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Characterization of pigment apparatus in leaves of *Salicaceae* Mirbel. species

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The photosynthetic pigments amount and their ratios in the leaves of native and introduced species of 5-7-year-old trees from the collection of the Bila Tserka National Agrarian University biological station and 30-40-year-old species of *Salicaceae* Mirbel. family in territories with different air pollution were investigated. The analysis of the parameters of the pigment apparatus showed that in the leaves of young trees both native and introduced species broad fluctuations of chlorophyll content (correspondingly, 1.32-2.95 and 0.86-2.22 mg/g of free weight) and carotenoids (respectively, 0.31-0.49 and 0.17-0.38 mg/g of free weight) were observed. Native and introduced species insignificantly differed in the ratio of chlorophylls *a/b* (from 3.1 to 3.3) and the change in chlorophylls *(a+b)*/carotinoids ratio between the most contrasting species (1.5-1.6 times). Thus, the analysis of parameters of pigment apparatus changes of different origin species showed that the young introduced plants *S. matsuda* Koidz. 'Tortuosa', *S. alatavica* Rar. ex Stschegl. *S. dasydados* Wimm., *S. elaegnos* Scop., *S. miyabeana* Seemen, and *S. ledebouriana* Trautv. successfully adapted to the dry conditions of the Right Bank Forest-Steppe of Ukraine.
A slight decrease of photosynthetic pigments content in adult trees leaves (by 6-11%) on the streets of Bila Tserkva, compared with those growing in the suburbs and near the square, and a narrow range of changes in the chlorophyll *a/b* ratio (3.2-3.4) indicate their successful adaptation to the conditions of the urban environment. The high stability of the pigment apparatus of the species *P. italica* (Du Roi) Moench., *P. nigra* L., *S. fragilis* L., *S. alba* 'Vittalina pendula', and *P. simonii* to the conditions of aerotechnogenic pollution by motor transport and it is recommended to use them in landscaping city streets.
Kew words: Salicaceae Mirbel; leaves; pigment apparatus; air pollution; chlorophylls content

Introduction

The planet urbanization increases the area of green plantations that are in the conditions of technogenic pollution. In particular, the plants of modern urban ecosystems are influenced by aerotechnogenic emissions, which affect on their morphological and physiological state. Among the main sources of pollution in the cities is a road transport, the number of which is steadily increasing. So in Ukraine at the beginning of this century, 50% of carbon monoxide, 38% of hydrocarbons, and 27% of nitrogen oxide were released into the atmosphere from the total amount of these substances for the country (National Report, ... 2001).
According to Yu.G. Feldman (1976), each car with exhaust emits about 200 different harmful components. The most dangerous among them are carbon monoxide and nitrogen (nitrous gases), hydrocarbons, benzopyrene, soot, dust, impurities of heavy metals, in particular such as Pb, Cu, Co, and Zn, which are classified as hazardous to 1-2 grades. An especial risk is the progressive pollution of the environment with plumbum compounds, which can accumulate in plant tissues and has a mutagenic effects. The highest levels of pollution are observed in crowded cars, at crossroads, at stops, near traffic lights and for slow movement in the crust. The mutagenic activity is manifested by organic matter contained in exhaust gases of car engines. Exhaust gases of diesel engines contain significantly more nitrogen oxides and 30-100 times more aerosols than gasoline engines. Extraction of these aerosols in organic solvents it was found that some aerosols have an

inconstant composition, and the extracts themselves contain more than 1000 chemical compounds. More than 200 of these have mutagenic activity (Sluchik, 2000; Dragan, 2003).

Bila Tserkva is an average industrial city on the territory of which there are enterprises that polluting the air pool. More than 300 different ingredients are emitted in the air, including organic and inorganic sources of dust, hydrocarbon compounds, lead, phenol, formaldehyde, nitrogen oxides, chromium, zinc, and nickel. The main polluting enterprises in the city are: OJSC "Belotserkovskaya Termal Power Station", CJSC "Rosava", OJSC "Tribo", JSC "Inter GTV", CJSC "FEROKERAM", and OJSC "Belotserkivteplomerezha" (http://bilatserkva.info/modules.php?name=Content&op=showpage&pid=164).

