CHARACTERISTICS OF THE SHAPE ASYMMETRY OF LEAF TIP AND BASE IN *POPULUS NIGRA* L. UNDER INDUSTRIAL DUMP CONDITIONS

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Abstract. The scope of this study is to reveale the nature and variability of shape asymmetry of leaf tip and base in *Populus nigra* L. under conditions of two types of industrial waste dumps: coal mine and overburden rock dumps. The coefficient of shape asymmetry of leaf tip ranges from 0.000 to 0.253 in the samplings of *P. nigra* (in ascending order): City Park → coal mine waste dumps → overburden rock dumps. The coefficient of tip shape asymmetry less than 0.050 is characteristic of most leaves of all analyzed samples. The coefficient of shape asymmetry of leaf base varies from 0.000 to 0.152. This value was statistically significantly higher in the leaves sampled in the overburden rock dumps. The asymmetry coefficient of base shape less than 0.015 is characteristic of most leaves of all analyzed samples. The shape asymmetry of leaf tip and base in *P. nigra* is fluctuating. Among three indices analyzed (overall shape pseudosymmetry index, and coefficients of shape asymmetry of leaf tip and base), the shape asymmetry coefficient of leaf base is the most informative indicator. It is most useful for differentiation of leaf blades sampled in less degraded ecosystems and those sampled in stressful ecosystems.
Keywords: *woody plants, morphological variation, fluctuating asymmetry*

Introduction

Plants are ideal organisms for studying genotypic and environmental influences on developmental stability. Because they may have numerous leaves, flowers, and stems, one can study variation in developmental stability in a single individual (Cowart and Graham, 1999).

Plants in man-disturbed environments are constantly affected by adverse environmental factors. Leaves are plant organs highly sensitive to environmental conditions, thus morphological changes are observed in leaves under the influence of various factors (Givnish, 1987; Stakovetskaya et al., 2012).

Many authors suggest that leaf morphology changes within the species are associated with the changed growth conditions (Givnish, 1978; Isakov et al., 1984; Zakharov, 2000; Bukharin et al., 2007; Niinemets et al., 2007; Bessonova, 2009; Migalina et al., 2009; Vogel, 2009; Khuzina, 2010; Zaitseva, 2012 etc.). Environmental factors influencing leaf development may have a significant effect on the features of its final structure and shape (Givnish, 1984; Migalina et al., 2009).

It is extremely difficult to study the influence of plant growth conditions on leaf shape directly and experimentally, but this influence can be traced in indirect way by plant material sampling in the sites with varying conditions (Isakov et al., 1984).

Asymmetry is one of the fundamental concepts of modern biology. There are three types of asymmetry: directional, fluctuating and antisymmetry (Palmer and Strobeck, 1986). Fluctuating asymmetry (FA) implies some minor and random (non-directional)

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deviations from a strict bilateral symmetry of biological objects. It is suggested to use this type of asymmetry to measure the developmental stability of an organism at the macroscopic level (Zakharov, 1987, 2001; Zakharov et al., 2000). The level of fluctuating asymmetry is minimal only under optimal environmental conditions and non-specifically increases in any stress conditions (Gelashvili et al., 2004). Some authors (Palmer and Strobeck, 1986, 2003; Palmer, 1994; Kryazheva et al., 1996; Møller and Swaddle, 1997; Cowart and Graham, 1999; Baranov and Gavrikov, 2008) suggest determining of fluctuating asymmetry as one of morphological methods to evaluate condition and dynamics of biological systems. The index of fluctuating asymmetry in this case is regarded to be an organism stability index. Directional asymmetry differs from fluctuating by the fact that the mean value of a feature for one side is higher than for the other. Antisymmetry takes place in the case when manifestation of the feature asymmetry is the standard, no matter what is the direction of the differences between sides. Statistically, it is reflected in the fact that frequencies deviate from normal distribution towards the negative kurtosis or bimodality (Palmer and Strobeck, 1986, 2003; Zakharov, 1987). The phenomenon of antisymmetry, as well as its direction, is to a great degree determined genotypically (Gelashvili et al., 2004), thereby rendering these forms of asymmetry useless for studies of developmental instability) (Graham et al., 1993). Fluctuating asymmetry allows defining developmental disorders based on the single genotype (Thoday, 1958).

