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ANATOMICAL AND PHYSIOLOGICAL CHANGES IN NEEDLES OF *PINUS NIGRA* J.F. ARNOLD REVEAL URBAN TRAFFIC AIR POLLUTION DRIVEN EFFECTS

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Abstract
The presence of trees in the urban area has many advantages for improving the environmental conditions and implicitly for the urban health and wellbeing. The urban environment, in turn, can be a polluting factor that can reduce trees health and physiological performances, due to various specific environmental constraints, including pollutants resulting from street traffic emissions. In this study, the changes of needles anatomical and physiological characteristics of the Pinus nigra species grown in urban area (Dendrological Park of the University of Agronomic Sciences and Veterinary Medicine from Bucharest and Road-Side urban trees) were quantified in order (1) to establish the effect of vehicles pollution on needles anatomy, (2) to establish the effect of vehicles pollution on needles gas exchanges, and (3) to evaluate the impact on assimilatory pigments content. We found that growth conditions have had affected all needle's analyzed attributes. Our results can be added to the existing ones as regard as the P. nigra vulnerability under urban environments and its use as a bioindicator of urban pollution.

Key words: *leaf, Pinus nigra, urban pollution.*

INTRODUCTION

The transformation of natural and agricultural land into urban areas is increasing every year, leading by all the related attributes the environmental degradation in relation to plant growth and increase plant stress (Czaja et al., 2020), as well as to the exacerbation of the climate change caused to urbanization (Hamdi et al., 2020; Ehrnsperger, & Klemm, 2022). As early as ten years ago, Solecki & Marcotullio (2013) point out that maintaining urban biodiversity levels may be key not only to urban residents, but also to global biodiversity in an urbanized future. Thus, the presence of trees in urban areas may be one of the strategies that can help strengthen the global response to climate change (Parsa et al., 2019; Lüttge & Buckeridge, 2020).

The ecological assessment of urban spaces is based on vegetation, which serves as an indicator of environmental conditions and ecological processes, acting both as bioattenuators and as bioindicators (due to their ability to absorb and degrade pollutants) (Jain et al., 2022). Plants are the ones that influence the environment and which in turn are influenced by it (Onete et al., 2010).

The presence of trees in the urban environment influences by multiple ways the microclimate (Leuzinger et al., 2010; Millward & Sabir, 2011; Gratani et al., 2016), can perform multiple ecological functions, can provide beneficial services for human well-being (Gerstenberg et al., 2016; Hall & Knuth, 2019; Machar et al., 2019; Fineschi & Loreto, 2020) and counteracting unfavorable conditions (Pace et al., 2021). If the right selection of trees species, proper planting and management of plants are ensured, urban biodiversity can grow and contribute to biological richness. In addition, trees are particularly suitable as indicators for prevailing environmental conditions (Lüttge & Buckeridge, 2020) and help to attenuating poor air quality etc. (Millward & Sabir, 2011; Sicard et al., 2018; He et al., 2020; Klingberg et al., 2022). The ability of trees to improve air quality in urban areas has also been demonstrated by measuring the accumulation of 32 poly-cyclic aromatic hydrocarbons (PAHs) in leaves of *Quercus palustris* and needles of *Pinus nigra*. A significant accumulation of low molecular mass

L-PAH (mainly gaseous) and high molecular mass PAHs (mainly particle-bound) was recorded in the 3-year-old pine needles, compared to the one-year-old ones, the pine proving superior to the oak from this point of view (Klingberg et al., 2022).

Moreover, the selection of species of urban greening must be done with attention with a view to also control and mitigate their pollution effects (Lyu et al., 2021).

There are many studies on the effects of pollution on plants, both as regard as the morpho-anatomical, as well as physiological and biochemical changes, as very well have already synthesized by Uka et al. (2017). For *Pinus sylvestris* species, Lin et al. (2001) shows a reduction in stomata density at both leaf surfaces by assuring a high concentration of carbon dioxide, although the number of stomata rows did not change significantly. Also, Fedorchak (2020) have highlighted the significant impact of industrial and vehicle exhaust emissions on assimilatory pigments in *Picea abies* and *Picea pungens*. It was suggested the possible involvement of the assimilatory pigment complexes of these species as bioindicators of air pollution status, available throughout the year.

