lower germinability and energy of pollen germination (manifested in form of small pollen tubes) (*Fig. 5*). The most drastic examples of changes of pollen grains were observed in the park III, in case of *Cedrus atlantica* and *Picea omorika,* and in the parks IV and V, in case of *P. nigra* (*Table 4; Fig. 6*).

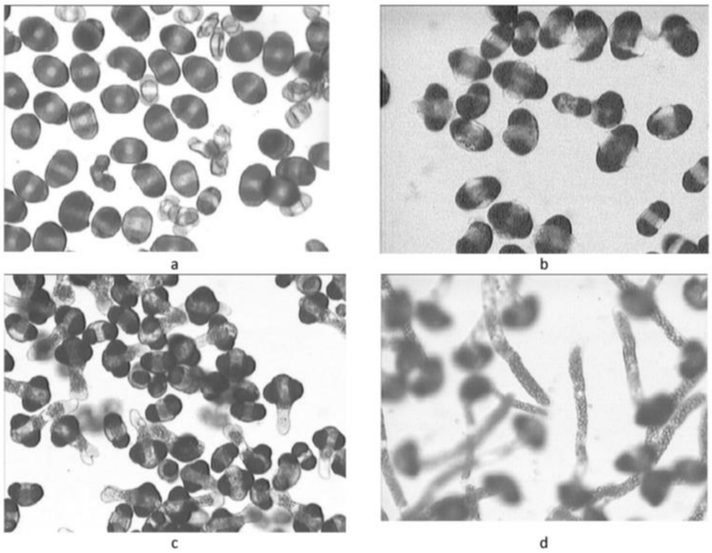

Figure 5. *Small, abnormal and non-vital pollen grains of Cedrus atlantica – V (a) and Picea omorika – V (b). Good germinability of Pinus nigra – IV (c) and C. atlantica – III (d) pollen grains*

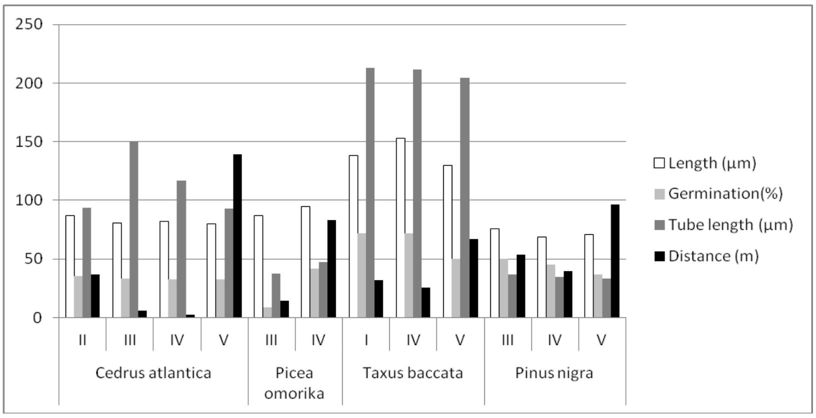

Figure 6. *Graphical illustration of average values of pollen grains length, length of pollen tubes and distance from traffic in Cedrus atlantica, Picea omorika, Taxus baccata, and Pinus nigra*

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In some analyzed species distances of particular trees from heavy traffic also were in significant correlation with needle dimensions (*A. concolor*, *A. nordmanniana and P. omorika*, r = -0.93, 0.99, and 0.56, respectively, *Fig. 7*), stomatal density (*A. alba*, *P. abies*, *P. omorika*, *P. pungens*, r = -0.99, 1.0, -0.70, and 0.71, resp., *Fig. 8*) and pollen vitality (germinability: *C. atlantica*, r = 0.73, *P. nigra*, r = 0.57 and *T. baccata*, r = 0.68, *Fig. 9;* length of pollen tubes: *A. pinsapo*, r = 0.99 and *P. menziesii*, r = 0.95, *Fig. 10*).