There are different methods and approaches to the studies of fluctuating asymmetry. Some of them are described by Graham et al. (2003).

There is a large amount of studies on fluctuating asymmetry in different plant species, yet this problem is still under-explored. The factors underlying fluctuating asymmetry, especially in plants, remain poorly understood (Wilsey et al., 1998; Wilsey and Saloniemi, 1999). Levels of fluctuating asymmetry may be partly determined by compensational growth, and the efficiency of such compensation may be affected by stress (Møller and van Dongen, 2003).

Applicability of fluctuating asymmetry manifestations to studies of plant developmental stability and biological indication of environment is debatable and further investigations are necessary.

For example, Wilsey and Saloniemi (1999) noted that there was no relationship between leaf FA and shoot growth in *Betula pubescens* Ehrh. ssp. *tortuosa*. The investigation of Mal et al. (2002) suggests that FA in *Lythrum salicaria* L. may be used as an ecological indicator to identify environmental stress caused by certain heavy metal pollutants.

Wuytacka et al. (2011) noted no direct relationship between FA in the leaves of *Salix alba* L. and air pollution that makes this biological indicator useless for air pollution monitoring.

Velickovica and Perisica (2006) obtained the first quantitative data on *Plantago major* L. indicating that plants living in the stressful sites are more symmetrical and leaf FA for plant species with wide ecological distribution such as *P. major* should be considered as an ‘index of habitat quality‘.

Leaf asymmetry appears to be a poor indicator of nutrient stress in young plants of *Acer platanoides* L. and *Betula pendula* Roth (Black-Samuelsson and Andersson, 2003).

There are indications of the relationship between leaf FA and its position within a plant. For example, Cowart and Graham (1999) indicate that leaf size and asymmetry in the common *Ficus carica* L. vary within a plant, depending on height (lower, middle,

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tip) and position (inside, outside). Leaves from the outside top are the largest, and those from the inside bottom are the smallest. Outer leaves, which may experience greater stress from cold, heat, sunlight, and desiccation, are more asymmetrical than inner leaves. Outer leaves, which may experience greater stress from cold, heat, sunlight, and desiccation, are more asymmetrical than inner leaves.

In the study of Premchamd et al. (1998) on the effect of salinity on leaf FA in *Glycine max* (L.) Merr., FA turned out a poor indicator of plant condition, whereas other measures of developmental instability were more informative.

One of the most general types of stress experienced by plants is water-limitation, which becomes particularly pronounced during periods of drought. Fair and Breshearsa (2005) noted that FA in *Quercus undulata* K. Koch are related to precipitation.

According to Hódarf (2002), the more symmetrical plants of *Quercus ilex* L. respond less to yearly variations in drought stress.

At the same time, it is evident from the study of Møller (1995) that plants of *Ulmus glabra* Huds. infected with mining insect *Rhynchaenus rufus* (Schrank, 1781) were characterized by a greater level of FA.

The study of Cornelissen and Stiling (2005) indicated that leaf miners might use leaf FA as a cue to plant quality. According to these data, asymmetric leaves of *Quercus laevis* Walter and *Quercus geminata* Small contained significantly lower concentrations of tannins and higher concentrations of nitrogen than symmetric leaves for both plant species.

Møller (1999) noted that the plants of *Ulmus glabra* Huds. with a higher level of FA were more susceptible to Dutch elm disease, than plants with more symmetrical leaves.

The parts of leaf are variable to a different degree. For instance, our studies on *Betula pendula* Roth growing in ecosystems transformed to a different degree have shown maximal variation of leaf basal part near the petiole, compared to other leaf parts.

Leaf length increases mainly due to intercalary growth. Leaf tip is the first to stop growing, and the base is the last. Accordingly, leaf tip is shaped first that is indicative of the general order of development of this organ, ‘from top to bottom’ (Nizhegorodtsev, 2010).