Biomonitoring of the environment is a major concern nowadays (Hutnik et al., 2014; Juranović-Cindrić et al., 2018; Nikolić et al., 2019; Schulz et al., 2019; Trstenjak et al., 2020). In this regard, the species used include conifer needles, lichens, mosses, bees, and their by-products, as well as snails and have been widely used in recent research (AL-Alam et al., 2019). As the authors summarize, those of conifers, which are usually kept for at least five years, are well suited and provide information on the emission of pollutants for longer periods (due to waxy leaves and increased affinity for polar compounds). It is worth noting that the most studied species are *Pinus pinea* and *P. nigra*, whose geographical distribution is particularly large and whose needles have proved to be the most examined.

Given the above, an additional challenge for practitioners is the selection of the most suitable tree species for the urban parks of the future, in relation to regional and local climatic conditions, as well as considering their degree of tolerance to stressors (Brune, 2016). For the optimal arrangement of green infrastructure, so that cities stay healthy and thriving, and to mitigate the effects of climate change, the principle of "the right plant in the right place and with the right management" must be considered (Hasan et al., 2017; Ferrini et al., 2020; Petrova, 2020; Velasco-Jiménezet et al., 2020) that will be translates into a better quality of life and reduction of urban pollution (Dobrescu & Fabian, 2017).

In the context of these concerns, the aim of this study was to characterize the effects of urban traffic (and not only) on *Pinus nigra* needles (1) anatomy, (2) gas exchanges and other relevant associated indicators and (3) assimilatory pigments content.

MATERIALS AND METHODS

Experimental site and biological material used

The studies were carried out during the year 2021, on the black pine *Pinus nigra* J.F. Arnold needles collected from trees grown in the Bucharest area, Romania. The needles taken from the trees growing in the Dendrological Park (DP) of University of Agronomic Sciences and Veterinary Medicine (UASVM) from Bucharest and, respectively, that of the Road-Side trees (RS), along Ion Mihalache Boulevard were analyzed in parallel. For anatomical observations, needles formed in the current year were used (CYN), and for physiological one's needles formed in the previous year, i.e. one-year-old needles (PYN) and needles formed in the current year (CYN) were analyzed.

Pinus nigra needle anatomy analysis

Acicular leaves from 1-year-old branches, taken at in June, were analyzed. Anatomical observations and measurements were carried out on cross-sections in the middle area of the leaves. The sections were clarified with chloral hydrate and stained with carmine-alum and iodine green, according to the classical method (Morlova et al., 1966). The following anatomical needle traits were measured: E = Epidermis; C = Cuticle; H = Hypodermis; M = Mesophyll; Rc = Resin canals; En = Endodermis; Tt = Transfusion tissue; Vb = Vascular bundles; X = Xylem; Ph = Phloem.

Observations, measurements, and photographs were taken with the LEICA DM 1000 LED optical microscope, LEICA DFC 295 video camera and LEICA S 8 APO stereomicroscope, using 4x, 10x lenses, existing in the endowment of the Research Center for Studies of Food Quality and Agricultural Products (UASVM Bucharest).

In situ analysis of gas exchanges at the needles level

The values of needles gas exchanges were measured *in situ* with the help of the portable system for photosynthesis (LCpro-SD ADC BioScientific) equipped with an infrared gas analyzer, immediately after the randomly detachment of those from the sun exposed exterior branches (2 m above the ground) of three individual trees, in environmental conditions specific to spring (late March), early summer (June) and early autumn (September), 2021. As Robakowski and Bielinis (2017) found, the rate of photosynthesis in detached 1-year-old *Abies alba* needles was not significantly different from that of those attached. Parallel determinations were performed for those DP and RS trees (6 needles randomly selected - n = 3), between hours 10:00 AM - 12:00 AM, for the following physiological indicators: the intensity of photosynthesis (A) (µmol CO_2 $m^{-2}s^{-1}$), the intensity of transpiration (E) (mmol H_2O $m^{-2}s^{-1}$), the respiration rate - R (µmol CO_2 $m^{-2}s^{-1}$), the stomatal conductance (g_s) (mol H_2O $m^{-2}s^{-1}$), and the intercellular concentration of carbon dioxide (CO_2i) (µmol CO_2 L^{-1} air). Along with these, the value of the photosynthetic photon flux density (PPFD) was recorded (data are not shown). Based on the obtained data, other indicators associated with gas exchanges were calculated, respectively: the instantaneous water use efficiency (WUE) (A/E), intrinsic water use efficiency (iWUE) (A/g_s), the quantum yield (φCO_2) (A/PPFD), as well as the instantaneous carboxilation efficiency (ICE) (A/ CO_2i).