But among the main sources of pollution in the city remains a motor transport, emissions of which make up more than 70% of all emissions. According to T.O. Hrabovs'ka (2014), who conducted a research in Bila Tserkva on the impact of motor vehicle emissions on the environmental state, the largest number was recorded on the Levanevsky street, near CJSC "Rosava". She found that on average 255 cars, 135 buses and, respectively, 135, 18, and 12 light, medium, and heavy duty trucks were transported per hour.

Under the conditions of the urban ecosystem, the trees form less organic matter than in country parks and forest parks (Ivanova, Kostyuchenko, 2011). Therefore, it is important to maintain the gas balance of the atmosphere by creating environmentally effective plantations. Improvement of the composition of the air is associated, in particular, with willow and poplar. Geography of the *Salicaceae* Mirbel family is closely connected with its ecological, biological and physiological features. Several authors believe that the high ecological plasticity of the species, forms and cultivars of the *Salicaceae* Mirbel family ensures their successful introduction and acclimatization to new conditions of localization. This can be due to the significant variability of the main metabolic processes of this family (Izdon, 1980; Hrozdova at al., 1986). In addition, a willow are also characterized by high growth rate, increased regenerative capacity and specific structure of the leaf parenchyma, allowing them to reproduce vegetatively, and create additional roots.

Assessment of wood species adaptation to environmental conditions can be carried out by morphological, physiological or biochemical indicators. Since ingress of toxins into the plant's internal tissues through the leaf, one important indicator of the physiological state of woody plants in the urban environment may be the reaction of their pigment system, which is closely related to one of the main processes in the plant organism - photosynthesis. Moreover, for deciduous tree plants, high photosynthetic activity is characteristic. Notes that the photosynthesis of sun leaves of hybrids poplars, close to the genus *Salix* L., is approaching the maximum photosynthesis of cultivated herbs, and most tree plants reach only 1/3 or 0.5of these parameters (Larcher, 1978; Prokopev 2001). Therefore, the study of the pigment apparatus adaptation to technogenic air pollution is an important aspect of studying the mechanisms of plant resistance under adverse environmental conditions.

The purpose of this study was to evaluate the introduction of *Salicaceae* family by changing of pigment apparatus parameters of young and adult trees.

Materials and methods

The determination of photosynthetic pigments content (chlorophyll *a* and *b*, as well as carotenoids) was carried out in leaves of 5-7-year-old plants and adults of 40-50-year-old trees of the *Salicaceae* family. The first of them grow in the collection of Bila Tserkva National Agrarian University (NAU) biological station, the second are in the urban streets with intensive movement (Levanevsky Street, Prince Volodymyr's Street), less polluted territory (near the plane), and the country green zone (artificial forest bays and ravines). The sampling of leaves, which have finalize their growth, was made from one year old shoots at a distance of 0.5 m from their edge in the second half of May. The numbers of leaves in each of the two repetitions was equal to 20, and then from them were selected on 5 middle leaves in 2 repetitions, on which they made the definition.

The chlorophyll content (*a* and *b*) and total carotenoids was determined by the non-capillary pigment extraction method for A.P. Wellburn (Wellburn, 1994). Pigment fixation was performed with dimethyl sulfoxide (DMSO): 100 mg of averaged sample of 5 leaves was poured into 10 ml of DMSO. Subsequently, the test tubes were incubated in a water bath with at of 67°C for 4 hours. One ml of the resulting solution was made up to 4 mL by adding DMSO. The extracts were transferred to a cuvette and the absorbance was read in a Spectrophotometer SF-26 (LOMO, Leningrad) at 649, 665, and 480 nm. Chlorophyll *a, b*, and carotenoids were calculated by the following formulae (Wellburn, 1994):

$C_a = 12{,}19\ A_{665} - 3{,}45A_{649}$

$C_b = 21{,}99A_{649} - 5{,}32A_{665}$

$C_{x+c} = (1000A_{480} - 2{,}14C_a - 70{,}16C_b)/220,$

where:

C_a, C_b – respectively, the content of chlorophylls *a* and *b*,

C_{x+c} – total carotenoids content,

$A_{480,\ 649,\ 665}$ – optical density of solutions of pigments at wavelengths of 480, 649 and 665 nm.