The scope of this study is to determine character and variability of shape asymmetry of leaf tip and base in *Populus nigra* L. growing in industrial waste dumps of two types, namely coal mine and overburden rock dumps.

P. nigra in the south-east of Ukraine is found in different types of habitats that enabled us to investigate its leaf morphological variation under different environmental conditions and applicability of this species as a biological indicator of environmental condition.

Materials and methods

Leaves were sampled during 2010 to 2012 summers from short shoots of the crown lower part of mature generative trees. Due to high hybridization with closely related species, we selected specimens with distinct species-specific morphological features. Leaves were collected in coal mine dumps and overburden rock dumps dating back to various times and located in industrial sites of the south-east of Ukraine. Ecotopes of coal mine waste dumps are characterized by acidic substrates; alkaline ones characterize those of overburden rock dumps. The textures, fertility, edaphotope salinity of these dumps are to a high degree similar.

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For control we used parameters of *P. nigra* leaves sampled in the Donetsk City Park. There the soils are less saline with pH ~7. On the whole, parkland is less exposed to pollutants than industrial sites.

The leaves were scanned and measured using ImageJ 1.43u program. The sample size was 380 leaves in coal mine waste dumps, 307 ones in overburden rock dumps, 264 ones in the City Park.

To assess variability of the shape asymmetry of leaf tip and base we used the numeric index, calculated using Gendels and Budantsev (1991) technique. The radius-vectors obliqued from the center (according to the mentioned method) at the angle of 20°. The numeric index was calculated for the left and right side of the leaf. Asymmetry coefficient of tip (or base) shape of the leaf blade was calculated using the formula:

$$K = \frac{/ I_L - I_R /}{(I_L + I_R)} \quad \text{(Eq.1)}$$

where K is a asymmetry coefficient of tip (or base) shape of the leaf blade, I_L and I_R are numeric values of this index for tip (or base), respectively, for the left and right side of the leaf blade.

To determine the nature of the shape asymmetry of leaf tip and base by the analyzed parameters we performed a significance test for the kurtosis and compared the index values calculated for the left and right sides by Wilcoxon criterion.

To assess informative value of analyzed measures for biological indication, we assessed general symmetry of the leaf shape within the mentioned samples and then conducted discriminant analysis. To estimate general leaf symmetry we used a program for pseudosymmetry level calculations in relation to mirroring for flat bilaterally symmetrical objects (BioPs) – biological pseudosymmetry.

Estimation of pseudosymmetry in the case is based on the expression of integral convolution:

$$\eta = \frac{\sum_{i,j} A_{i,j} \times B_{i,j}}{\sum_{i,j} A_{i,j}^2} \quad \text{(Eq.2)}$$

where η is a symmetry level, A is a matrix of original image intensities, B is a matrix of intensities, obtained as a result of reflection of A matrix against a drawn plane. As we deal with a sum of positive values defining intensity pixels, the range of symmetry change level is within the limits from zero (for absolutely non-symmetrical object) to one (for absolutely symmetrical one). In the procedure of calculation of pseudosymmetry index pixel intensities were not taken into account and nonhomogeneity of the object was ignored. The intensity of background pixels is 0, and the intensity of object pixels is 255. In other words, an object in this program is a dark spot against the white background with a symmetry plane drawn across it (Nizhegorodtsev, 2010). Thus, invariance degree of congruency (shape) was taken into account and color intensity of the leaf was ignored. The reflection plane was chosen by several points of main vein.