The assimilating pigments content of the needles

The Arnon spectrophotometric method was used for the quantitative determination of assimilating pigments. On briefly, the needles were taken from the tree, placed in Eppendorf's tubes and transported quickly to the laboratory, where the extraction in acetone 80% (Lichtenthaler, 1987) has been done. Then, the absorbance of the clarified extracts was measured using the spectrophotometer (Helios Alpha Thermo Scientific) at three wavelengths (663 nm to determine chlorophyll *a* - Chl *a*; 646 nm for chlorophyll *b* - Chl *b* and 470 nm for carotenoids). Afterwards, the content of assimilating pigments was calculated by using the specific formulas reported by Lichtenthaler (1987). The obtained values were finally expressed as mg $100g^{-1}$ fresh weight (FW).

Statistical analysis

The reported data of all parameters represent the mean ± standard error (SE). Statistically significant differences between the mean values were assessed using one-way analysis of variance (ANOVA). Then, the paired-samples Student T test (2-tailed) were accomplished and the significant differences among variants have been considered at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Pinus nigra needle anatomy

The leaf anatomy data of *P. nigra* are shown in Table 1 and these results are according to those reported in the literature (Andrei, 1978; Georgescu et al., 1999; Alvarez, 2009; Dörken & Stützel, 2012).

Very strongly statistically significant differences are observed ($P < 0.001$) in the case of needles collected from Street Trees for epidermis (23.4 µm), cuticle (4.40 µm), transfusion tissue (585.37 µm) and phloem (61.41 µm) compared to the values characteristic of those taken from the Dendrological Park trees (15.33; 2.87; 435.27 and respecttively, 35.79 µm). There were also significantly higher values ($P < 0.01$) for the vascular bundles (RS -126.59 vs. DP - 103.01 µm), as well as for the hypodermis ($P < 0.05$) (RS – 34.07 vs DP-16.29 µm). In the case of mesophyll, resin canals and xylem, there were no statistically significant differences ($P > 0.05$).

In a cross section, the *P. nigra* leaf has a semicircular contour, with a flat upper face being formed by the epidermis, the mesophyll and the central cylinder (Figure 1a, Figure 1 b, and Figure 1c).

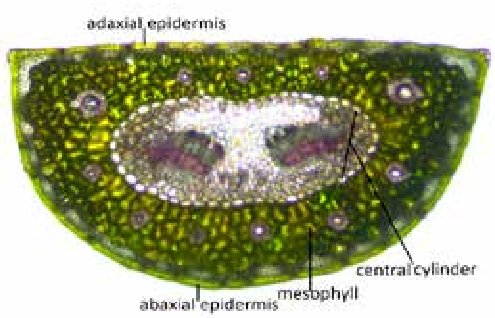

Figure 1a. Transversal section on the leaf of *Pinus nigra*

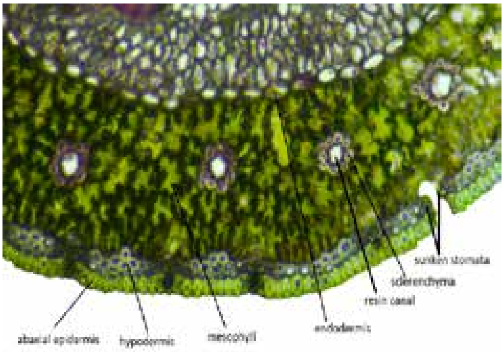

Figure 1b. Transversal section, detail of the leaf anatomy of *Pinus nigra*

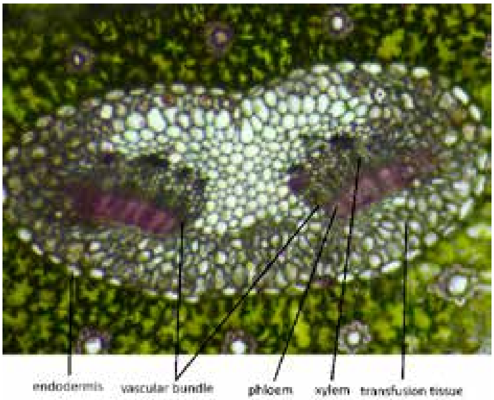

Figure 1c. Transversal section, detail of the central cylinder of the leaf of *Pinus nigra*

From the analysis of the literature, tissue thickness differs depending on the area and climatic factors (Tiwari et al., 2013; Crivellaro & Schweingruber, 2015). The epidermis is made up of cells with thick, low lumen walls in the shape of the letter X, covered by a layer of cuticle. The hypodermis consists of 1-3 layers of cells, with lignified, sclerified walls, being thicker in the two edges of the leaf, while mesophyll consists of septate cells.