Results

Analysis of chlorophyll content (*a* + *b*) in the leaves of 5-7 year old plants of the family *Salicaceae* from the collection of the Bila Tserka NAU biological station showed that the content of this basic photosynthetic pigment ranged from 0.86 ± 0.02 to 2.95 ± 0.06 mg/g fresh weight (Fig. 1a).

Wherein, the fluctuations boundaries of values both native (1.32-2.95 mg /g fresh weight) and introduced species (0.86-2.22) were quite broad. In most species (*S. alatavica* Rar. ex Stschegl., *S. miyabeana* Seemen, *S. ledebouriana* Trautv., *S. dasyclados* Wimm., *S. caprea* L. *S. purpurea* L., *S. elaegnos* Scop., *P. trichocarpa,* and *P. hybrida 'Weresina'*) the average total chlorophyll fluctuated within the range of 1.5-2.2 mg/g fresh weight.

Similar patterns were observed for the total carotenoids content (Fig. 1 b). The average variations boundaries for most species, regardless of their origin, were 0.35-0.40 mg/g fresh weight. The smallest carotenoids content, as for chlorophyll content (*a* + *b*), was observed, in leaves of poplar (*S. hybrida* 'Inger').

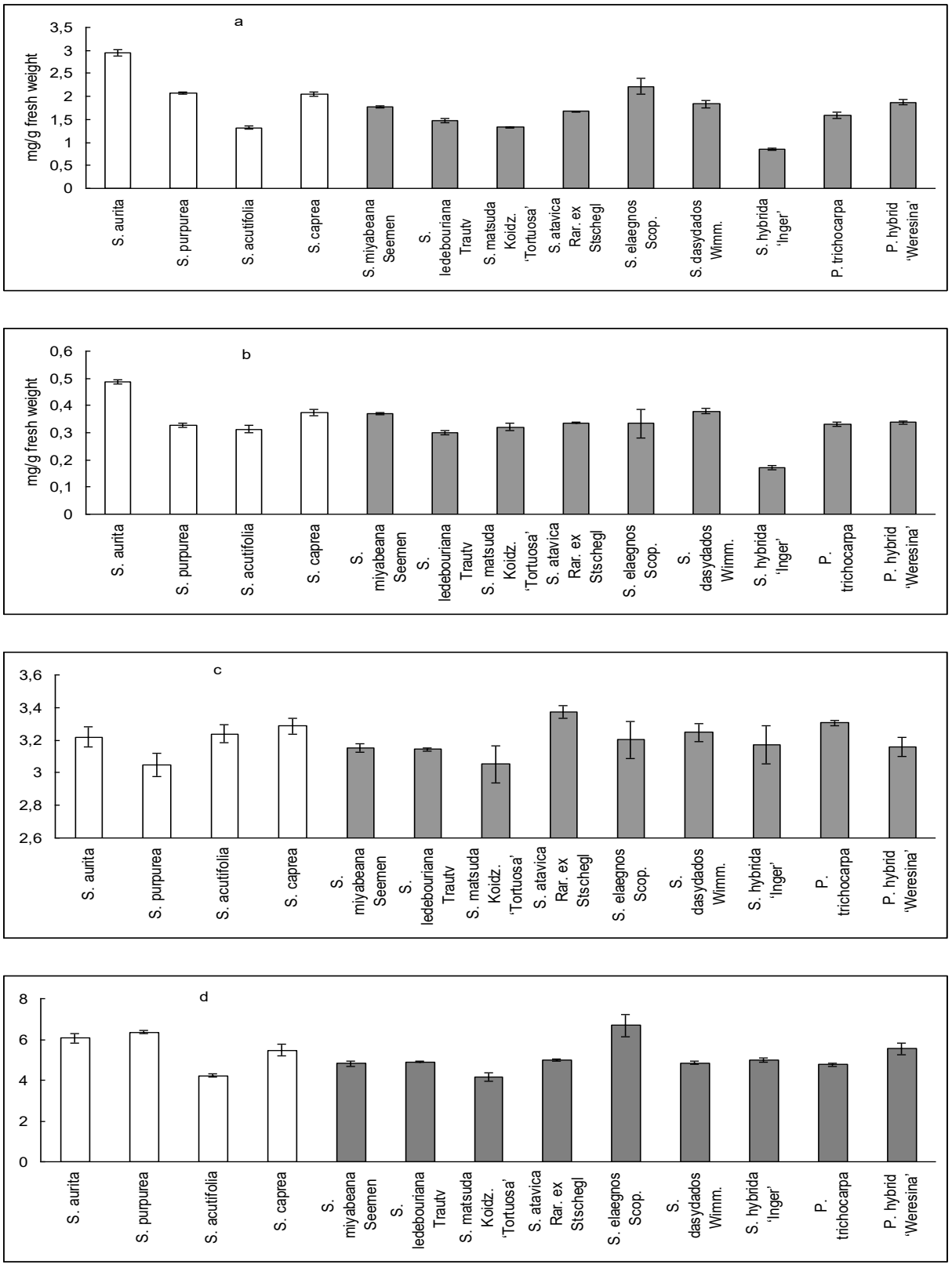

Fig. 1. The photosynthetic pigments content (a - chlorophyll (a+b), b - total carotenoids) and their ratios (c - chlorophyll a/b, d - chlorophyll (a+b)/carotenoids) in leaves of 5-7-year-old trees *Salix* and *Populus* at Bila Tserkva NAU biological station.
Note: bright signs are native species, dark – introduced ones.

Degree of resistances of plant photosynthetic apparatus to unfavorable factors also may be estimated by the relationships between individual forms of chlorophylls and between chlorophylls and carotenoids (Tyutereva, Voitsekhovskaya, 2011a, 2011b; Bukharin at al., 2013; Kochubei et al., 2014). The chlorophyll *a/b* ratio in native species ranged from 3.05 to 3.29, in the introduced species - from 3.05 to 3.37 (Fig. 1c). The close interval of the values of this ratio in different of origin species indicates the near adaptive capacity of their pigment apparatus to the environment. The difference between the individual species by chlorophyll (*a+b*)/carotenoids ratio was greater than chlorophyll *a/b* ratio.