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Morphological diversity of samples was analyzed basing on representation of leaves of different gradations, distributed according to asymmetry indices. Morphological diversity was estimated using Shannon index. This index was calculated according to a formula:

$$H = -\sum_{i=1}^{N} p_i \ln p_i \quad \text{(Eq.3)}$$

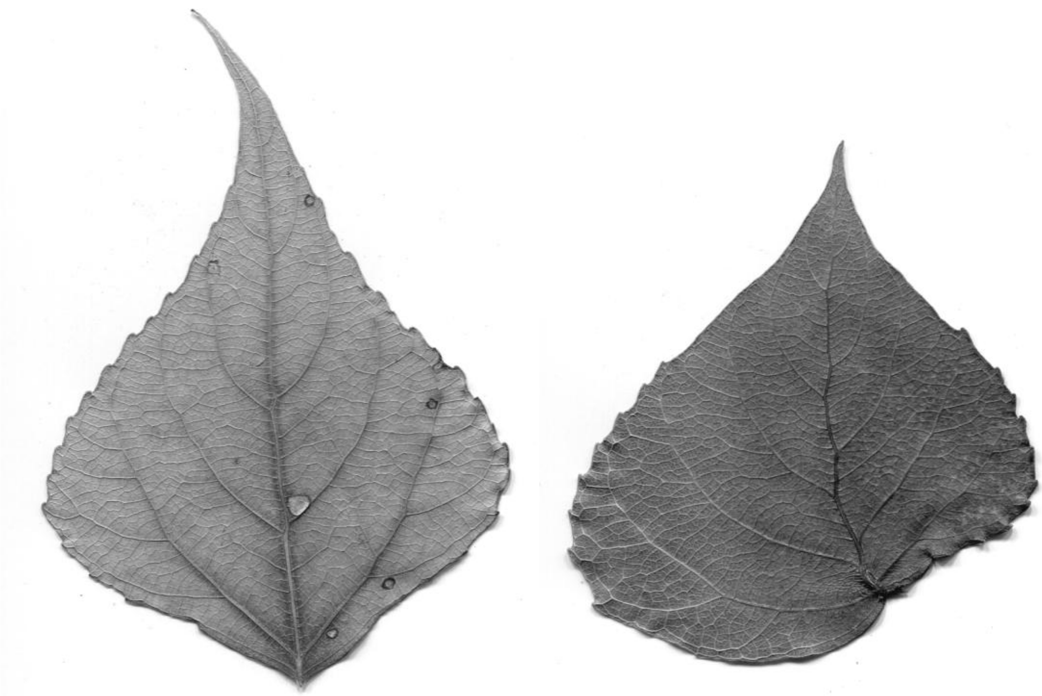
where H is Shannon's index, p_i is the percentage of leaves from the sample, which belong to i-gradation of a parameter, N – total number of gradations of the analyzed parameter.

Significance test for the differences in Shannon's index is conducted using Hatcheson method according to calculation formulas indicated by Magurran (1988).

Statistical data processing was carried out using the STATISTICA 6.0 package.

Results

The coefficient of shape asymmetry of leaf tip ranged from 0.000 to 0.253 for all of the analyzed samples. The coefficient of shape asymmetry of leaf base ranged from 0.000 to 0.152. Leaves with the maximum values of asymmetry coefficient of tip shape and with the maximum values of coefficient of base shape asymmetry are shown in *Figure 1.*



a b

Figure 1. *Leaf blades of Populus nigra L.: a – with the maximum values of coefficient of tip shape asymmetry (0.253); b – with the maximum values of coefficient of base shape asymmetry (0.152)*