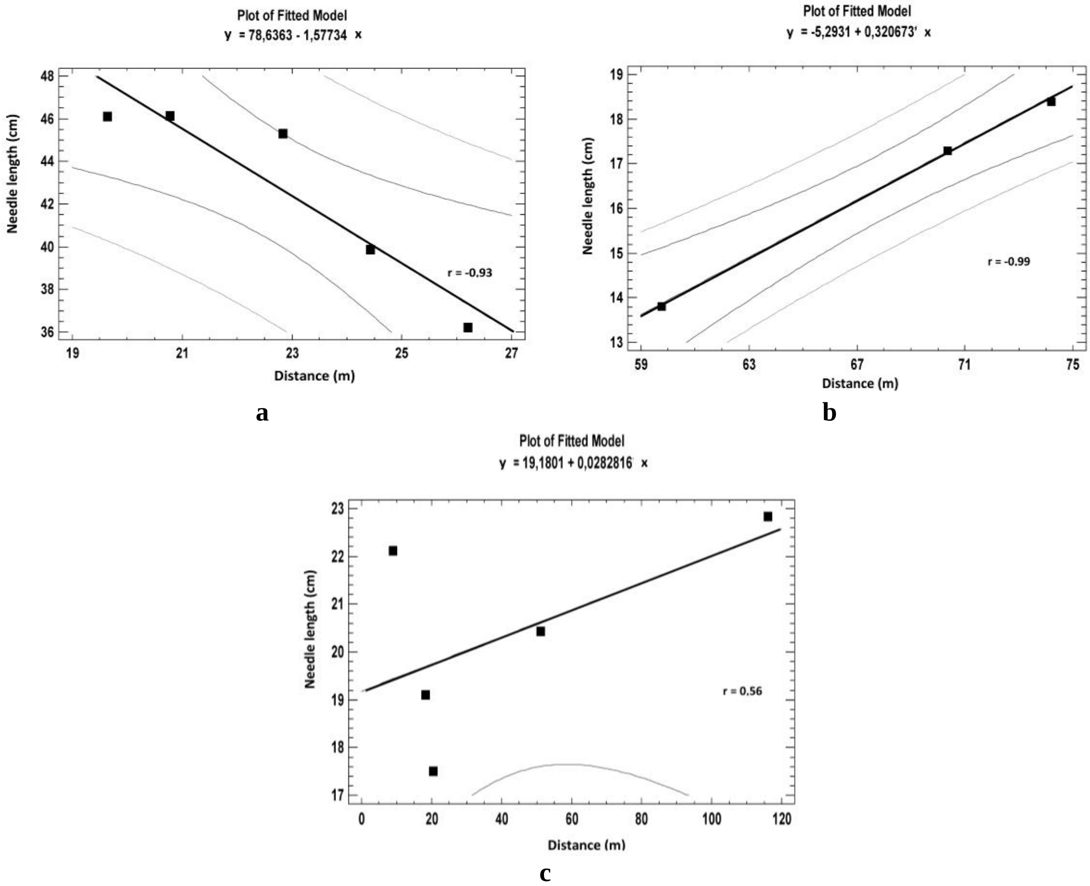
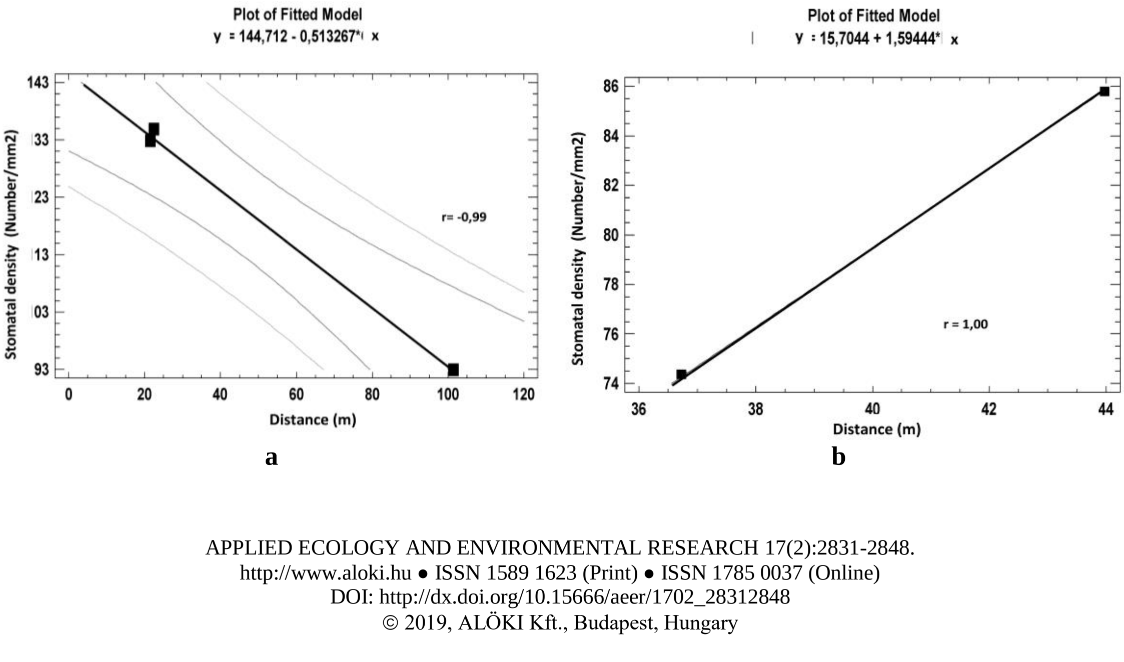