The highest values exceeded the smallest in 1,5-1,6 times both for native (respectively 4,23 and 6.35), and for introduced species (4.16 and 6.68 - Fig. 1d). It should be noted that for the variety *S. hybrida* 'Inger', differing in the low concentration of pigments, the ratio between photosynthetic pigment forms did not differ significantly from other members of family *Salicaceae*. Consequently, fluctuations in the photosynthetic pigments content and their ratios in near range in native and introduced species indicate that the young introduced species family *Salicaceae* have successfully adapted to the soil-climatic conditions of the Right Bank Forest-steppe of Ukraine, which, in addition, has been characterized by aridization of climate in recent years. Creating environmentally efficient green plantations in modern cities requires the identification of plants resistant to technogenic pollution. Particular damage is experienced by plantations that grow near motor transport parking lots, filling stations, and industrial facilities. To compare the stability of the pigment apparatus of native and introduced species of genus *Salix* to the pollutants, changes in the content of photosynthetic pigments and their ratios in 30-40-year-old trees were analyzed.

The level of technogenic action on these trees was different. Plants located on Levanevsky Street (with intensive traffic) and Prince Volodymir's Street (intensive motor transport, near the refueling cars) are more negatively affected than plantations in the square (near the plane) and the country green zone (artificial forest bays and ravines). The highest chlorophyll content among native species was *S. alba* 'Vittalina pendula' (2.70 ± 0.02 mg/g fresh weight in the park and 2.59 ± 0.01 in the street with an intensive traffic flow), among the introduced - *P. italica* (respectively, 2.07 ± 0.05 and 1.95 ± 0.07) (Table 1).

The content of this pigment in leaves of other species was 1.3-1.8 times smaller. On average, the chlorophyll content in the leaves of trees growing on contaminated territories was by 6-11% higher than those growing in the suburbs of Bila Tserkva (in 2-3 km of hard-coated roads) and near the square, except for *P. simonii*. Unusual changes of chlorophyll content in the country zone at the NAU biological station may be related to the age characteristics of model trees. The age of *P. simonii* at the biological station is 7 years, and in the city of Bila Tserkva on Levanevsky Street are 40-year-old trees. Changes in the carotenoids content were similar (Table 1).