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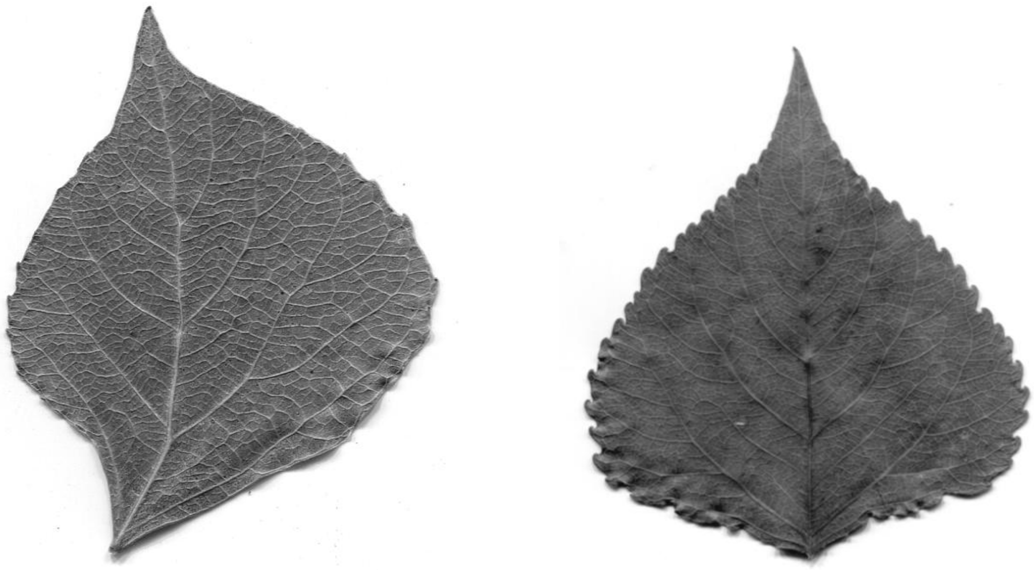
Pseudosymmetry index (invariance level) of general leaf shape for *P. nigra* samples ranged from 0.5485 to 0.9796. Leaves with the minimal and maximal values of coefficient are shown in *Figure 2*.



a b

Figure 2. *The leaves of Populus nigra L. with minimal and maximal values of pseudosymmetry of the general shape: a) minimal (0.5485); b) maximal (0.9796)*

Waste dumps of coal mines

The coefficient of shape asymmetry of *P. nigra* leaf tip varied from 0.000 to 0.154, the coefficient of variation was 91.7 % and the mean value was 0.043 ± 0.0037 (from now on the confidence interval is specified for P = 0.05). In the analyzed sample, leaves with a asymmetry coefficient of tip shape less than 0.050 constituted 67.1 % (including 16.4 % of those with the value of 0.000), those with a coefficient of 0.050 to 0.099 constituted 21.0 %, those with a coefficient of 0.100 to 0.149 constituted 11.2 %, those with a coefficient of 0.150 and more constituted 0.7 % of the sample (*Fig. 3*).

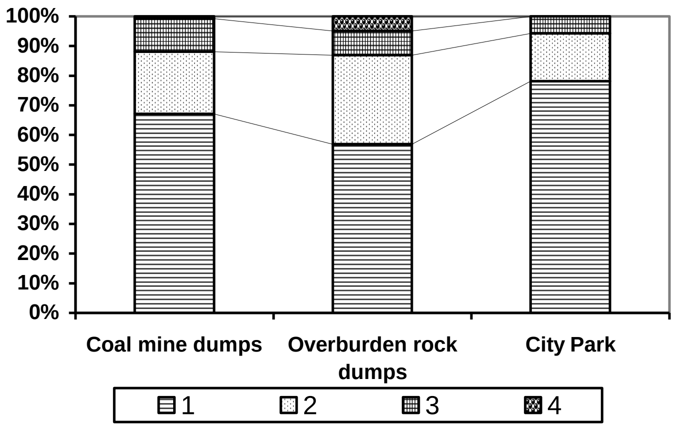


Figure 3. *Distribution of Populus nigra L. leaves sampled in different ecosystems according to the values of the asymmetry coefficient of tip shape: 1) less than 0.050; 2) 0.050–0.099; 3) 0.100–0.149; 4) 0.150 and more*

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The coefficient of shape asymmetry of leaf base varied from 0.000 to 0.064, the coefficient of variation was 81.9 %, and the mean value was 0.015 ± 0.0014. Leaves with a coefficient of base shape asymmetry less than 0.015 constituted 58.6 % (including 21.1 % of those with the value of 0.000), those with a coefficient of 0.015 to 0.029 constituted 31.6 %, those with a coefficient of 0.030 to 0.044 constituted 7.2 %, those with a coefficient of 0.045 to 0.059 constituted 1.9 %, those with a coefficient of 0.060 and more constituted 0.7 % of the sample (*Fig. 4*).