Table 1. Anatomical characteristics of current year needles (CYN) of *Pinus nigra* from Dendrological Park (DP) and Road-Side (RS) trees. Mean value ± Standard Error. N=7

	Specification	*Pinus nigra* DP	*Pinus nigra* RS	P value
Thickness (µm)	E = Epidermis	15.33± 0.56	23.43± 1.48***	<0.001
	C = Cuticle	2.87±0.21	4.40±0.21***	<0.001
	H = Hypodermis	16.29±0.98	34.07±5.65*	<0.05
	M = Mesophyll	113.34±10.37	119.97±.89ns	>0.05
	En = Endodermis	18.07±0.77**	13.83±0.97	<0.01
	Tt = Transfusion tissue	435.27± 14.21	585.87±12.39***	<0.001
	Vb = Vascular bundles	103.01±1.84	126.59±4.95**	<0.01
	X = Xylem	67.66±2.38ns	65.17±2.46	>0.05
	Ph = Phloem	35.79±2.59	61.41±2.62***	<0.0001
Diameter (µm)	Rc = Resin canals	30.70±2.55ns	25.57±1.25	>0.05

Resin canals are also present, and their size is influenced by the number of secretory cells and sclerenchyma fibers (Ghimire, 2014). In the middle of the leaf there is the central cylinder surrounded by a primary endoderm, under which is the transfusion tissue and, the xylem and the phloem are located. Between the conducting fascicles there is a parenchyma comprised of parenchymal cells.

Stomata are located below the external level of the epidermis, having a large substomatal chamber (Figure 1c). Stomata number was proved to be significantly lower at a high carbon dioxide (CO_2) concentration (Lin et al, 2001; Domec et al., 2015). Nikolić et al. (2019) showed that for *P.nigra* (trees with shorter needles) a higher stomatal density was observed as against other 11 conifers studied species. Besides, the size of the needles as well as pollen vitality were also correlated with the distance from the heavy traffic.

Gas exchanges at the needles level at field site and associated indicators

The obtained results regarding the gas exchanges specific to the two studied variants and their seasonal dynamics are presented in Table 2.

Maximum photosynthesis rate (A) (5.66 µmol CO_2 m^{-2} s^{-1}) was determined in June, for previous year leaves (PYN), in the conditions of the Dendrological Park (DP) and at the opposite pole are the specific values for the leaves of the same age (1.11 µmol CO_2 m^{-2} s^{-1}), determined in September, but grown in the environmental conditions specific to street traffic (RS). We also noticed the maximum values of A during the early spring (RS - 5.37 and DP -5.01 µmol CO_2 m^{-2} s^{-1}, respectively), when the transpiration rate and stomatal conductance had low values, with non-significant differences between the two sites.

Needles transpiration (E) and stomatal conductance (g_s) also declined with needle age. In addition to the obviously lower values of A in the case of SR tree leaves, there is also a decrease in the process rate as the age of the needles increases.

Table 2. Seasonal course of photosynthesis rate (A), transpiration rate (E), stomatal conductance (g_s), intercellular carbon dioxide concentration (CO_2 i), respiration rate (R) and other associated parameters of *Pinus nigra*: previous year needles (PYN) and current year needles (CYN) from Dendrological Parc (DP) and Road- Side (RS) trees. Data are the Mean values ± Standard Errors. N = 3