Most of the literature data on the impact of industry and motor vehicle emissions on the content of pigments in leafy woody plants indicate a decrease in their concentration, which depends on both the species and the degree of air pollution. Thus, the chlorophyll content in the leaves of white willow in the territory with higher pollution was 35-37% lower than in a zone with less pollution, and carotenoids - 11-14% (Rostunov et al., 2017). In aspen, the difference in green and yellow photosynthetic pigments amount between plants from areas with contrast air pollution was much smaller (Rostunov et al., 2017). N.A. Ivanova and R.N. Kostyuchenko (2011) found that the chlorophyll content in the leaves of some species of the genus *Salix* in forest cenoses, compared with the flood of the river Ob, decreased by 50%, and in the industrial zone - by 75%. Major changes in the chlorophyll content in species and hybrids of the genus *Populus* are shown in the work Dani'lchuk O.V. (2013): at the industrial sites of the mining and processing plant of Kryvyi Rih chlorophylls *a*, *b* and their amounts decreased by 2-5 times.

Table 1. The photosynthetic pigments content, mg/g fresh weight, and their ratio in the leaves of adult trees *Salix* and *Populus* in city of Bila Tserkva and the suburban green zone.

Origin	Species	Location	Content of		Ratio	
			chlorophyll (*a+b*) (Chl)	carotenoids (Car)	a/b	Chl/Car
Native	*S. fragilis*	1	1.50±0.04	0.35±0.02	3.23±0.05	4.31±0.36
		2	1.41±0.02*	0.32±0.01	3.21±0.08	4.46±0.22
	S. alba 'Vitta-	1	2.70±0.02	0.61±0.01	3.34±0.05	4.45±0.06
	lina pendula'	2	2.59±0.01*	0.58±0.01*	3.36±0.01	4.50±0.02
	P. nigra	1	1.62±0.03	0.35±0.01	3.37±0.01	4.67±0.03
		2	1.46±0.01*	0.31±0.01*	3.39±0.04	4.77±0.09
Introduced	*P. italica*	1	2.07±0.05	0.46±0.01	3.33±0.05	4.56±0.02
		2	1.95±0.07	0.42±0.02*	3.35±0.05	4.66±0.08
	P. simonii	1	1.42±0.01	0.30±0.01	3.41±0.18	4.80±0.12
		2	1.53±0.01*	0.31±0.01	3.27±0.07	4.96±0.01*

Note: *S. fragilis*: 1 - phytomelioration plantings in the ravine, 2 - Prince Volodymyr's Street, Bila Tserkva; *S. alba* 'Vittalina 'Vittalina pendula': 1 - square near the plane, Bila Tserkva, 2 – Levanevsky Street, Bila Tserkva, *P. Nigra*: 1 - forest belt in 2 km from the city, 2 - Bila Tserkva, Levanevsky Street; *P. italica* 1 - forest belt in 3 km from the city, 2 - Bila Tserkva, Levanevsky Street; *P. simonii*: 1 - NAU biological station, 2 – Levanevsky Street, Bila Tserkva. * - the difference with the corresponding control is significant at p≤0.05

We found a lower degree of changes in photosynthetic pigments content (by 6-11%) of the species of the family *Salicaceae* shows their successful adaptation to the urban environment conditions of the city of Bila Tserkva.

In support of this assumption, attests the chlorophyll *a/b* ratio. For all studied species and conditions, it varied in the narrow range: from 3.2 to 3.4. It is known that photosystems I and II (respectively, PS I and PS II) are organized into large supercomplexes with different components of the antenna peripheral part - light-harvesting complexes (LHC). All LHC I proteins contain more chlorophyll *a* than *b* (*a/b* = 3-4), whereas LHC II contains chlorophyll *a* and *b* approximately equal proportions (Melis, 1991; Biswal et al., 2012). Therefore, it is believed that the chlorophyll *a*/*b* ratio indirectly reflects the ratio of the number of photosystems I and II in the thylacoid membrane and characterizes the size of the peripheral antenna of photosystem II (Biswal et al., 2012, Kochubei et al., 2014). Consequently, the nearby values of chlorophyll *a*/*b* ratio in the streets and in the suburbs may indicate the preservation of the photochemical activity of the leaves and the intensity of their photosynthesis in *Salicaceae* Mirbel species in conditions of significant technological pollution.