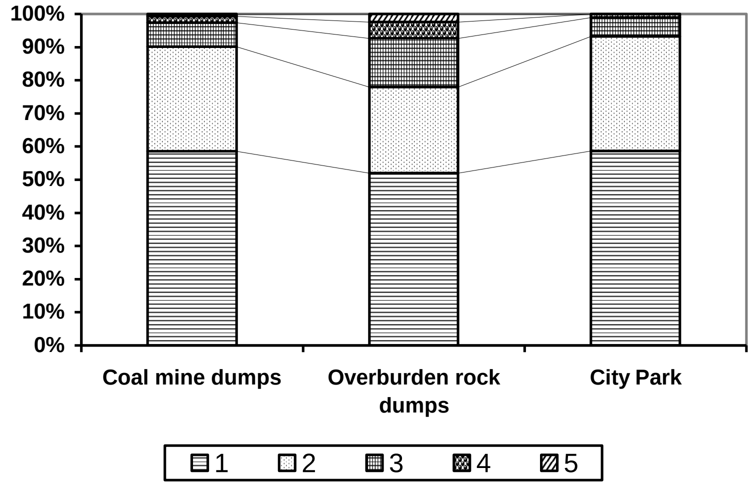

Figure 4. *Distribution of Populus nigra L. leaves sampled in different ecosystems according to the values of shape asymmetry coefficient of leaf blade base: 1) less than 0.015; 2) 0.015–0.029; 3) 0.030–0.044; 4) 0.045–0.059; 5) 0.060 and more*

Pseudosymmetry index of leaf general shape of *P. nigra* ranged from 0.7119 to 0.9796, coefficient of variation is 4.55 %, and mean value is 0.9267 ± 0.00421. Leaves with pseudosymmetry index of general shape 0.7000–0.7999 constituted 2.1 % of overall sample, those with index of 0.8000–0.8999 – 13.2 %, 0.9000 and more – 84.7 % (*Fig. 5*).

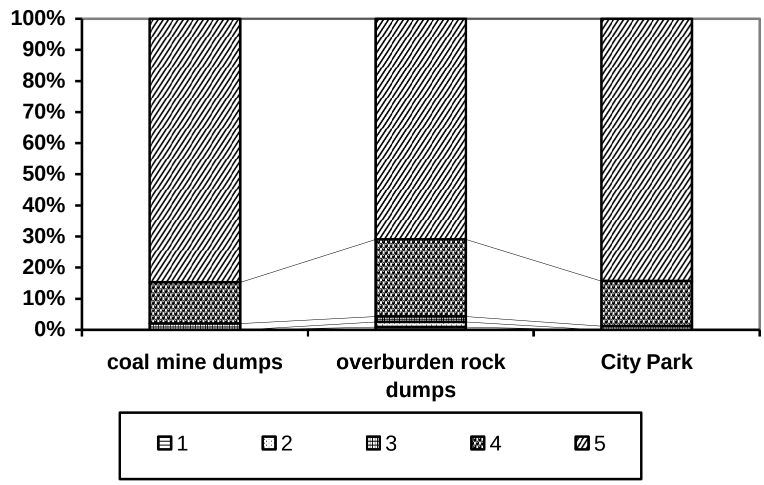

Figure 5. *Distribution of Populus nigra L. leaves sampled in different ecosystems according to the values of asymmetry coefficient of leaf general shape: 1) less than 0.6000; 2) 0.6000–0.6999; 3) 0.7000–0.7999; 4) 0.8000–0.8999; 5) 0.9000 and more*

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Overburden dumps

The value of shape asymmetry coefficient of the *P. nigra* leaf blade tip varied from 0.000 to 0.253, the coefficient of variation is 85.1 %, and the average value is 0.055 ± 0.0050. Leaf blades with a coefficient of tip shape asymmetry less than 0.050 constitute 56.9 % of the sample (including 5.7 % of in the leaves with asymmetry coefficient of 0.000), those with a coefficient of 0.050 to 0.099 constitute 30.1 %, those with a coefficient of 0.100 to 0.149 constitute 8.1 %, those with a coefficient more than 0.150 constitute 4.9 % of the sample (*Fig. 3*).