March		June				September			
PYN		PYN		CYN		PYN		CYN	
DP	RS	DP	RS	DP	RS	DP	RS	DP	RS
Photosynthesis rate (A) (µmol CO_2 m^{-2} s^{-1})									
5.01 ±1.06	5.37 ±1.32^{ns}	5.66 ±0.21**	2.00 ±0.12	4.83 ±0.82^{ns}	3.58 ±0.59	4.34 ±0.15*	1.11 ±0.10	4.59 ±0.03*	2.88 ±0.09
Transpiration rate (E) (mmol H_2O $m^{-2}s^{-1}$)									
1.42 ±0.24	1.57 ±0.26^{ns}	4.28 ±0.63*	1.20 ±0.19	5.73 ±0.95*	2.19 ±0.28	2.22 ±0.03*	1.29 ±0.01	2.47 ±0.05**	1.66 ±0.04
Stomatal conductance (g_s) (mol H_2O $m^{-2}s^{-1}$)									
0.079 ±0.017^{ns}	0.075 ±0.018	0.082 ±0004**	0.029 ±0.003	0.152 ±0.024^{ns}	0.115 ±0.005	0.051 ±0.013^{ns}	0.046 ±0.01	0.07 ±0.01^{ns}	0.055 ±0.013
Intercellular carbon dioxide (CO_2) concentration (CO_2 i) (µmol CO_2 L^{-1} air)									
325 ±8.55	294 ±14.80	303 ±0.9	329 ±4.3	331 ±24.5	377 ±10.2	241 ±0.1	309 ±35.5	241 ±40.5	262 ±34.4
Respiration rate (R)(µmol CO_2 m^{-2} s^{-1})									
2.62 ±0.41	4.54 ±0.65*	3.42 ±0.25*	2.28 ±0.09	14.01 ±0.55***	9.81 ±0.54	5.92 ±0.14	8.03 ±0.19^{ns}	4.97 ±8.34	8.34 ±0.57*
Water use efficiency (WUE)(A/T) (µmol CO_2 m^{-2} s^{-1}/ mmol H_2O $m^{-2}s^{-1}$)									
3.528	3.420	1.322	1.667	0.843	1.635	1.955	0.860	1.858	1.735
Quantum yield (φCO_2) (A/PPFD) (µmol CO_2 m^{-2} s^{-1}/ µmol photons $m^{-2}s^{-1}$)									
0.0042	0.0044	0.0047	0.0017	0.0040	0.0030	0.0036	0.001	0.0038	0.0024
Instantaneous carboxylation efficiency (ICE)(A/CO_2i) (µmol CO_2 m^{-2} s^{-1}/(µmol CO_2 L^{-1} air)									
0.0154	0.0183	0.0187	0.0061	0.0146	0.0095	0.0180	0.0036	0.0190	0.0110
Intrinsec waster use efficiency (IWUE) (A/g_s) (µmol CO_2 m^{-2} s^{-1}/ mol H_2O $m^{-2}s^{-1}$)									
63.412	71.60	69.024	68.966	31.776	31.13	85.098	24.13	65.571	52.36

Values have been compared in paired for the same leaf age and different growing conditions.
Differences significance is specifically marked at a probability of $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and $P>0.05$ (non-significant – ns).

Except for the March data, there is a decrease in the PYL of about 1.3 times, from June to September (DP), and about 1.8 times, respectively, in RS conditions. The trend was similar for CYN, except that the decrease was slower: 1.11 times for those in the park, and 1.24 times for those on the street.

As regard as the intercellular concentration of carbon dioxide (CO_2 i) the values were between 241µmol CO_2 L^{-1} air for the PYN, September, DP and respectively 377 µmol CO_2 L^{-1} air for CYN, June, RS. It is observed that except for the spring period, in all other cases, for the leaves of the RS trees the determined values are higher, compared to the values measured for the leaves of the trees in the park, if values are compared in pairs. Respiration rate varied between 2.28 µmol CO_2 m^{-2} s^{-1} (June, PYN, RS) and 14.01 µmol CO_2 m^{-2} s^{-1}) (June, CYN, DP). Significantly higher values were recorded for street trees: 4.54 µmol CO_2 m^{-2} s^{-1} (March, PYN) and September (CYN) (8.34 µmol CO_2 m^{-2} s^{-1}) as against the respiration rate in DP conditions.

Maximum values of WUE have been calculated in March (3.528 – PYN, DP), while during the seasons, the values decreasing were noticed till 0.843 (June, CYN, DP), in close relation with the specific highest transpiration rate. Moreover, the φCO_2 was highest in June (0.0047) (PYN, DP), while at the opposite pole was the value of 0.001 (September, PYN, RS), in relation with the highest carbon assimilation rate, and the lowest one, respectively.

Concerning the ICE, excepting the early spring period, when the differences between the two site were low, thereafter, at the pollution site the values were evidently less than at the park site. For instance, the lowest one was 0.0036 (PYN, September, RS), as against 0.0180 (PYN, September, DP).

As a measure of the potential water cost to maintain a given rate of photosynthesis per unit leaf area the IWUE was calculated. A maximum value was obtained in autumn (85.098) for PYN, DP, respectively the lowest one (24.13) in the case of PYN, RS for the same season.