Figure 7. *Correlation between needle length and distance from traffic in Abies concolor (a), A. nordmanniana (b) and Picea omorika (c)*



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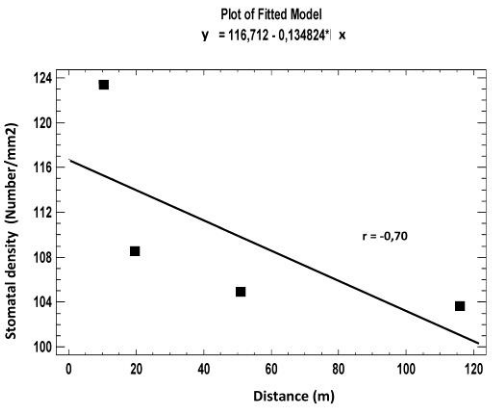
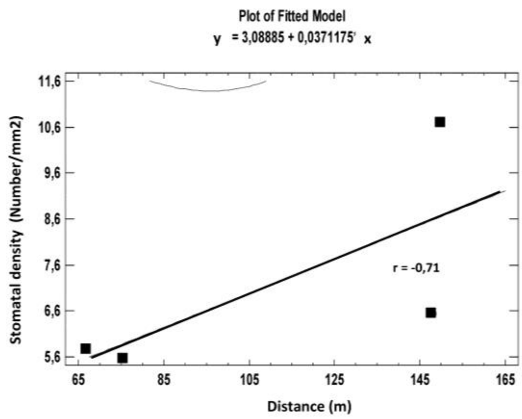


c



d

Figure 8. *Correlation between stomatal density and distance from traffic in Abies alba (a), Picea abies (b), Picea omorika (c) and P. pungens (d)*

Table 4. *Results of morpho-physiological analysis of pollen grains and distance of trees from traffic*