The value of the shape asymmetry coefficient of leaf blade base ranges from 0.000 to 0.152, the coefficient of variation is 107.5 %, and the mean value is 0.020 ± 0.0017. Leaf blades with a coefficient of base shape asymmetry less than 0.015 constitute up to 52.0 % of the analyzed samples (including 13.8 % of those with the coefficient value of 0.000), those with a coefficient of 0.015 to 0.029 constitute 26.0 %, those with a coefficient of 0.030 to 0.044 constitute 14.7 %, those with a coefficient of 0.045 to 0.059 constitute 4.9 %, those with a coefficient of 0.060 and more constitute 2.4 % of the sample (*Fig. 4*).

The value of pseudosymmetry index of leaf general shape varied from 0.5485 to 0.9681, the coefficient of variation is 7.0 %, and the average value is 0.9097 ± 0.00714. Leaves with pseudosymmetry index of general shape less than 0.6000 constitute 0.9 % of the sample, those with a coefficient of 0.6000 to 0.6999 constitute 1.7 %, those with a coefficient of 0.7000 to 0.7999 – 1.7 % of the sample, those with a coefficient of 0.8000 to 0.8999 constitute 24.8 %, and those with a coefficient more than 0.9000 – 70.9 % (*Fig. 5*).

City Park

The value of shape asymmetry coefficient of the *P. nigra* leaf blade tip varies from 0.000 to 0.111, the coefficient of variation is 97.2 %, and the average value is 0.029 ± 0.0025. Leaf blades with the value of a coefficient of leaf blade tip asymmetry less than 0.050 constitute 78.2 % (including 20.7 % of those with the value of 0.000), those with a coefficient of 0.050 to 0.099 constitute 16.1 %, and those with a coefficient value of 0.100 to 0.149 constitute 5.7 % of the sample (*Fig. 3*).

The value of shape asymmetry coefficient of leaf blade base ranges from 0.000 to 0.045, the coefficient of variation is 71.0 %, and the average value is 0.015 ± 0.0013. Leaf blades with the asymmetry coefficient of base shape less than 0.015 constitute 58.7 % (including 18.4 % of those with the value of 0.000), those with a coefficient of 0.015 to 0.029 constitute 34.5 %, those with a coefficient of 0.030 to 0.044 constitute 5.7 %, and those with a coefficient of 0.045 to 0.059 constitute 1.1 % of the sample (*Fig. 4*).

The value of pseudosymmetry index of leaf general shape varied from 0.7792 to 0.9673, the coefficient of variation is 3.71 %, and the average value is 0.9268 ± 0.00417. Leaves with pseudosymmetry index of general shape with a coefficient of 0.7000 to 0.7999 constitute 1.2 % of the sample, those with a coefficient of 0.8000 to 0.8999 constitute 14.5 %, and those with a coefficient more than 0.9000 – 84.3 % (*Fig. 5*).

Shannon's index values that represent morphological diversity of sampled in different ecosystems *P. nigra* leaf blades by asymmetry indexes are showed in *Table 1*.

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Table 1. *Shannon's index values that represent morphological diversity of sampled in different ecosystems Populus nigra L. leaf blades by asymmetry indexes*

Leaf blade asymmetry index	**Ecosystem**		
	Coal mine dumps	**Overburden rock dumps**	**City Park**
	Shannon's index values, nats		
Shape asymmetry coefficient of leaf blade tip	0.874	1.034	0.651
Shape asymmetry coefficient of leaf blade base	0.945	1.209	0.896
Pseudosymmetry index of leaf general shape	0.488	0.769	0.477

Discussions

Most leaf blades of all analyzed samples are characterized by the tip shape asymmetry coefficient less than 0.050. According to significance estimates of differences of the analyzed parameters using Student t-test ($P < 0.05$), the values of shape asymmetry coefficient of leaf tip were statistically significantly different when comparing pairs of analyzed samples, and they increased in the ecosystems as follows: City Park → waste dumps of coal mines → overburden dumps.