The results agree with those obtained by Kinerson et al. (1974) in *Pinus taeda*, respecttively the highest A at those of one-year, during the late spring, corresponding to the greatest growth of the shoot, and of course, an obvious need for assimilated carbon for growth support in developing shoots and needles. Also, A declined for CYN as these began to mature (Ellisworth, 2000). For *A. alba*, Robakowski and Bielinis (2017) pointed out that with needle age, the structural adaptations that take place will influence too the rate of physiological processes, including an increase in internal resistance to CO_2. Thus, the rate of A decreased by 2 µmol CO_2 m^{-2} s^{-1}, while CYN recorded the highest A values (12 µmol CO_2 m^{-2} s^{-1}). Also, the rate of E, the WUE, as well as the g_s had lower values as the age of the leaf increased. On the other hand, at *P. sylvestris*, González-Rodríguez et al. (2019) emphasized an obvious decrease from late-spring till summer, reaching close to zero, in the case of A and E, respectively.

Regarding the possibilities of water conservation and increasing the WUE, according to the data obtained by Salazar-Tortosa et al. (2018), A values for *P. sylvestris* was maximum in June ((4.3 µmol CO_2 m^{-2} s^{-1}), had been similar those of other *Pinus* species, then a sharp decrease took place from June to July (2.85 µmol CO_2 m^{-2} s^{-1}). In the case of *P. nigra* and *P. halepensis* there were no significant differences between periods. Values of g_s have had a similar trend. Therefore, for *P. sylvestris* there were recorded the maximum iWUE values for both analysis data. Tomášková et al. (2017) state that *P. nigra* which grows well in southern Europe, the Mediterranean area and northern Africa, requires a continental climate compared to *P. sylvestris* and behaves better in extreme conditions. It can reduce the daily amount of E by 21% in one-year- needles and up to 50% in those of two years. And the studies conducted by Forner et al. (2018) highlighted the fact that the highest intra- and inter-annual plasticity in terms of WUE was observed in *P. nigra*, which maintained a higher water saving strategy. Naturally, the genus *Pinus* includes different species, with different needles lengths and with specific characteristics, which impact their anatomical traits (Wang et al., 2019). On this topic, additional research was conducted by Kuusk et al. (2018) on the characteristics of young (primary) and adult (secondary) (heteroblasts) needles in Mediterranean pines *P. halepensis*, *P.pinea* and *P.nigra*. There was highlighted that in the adult leaves, the fraction of supporting tissues increased positively with the known classification of drought tolerance of species (*P. halepensis* > *P. pinea* > *P. nigra*). In all species and ages of needles and plants, a negative correlation was observed between mesophyll volume fractions and structural tissues, showing a trade-off between investments in biomass and different needles functions. It is emphasized the ecological advantage of the juvenile morphophysiotype is to maximize the carbon gain when setting up plantations, while the characteristics of the adult needle improve the tolerance to environmental stress of plants stabilized at the final site.

An increase in the level of air pollution caused by heavy traffic can change the behavior of plants in terms of their ability to CO_2 assimilation, which has repercussions on other morphological, physiological, and biochemical characteristics.

Thus, changes in leaf thickness can affect stomatal movements, can lead to a decrease in g_s and therefore can reduce the process of A. The deposition of dust on the surface of the leaf decreases the intensity of the E and as an immediate consequence will be the increase of the internal temperature of the leaf (Singh și al. (2017). On *Lagerstroemia speciosa* trees on roadside, Singh et al. (2017) highlighted: a reduction of A with 36.7%, of E with 42.14%, of g_s with 66.85%. Also, an increase of stomatal resistance by 212.2%, of leaf thickness with 40.54%, as well as WUE with 9.4% have been noticed. In the same time, increased of lead (179.31%) and proline content (15.61%) were recorded as against to the trees exposed to less traffic area.

The assimilating pigments content of the *P.nigra* needles

Figure 2 shows the quantitative variability of the different categories of assimilating pigments. As expected, the highest values were recorded for chlorophyll *a* (Chl *a*), followed by chlorophyll *b* (Chl *b*) and carotenoids (C+X), respectively.