Park No.	Tree No.	Pollen analyses					Distance of trees from traffic (m)
		Morphological			Physiological		
		Dimensions		Shape (%)	Vitality		
		Length (µm)	Width (µm)		Germination (%)	Length of pollen tubes (µm)	
Abies alba							
III	96	149.47	103.31	69.45	48.45	39.77	100.3
Mean ± SD		**149.47 ± 0.00**	**103.31 ± 0.00**	**69.45 ± 0.00**	**48.45 ± 0.00**	**39.77 ± 0.00**	**100.3 ± 0.00**
Abies nordmanniana							
I	149	123.76	88.66	72.17	62.91	44.59	74.1
	150	139.73	96.07	69.01	51.27	52.01	70.3
Mean ± SD		**131.75* ± 11.29**	**92.36* ± 5.24**	**70.59* ± 2.23**	**57.09 ± 8.23**	**47.74* ± 5.25**	**72.2 ± 2.69**
Abies pinsapo							
IV	8	130.16	95.40	73.63	47.00	27.15	25.6
	373	142.39	95.86	68.17	46.81	51.03	96.8
	375	138.80	94.38	67.39	60.26	50.57	92.2
Mean ± SD		**137.12* ± 6.29**	**95.21 ± 0.76**	**69.73* ± 3.40**	**51.36 ± 7.71**	**42.92* ± 13.66**	**74.8 ± 39.85**
Cedrus atlantica							
V	26	79.00	54.13	68.60	18.36	71.38	86.8
	80	81.27	60.50	74.70	24.13	108.85	44.3
	Mean ± SD	*80.14 ± 1.60*	*57.32* ± 4.50*	*71.65*± 4.31*	*21.25* ± 4.08*	*92.79* ± 26.50*	*67.0 ± 30.05*
IV	59	83.03	52.73	63.66	31.40	110.39	148.2
	68	80.64	54.99	68.43	32.99	111.46	161.9
	83	82.27	50.86	61.90	52.53	128.37	133.1
	Mean ± SD	*81.98 ± 1.22*	*52.86* ± 2.07*	*64.66* ± 3.38*	*38.97* ± 12.03*	*116.94* ± 10.09*	*147.7 ± 14.41*

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III	322	79.02	51.11	64.78	3.70	101.90	10.8
	329	84.26	56.70	67.46	33.58	157.80	32.2
	335	78.04	53.17	74.20	12.86	174.34	34.9
	Mean ± SD	*80.44* ± 3.34*	*53.66* ± 2.83*	*68.81* ± 4.85*	*16.72* ± 15.31*	*150.33* ± 37.96*	*26.9 ±13.20*
II	47	86.91	55.69	64.17	3.51	93.79	4.5
	Mean ± SD	*86.91 ± 0.00*	*55.69 ± 0.00*	*64.17 ± 0.00*	*3.51 ± 0.00*	*93.79 ± 0.00*	*4.5 ± 0.00*
Mean ± SD		**81.61* ± 2.85**	**54.43* ± 3.00**	**67.54* ± 4.52**	**23.67* ± 15.85**	**119.82* ± 31.75**	**61.5 ± 61.09**
Picea abies							
III	382	114.54	74.07	64.63	24.59	75.36	62.1
Mean ± SD		**114.54 ± 0.00**	**74.07 ± 0.00**	**64.63 ± 0.00**	**24.59 ± 0.00**	**75.36 ± 0.00**	**62.1 ± 0.00**
Picea omorika							
IV	362	94.91	57.85	61.18	28.19	36.89	115.9
	367	96.99	56.43	58.31	19.54	53.26	82.4
	495	92.50	52.84	57.58	77.30	46.59	51.2
	Mean ± SD	*94.80* ± 2.25*	*55.71* ± 2.58*	*59.02* ± 1.90*	*41.68* ± 31.15*	*47.61* ± 8.23*	*83.1 ± 32.36*
III	1	86.97	53.70	61.94	2.88	27.0	9.3
	4	86.94	56.23	64.94	14.51	37.82	19.7
	Mean ± SD	*86.95 ± 0.02*	*54.96* ± 1.79*	*63.44* ± 2.12*	*8.70* ± 8.22*	*32.41* ± 7.65*	*14.5 ± 7.35*
Mean ± SD		**91.66* ± 4.58**	**55.41 ± 2.07**	**60.79* ± 2.96**	**28.48*± 28.78**	**40.31*± 10.03**	**55.7 ± 44.17**
Pinus nigra							
V	39	73.21	45.04	61.61	83.51	37.28	101.0
	45	72.19	43.96	60.96	78.27	32.81	95.1
	46	68.23	43.34	63.60	36.67	27.65	93.1
	Mean ± SD	*71.21* ± 2.63*	*44.11 ± 0.86*	*62.06* ± 1.37*	*66.15* ± 25.66*	*33.48* ± 4.82*	*96.4 ± 4.11*
IV	2	69.28	46.76	67.64	63.02	41.51	41.6
	4	64.60	40.53	62.98	22.71	27.45	34.9
	6	72.48	44.92	62.22	50.56	33.37	44.0
	Mean ± SD	*68.78* ± 3.96*	*44.07* ± 3.20*	*64.28* ± 2.93*	*45.43* ± 20.64*	*35.11* ± 7.06*	*40.1 ± 4.72*
III	9	79.42	52.60	66.42	52.49	38.46	50.5
	10	69.15	43.83	63.35	42.74	28.81	53.6
	11	78.70	51.00	64.89	54.41	40.65	56.5
	Mean ± SD	*75.76* ± 5.73*	*49.14* ± 4.67*	*64.89* ± 1.53*	*49.88 ± 6.26*	*37.18* ± 6.30*	*52.2 ± 3.00*
Mean ± SD		**71.92* ± 4.82**	**45.78* ± 3.82**	**63.74* ± 2.21**	**53.82* ± 19.24**	**34.92* ± 5.51**	**46.1 ± 25.68**
Pseudotsuga menziessi							
V	205	97.98	85.37	87.71	76.58	140.19	53.6
	Mean ± SD	*97.98 ± 0.00*	*85.37 ± 0.00*	*87.71 ± 0.00*	*76.58 ± 0.00*	*140.19 ± 0.00*	*53.6 ± 0.00*
III	46	126.56	100.32	80.02	67.42	147.70	78.2
	383	132.76	102.78	78.48	54.97	143.64	71.6