An important characteristic of photosynthetic apparatus state is also chlorophyll (*a+b*) to carotenoids ratio. Carotenoids perform such important functions as the absorption of light from the part of the solar spectrum where chlorophyll does not absorb, and their oxidized forms (xantophylls) play a major role in protect LHC from photooxidation by quenching triplet excited states of chlorophyll molecules (Pryadkina, 2005; Ladygin, 2015). It is believed that in unfavorable conditions their content increased (Maslova, Popova, 1993). In particular, the growth chlorophyll (*a+b*)/carotenoid ratio was observed on highly polluted areas of Arzamas in plant species *Tilia cordata* Mill., *Populus tremula* L., *Salix fragilis* L., and *Salix alba* L. (Rostunov, 2017).

Chlorophyll (*a+b*)/carotenoids ratio in the leaves of adult trees *Salix* and *Populus* in city and the suburban green zone varied from 4.31 to 4.96 (Table 1). Almost this range of changes in this ratio (3.3-4.9) was observed in the leaves of artificial clones *S. alba, S. Nigra* at 2 cadmium levels (Nikolić et al., 2015), whereas in the hybrid S. purpurea × S. triandra ×S. viminalis 2, when treated with zinc, it increased significantly – from 7 to 13 (Borowiak et al., 2015). In 4 researched by us species, the level of territory pollution did not significantly affect of chlorophyll (*a+b*)/ carotinoids the ratio, and only *P. simonii* the difference was significant. Thus, according to pigment changes analysis, it was found that all investigated species successfully adapted to the conditions of technogenic pollution in the city of Bila Tserkva. This is confirmed the results of our previous researches that showed that in the conditions of the most polluted industrial and linear landscapes of the urbecosystem of Bila Tserkva, the highest viability and, respectively, the most promising species and hybrids, is *P. italica* (Du Roi) Moench., *P. simonii* Corr., *P. x canescens* (Ait.) Smith, *P. tremula* L., *P. x beroliensis* Dippel (Ischuk, 2015a; 2015b; 2016).

Conclusions

A comparative analysis of photosynthetic pigments amounts in the leaves of 5-7-year-old plants of the family *Salicaceae* from the collection of Bila Tserkva NAU biological station showed that the boundaries of fluctuations chlorophyll (a + b) and carotenoids were rather broad in native (respectively, 1.32-2.95 and 0.31-0.49 mg/g free weight) and introduced species (0.86-2.22 and 0.17-0.38). The most contrasting native species differed in chlorophylls (a + b)/carotenoids ratio 1.5 times, and introduced - 1.6 times. The chlorophylls a/b ratio varied within the limits from 3.1 to 3.3 regardless of origin species. This indicates that the introduced plants (*S. matsuda* Koidz. 'Tortuosa', *S. alatavica* Rar. ex Stschegl. *S. dasydados* Wimm., *S. elaegnos* Scop., *S. miyabeana* Seemen, and *S. ledebouriana* Trautv.) successfully adapted to soil-climatic conditions of the Right Bank Forest-Steppe of Ukraine, with increased aridity.

A slight decrease (by 6-11%) of photosynthetic pigments content in the leaves of 30-40-year-old species of the family *Salicaceae* in the contaminated areas of Bila Tserkva, as compared to those growing in the suburbs and near the square, confirm their successful adaptation to the conditions of the urban environment. The narrow range of changes chlorophyll a/b ratio in the streets and in the suburbs (3.2-3.4) also confirms the preservation of photosynthetic activity of the leaves of the species of this family in conditions of significant technological pollution. Based on the results of the pigment apparatus analysis recommended the use of the species *P. italica* (Du Roi) Moench., *P. nigra* L., *S. fragilis* L., *P. simonii*, and *S. alba* 'Vittalina pendula' for the planting of streets Bila Tserkva.

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