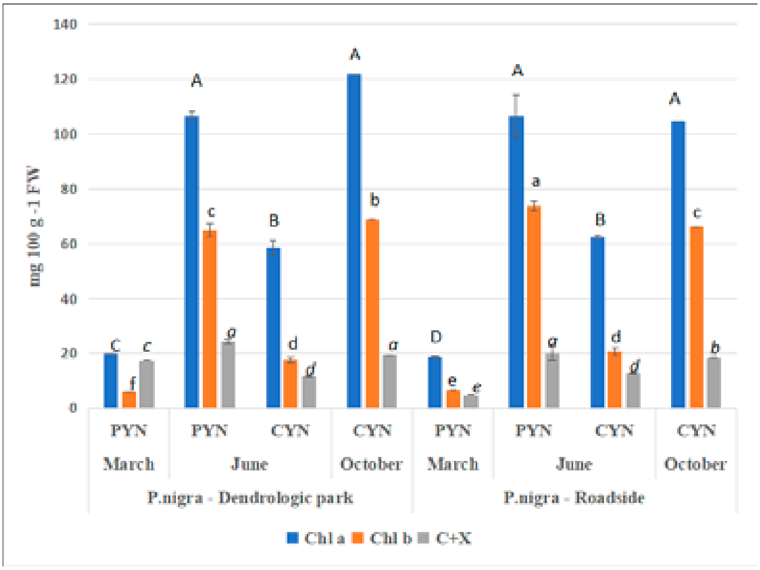

Figure 2. Assimilating pigments variation in *Pinus nigra* needles
Legend: Chl a= chlorophyll a; Chl b= chlorophyll b; C+X= carotenoids; PYN= previous year needles; CYN= current year needles. Columns are means ±standard errors. Different capital letters signify significant differences for Chl a; lowercase letters mean significant differences for Chl b. Italic lowercase letters mean significant differences for C + X. $P < 0.05$.

From the point of view of statistical analysis, the comparison of the data was performed separately, by categories, starting from the highest value. We note that in the case of Chl *a*, the highest values were determined in autumn, for those formed in the PYN (121.68 mg 100 g $^{-1}$FW), while the minimum values of 18.77 mg 100 g $^{-1}$FW were also characteristic for PYN, at the beginning of spring, and the differences were statistically significant ($P < 0.001$). If we compare the values for those trees in the DP and those on RS, we notice that at the beginning of summer, for PYN and CYN, respectively, there were no statistically significant differences ($P > 0.05$). But, it is obvious that between needles of different ages, the value differences are statistically significant.

At the same time, in the spring weather for PYN collected from the park, the values are significantly higher, compared to those specific to leaves taken from street trees ($P < 0.01$).

The maximum value of Chl *b* (73.85 mg 100 g $^{-1}$FW) was recorded in June at PYN, RS, while the minimum value (6.19 mg 100 g $^{-1}$FW) was calculated at the beginning of spring, for PYN, DP ($P < 0.001$). We also observed significantly higher values for PYN taken from RS trees (6.67 mg 100 g $^{-1}$FW) in March, compared to those in the DP (6.19 mg 100 g^{-1} FW) ($P < 0.001$). In early spring, higher values for PYN were recorded both in DP (64.83 mg 100 g $^{-1}$FW) and in RS (73.85 mg 100 g^{-1}FW), conditions, but statistically significantly higher for RS ($P < 0.05$). For CYN, at the beginning of the summer the values were lower for both studied variants and there were no statistically significant differences ($P > 0.05$). Data obtained in autumn, for CYN, in the case of both studied sites were significantly higher as against in June, and, in addition, for DP the value of 68.76 mg 100 g^{-1}FW, was significantly higher than 66.25 mg 100 g $^{-1}$FW, determined for needles taken from street trees.

The content of carotenoid pigments was between 24.24 mg 100 g^{-1}FW (needles from the previous year, June, DP) - 4.76 mg 100 g $^{-1}$FW (needles from the previous year, March, RS). In June, there were no significant differences for the two studied variants. In autumn, however, the amount of carotenoid pigments decreased significantly for leaves taken from SR trees, from 19.53 mg 100 g $^{-1}$ FW (DP) to 18.53 mg 100 g $^{-1}$FW (RS) ($P < 0.001$).

González-Rodríguez et al. (2019) studying the species *Pinus canariensis* mentions that the maximum values of Chl content were recorded in spring (578 μmol m^{-2}), then decreased during the year, reaching minimum values in winter (a decline of 15 %). The recovery of Chl was easily carried out the following spring, with significant changes for Chl *b*, and not for Chl *a*, respectively an increase of them with the decrease of the temperature. For C+X, the same tendency was noticed, with minimum value determined in spring. Their content increased throughout the seasons and that is interesting is the higher content of β-carotene during the winter and no recovery in the following spring.