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	384	144.74	102.60	71.80	80.16	142.62	62.6
	Mean ± SD	*135.94* ± 9.24*	*102.14 ± 1.37*	*76.27* ± 4.37*	*67.51* ± 12.59*	*144.65 ± 2.69*	*70.8 ± 7.83*
Mean ± SD		**125.40* ± 19.84**	**97.49* ± 8.34**	**79.44* ± 6.53**	**69.78* ± 11.24**	**143.54 ± 3.13**	**62.2 ± 10.72**
Taxus baccata							
V	242	111.26	99.32	89.56	50.50	194.81	66.2
	243	148.42	129.00	86.30	49.88	214.13	67.4
	Mean ± SD	*129.84* ± 26.28*	*113.71* ± 20.99*	*87.93* ± 2.30*	*50.19 ± 0.44*	*204.47 ± 13.66*	*66.8m ± 0.85*
IV	791	150.07	131.50	87.70	75.40	201.62	27.1
	792	179.32	156.71	87.68	69.82	215.82	25.0
	793	129.89	116.56	90.25	71.02	216.94	24.7
	Mean ± SD	*153.10* ± 24.85*	*134.89* ± 20.29*	*88.54 ± 1.48*	*72.08 ± 2.94*	*211.46 ± 8.54*	*25.6 ± 1.31*
I	340	134.72	115.35	86.60	74.68	213.03	61.5
	669	139.98	124.83	89.73	69.76	212.78	3.2
	Mean ± SD	*138.23 ± 3.72*	*121.67 ± 6.70*	*88.69 ± 2.21*	*72.22 ± 3.48*	*212.91 ± 0.18*	*32.3 ± 41.22*
Mean ± SD		**142.72* ± 21.01**	**125.50* ± 17.73**	**88.37 ± 1.58**	**65.87* ± 10.94**	**209.88 ± 8.33**	**38.2 ± 25.41**