As regard as the Chl fluorescence, the maximum values of quantum efficiency of photosystem II (PSII) photochemistry (F_v/F_m) were measured during late spring and early summer (0.82) and a markantly reduction was noticed in autumn and winter (0.65).

In the case of *P. sylvestris* plants exposed to technogenic pollution, Kalugina et al. (2018) noticed several changes, such as: the amount of Chl *a*, Chl *b* and C+X decreased by the mass of a needle by 23, 40 and 42%, respectively. At the same time, as a protective reaction, there was an increase in the amount of ascorbic acid in the needles by 48%; the amount of water-soluble phenolic compounds by 29%; of the report Chl *a* /Chl *b* by 35%; the ratio between green and yellow pigments by 40%; and the level of water and alcohol soluble proteins, by 40 and 30%, respectively.

For the leaves of *P. abies* and *P. pungens* from industrially polluted places and exposed to exhaust fumes, the Chl *a* content decreased by 27.2% and 25.0%, respectively, and the Chl *b* content by 17.9% and 20.0% since May, until September, compared to the control. At the same time, the carotenoids content increased by 26.1% and 24.0%, respectively, as a protective measure. Also, the total Chl decreased by 24.4% and 23.6%, respectively; the Chl *a* / Chl *b* ratio with 11.4% and 12.3%; the ratio (Chl [*a* + *b*] / carotenoids) decreased by 30.1% and 38.0%, respectively (Fedorchak, 2020).

There are genetic differences between sensitive and pollution-resistant pine trees, and it is necessary to exploit the cultivation potential of the latter, both by exploring the native plant material and by enriching the genetic diversity (Chudzińska et al., 2014). Moreover, research on *Pinus pinaster* carried out by Acquaviva et al. (2012) highlighted some physiological indicators (e.g. antioxidant metabolites, heat shock proteins 70 and hemoxygenase) as being useful to monitor environmental contamination in a region and to better understanding the mechanisms implied in plant's protection and stress tolerance.

Recently, the ability of *P. nigra* to remove pollutants from the environment in urban area have been noticed (Przybysz et al., 2018; Petrova, 2020). In fact, since ten years ago, following studies conducted by Sawidis et al. (2011) in three European cities (polluted and unpolluted areas) for *P. nigra* and *Platanus orientalis*, black pine has proven to be a good accumulator of heavy metals, especially in the bark, and as such has shown its increased efficiency as a bioaccumulator of urban pollution, even though both species can be considered for comparative studies of this type.

Thus, searching for and planting trees with higher pollutant removal efficiency could be crucial to improving the quality of the urban environment.

Last but not least, the various local efficient and reasonable government regulation policies to control heavy traffic (consecutive and intermittent policies) have proven to be effective to alleviate vehicles pollution emissions continuously (Sun et al., 2022). At the same time, the promotion of the public transport can have beneficial effects on reducing urban air pollution for local residents, given the major contribution of passenger cars to the emission of particulate matter (PM1).

CONCLUSIONS

Based on the findings of the present study, it may be concluded that environmental conditions significantly ($P<0.05$) affect the *P.nigra* needles anatomy and physiological behavior, too. These differences are related also to seasonal adaptation of the foliage.

Urban traffic caused a significant increase ($P <0.05$) in the values of the thickness of the epidermis, cuticle, transfusion tissue, vascular tissues and hypodermis of *P. nigra* leaves, compared to the values determined for the leaves collected for the trees in the Dendrological Park.

Specific gas exchanges (photosynthesis rate, transpiration rate, stomatal conductance and intercellular carbon dioxide), but also their associated indicators (the instantaneous water use efficiency, intrinsic water use efficiency, the quantum yield, as well as the instantaneous carboxilation efficiency) were negatively affected by the urban traffic.

In addition, our study indicates that with the aging of pine needles, the structural adaptations and assimilatory pigments evolution occur. Also, changes induced by pollutants, influence physiological functions of the needles.

These results provide valuable information for the management of *P.nigra* species by using relevant anatomical and physiological indicators with a view to monitor environmental contamination in a region and for better understanding the mechanisms involved in plant protection and stress tolerance. It is necessary to exploit pollution-resistant species and apply management practices focused on both the use of native biological material and the improvement of genetic diversity (Chudzińska et al., 2014).

In order to improve the ecosystem services destined to regulate the urban vegetation, the search and planting of trees with increased capacity to remove pollutants from the environment can be an essential way to improve the quality of the urban environment (Petrova, 2020).

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