*****Significant differences (*p < 0.05*)

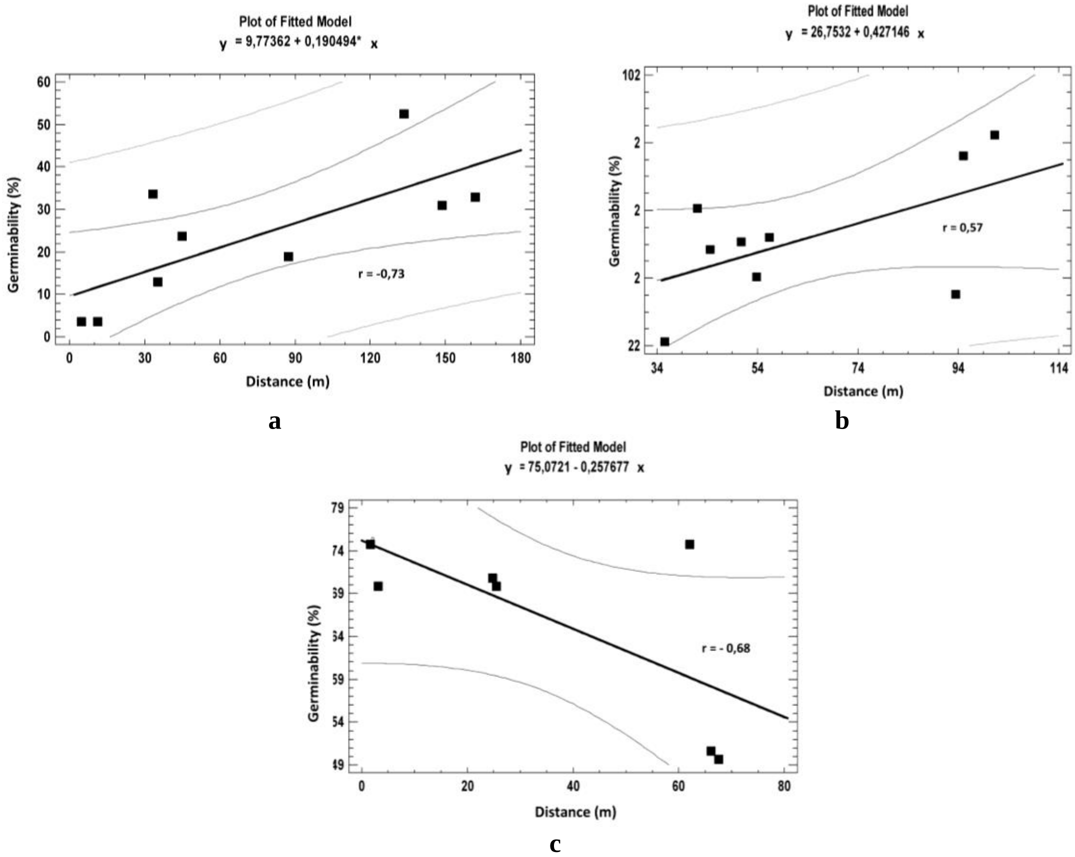

Figure 9. *Correlation between germinability and distance from traffic in Cedrus atlantica (a), Pinus nigra (b), and Taxus baccata (c)*

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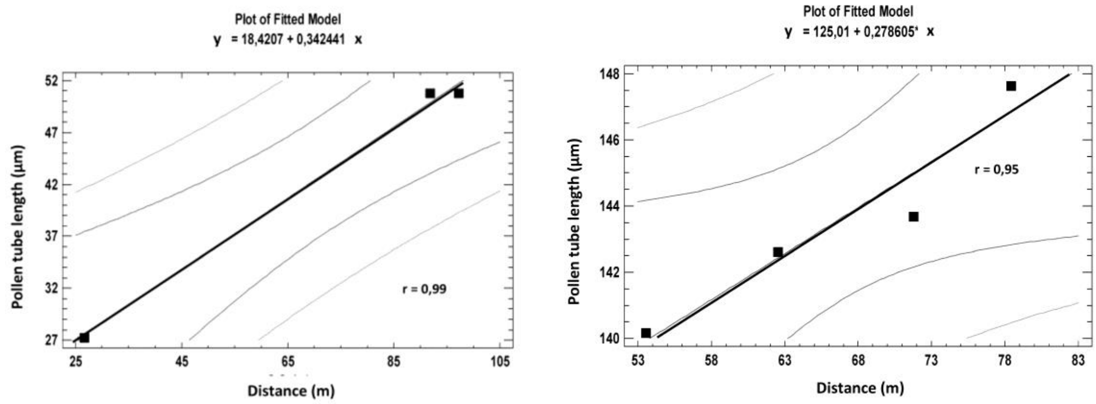

a b

Figure 10. *Correlation between pollen tube length and distance from traffic in Abies pinsapo (a), and Pseudotsuga menziesii (b)*

Discussion

Conifer foliage is a useful indicator in bio-monitoring of air pollution (*Pinus ponderosa*). Several of investigated species Vukićević (1987) considered resistant to urban conditions (*A. concolor*, *A. nordmanianna*, *A. pinsapo*, *C. atlantica* and *P. omorika*). The average needle length and width for all twelve investigated species are in agreement with the published results of Vidaković (1982) and Vukićević (1987). The needle length and width of *A. alba* from park V also agree with previous results obtained by Pawlaczyk et al. (2005) and Pawlaczyk and Bobowicz (2008). The average needle length and width of *P. omorika* are also in agreement with the results obtained in natural habitats (Milovanović et al., 2005; Radovanović et al., 2014; Nikolić et al., 2015). Furthermore, dimensions of *Pinus nigra* from park V are in agreement with the results obtained by Matziris (1984) and Borzan et al. (2002). The values of needle length and leaf area in our *A. pinsapo* from park IV are higher than those already reported by Sękiewicz et al. (2013). The needle length of *Cedrus atlantica* also agrees with the results obtained by Jasińska et al. (2013), while needle width was about 25% higher. Needle width of *Pinus sylvestris* was higher in CO_2 polluted air, as a result of an increase of messophyl tissue (Lin et al., 2001). In some other conifers, air-polluted needles are shorter, with lower number of stomata (*Pinus pinaster*, Wahid et al., 2006). Effects of drought could be found even at the level of needle anatomy, where stomatal density increased as needle length decreased (*Pinus canariensis*, Grill et al., 2004).

Dimensions of trees and their leaves could depend on soil nutrition. Addition of nitrogen increased length, width and number of needles per shoot in *P. menziesii* (Brix and Ebell, 1969).

In *A. alba*, *A. nordmanniana* and *A. pinsapo* stomatal density was higher (*Table 1*) than in literature (Robakowski et al., 2004; Meidner and Mansfield, 1968; Sancho-Knapik et al., 2014, resp). The same situation was found in *P. abies* and *P. pungens* (Dixon et al., 1995 and Meidner and Mansfield, 1968, resp.). In *T. baccata* stomatal density is lower in park V than in other investigated parks, but similar with literature results (Stefanović, 2015).

Our results of pollen length of *P. abies* and *P. omorika* were slightly higher than those reported by Jia et al. (2014), but germination was significantly low, especially of *P. omorika* in park III (*Table 2*). In our previous investigations germination of *P.*

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omorika pollen was 54-68% (max. 94%) (Batos and Nikolić, 2013). In case of *P. menziesii*, the average values of pollen size were similar to those reported in the literature (Ho, 1968). The air pollution changed the properties of leaves and pollen grains through chemical changes. An increased content of lead in the air influenced lower pollen germination and pollen tube growth in *Pinus strobus* and *P. resinosa* (Cox, 1988). In case of *Abies alba*, contaminated pollen decreased vitality and germination up to 50%, which depended on genotype-specific response to air pollution (Kormuťák, 1996 and refs. cited therein). The same situation is found in our results of *A. alba* (*Table 2*), where decreased germinability and length of pollen tubes was also up to 60% and 50 μm, respectively. Furthermore, in some species (*P. omorika, P. nigra*, etc.) we found some abnormalities in dimensions, shape and vitality of pollen grains, which occurred slightly more frequently in more heavily contaminated parks (case of *C. atlantica*, *P. omorika*, etc., *Table 2, Figs. 5* and *6*).

We have to underline that presented results of morpho-anatomical and pollen propertiesof conifers in urban area have never been explored so detailed in the past.

Conclusions

Based on the presented results of morpho-anatomical and morpho-physiological characteristics of conifer needles and pollen, respectively, it can be concluded that the consequences of air pollution are more apparent in case of pollen. In city parks with heavy traffic (V, IV, III), pollen grains of trees were smaller and less vital. Very often results were in correlation with distance of trees from traffic. Some of examined conifer species are especially sensitive to air pollution caused by vehicles (*Picea omorika*, *Cedrus atlantica*, *Taxus baccata*, *Pinus nigra*), especially in pollen properties. The level of air contamination, as well as other atmospheric conditions (temperature, humidity, wind direction and strength, etc.) could vary among different years, too. All the above factors and their influence on growth, development and blooming of trees, should be taken into consideration in landscape architecture and horticulture works in future.

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