Most leaf blades of all analyzed samples are characterized by the base shape asymmetry coefficient less than 0.015. The values of asymmetry coefficient of base shape are statistically significantly different when comparing the following pairs of samples: coal mines dumps with overburden dumps, overburden dumps with the City Park. This parameter is higher for the leaf blades of the plants growing in overburden dumps.

Most leaf blades of all analyzed samples are characterized by the pseudosymmetry index of leaf general shape more than 0.9000. The values of pseudosymmetry index of leaf general shape are statistically significantly different comparing samples of overburden rock dumps with two other analyzed samples. As the result of comparing of shape pseudosymmetry coefficient of the *P. nigra* leaf general shape values between coal mine dumps and City Park no significant statistical difference was established.

The maximum values of the analyzed asymmetry parameters are registered in leaves sampled in overburden dumps that is obviously related to alkaline reaction of the substrate.

The maximum values of morphological diversity of leaf blades by asymmetry parameters counted using Shannon's index are also registered in leaves sampled in overburden dumps.

There were no cases when empirical values of the kurtosis of numeric index for the tip and base of leaf left and right sides surpassed the critical one, thus antisymmetry is not found (*Table 2*). Using Wilcoxon test we have not found any statistically significant differences in the value of numeric index of tip and base for left and right sides of the leaf, thus the statistically significant directional asymmetry is not found (*Table 3*).

Table 2. *Empirical values of numeric indexes kurtosis for left and right sides of tip and base of Populus nigra L. leaf*

Analyzed part of leaf blade	**Ecosystem**		
	Coal mine dumps	**Overburden rock dumps**	**City Park**
Tip	-0.524	-0.226	0.259
Base	-0.798	0.723	-0.297

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Table 3. *Wilcoxon test values received by comparing of tip and base numeric indexes of left and right sides of Populus nigra L. leaf blade*

Analyzed part of leaf blade	**Ecosystem**		
	Coal mine dumps	**Overburden rock dumps**	**City Park**
Tip	14990.5	12075.0	10740.5
Base	12904.5	12306.0	10050.0

On this basis it is possible to make a conclusion about fluctuating character of shape asymmetry of *P. nigra* leaf blade tip and base.

Previously reported gives the possibility of using *P. nigra* shape asymmetry coefficient of leaf blade tip and shape asymmetry coefficient of leaf blade base to determine resistance level of this species, compliance of plantations to various technogenic loads, and makes them perspective for indicating of environment state in general.

Conducted discriminant analysis showed following results. From the analysis of discriminant functions coefficients it should be noted that for the first discriminant function shape asymmetry coefficient of leaf blade base is most significant. The impact of other two variables is considerably lower (*Table 4*). According to the values of mean canonical variables the first discriminant function separates City Park sample from two other samples (*Table 5*).

The second discriminant function is marked mostly by shape asymmetry coefficient of leaf blade tip, impact of pseudosymmetry index of leaf general shape is lower, but at the same time it is also valuable comparing with the smallest impact of shape asymmetry coefficient of leaf blade base (*Table 4*). This discriminant function separates mostly sample of coal mine dumps and also other samples, but less qualitative (*Table 5*).

Table 4. *Standardized coefficients for canonical variables, received as a result of discriminant analysis*

Variables (asymmetry indexes of *Populus nigra* L. leaf blade)	**Root 1**	**Root 2**
Pseudosymmetry index of leaf general shape	-0.04903	-0.454729
Shape asymmetry coefficient of leaf blade tip	-0.02986	0.792767
Shape asymmetry coefficient of leaf blade base	-1.00363	0.078718

Table 5. *Means of canonical variables, received as a result of discriminant analysis*

Ecosystem	**Root 1**	**Root 2**
Coal mine dumps	1.33742	-0.175946
Overburden rock dumps	0.20904	0.308783
City Park	-2.63625	-0.122323

This interpretation is confirmed by a scattering diagram (*Fig. 6*).

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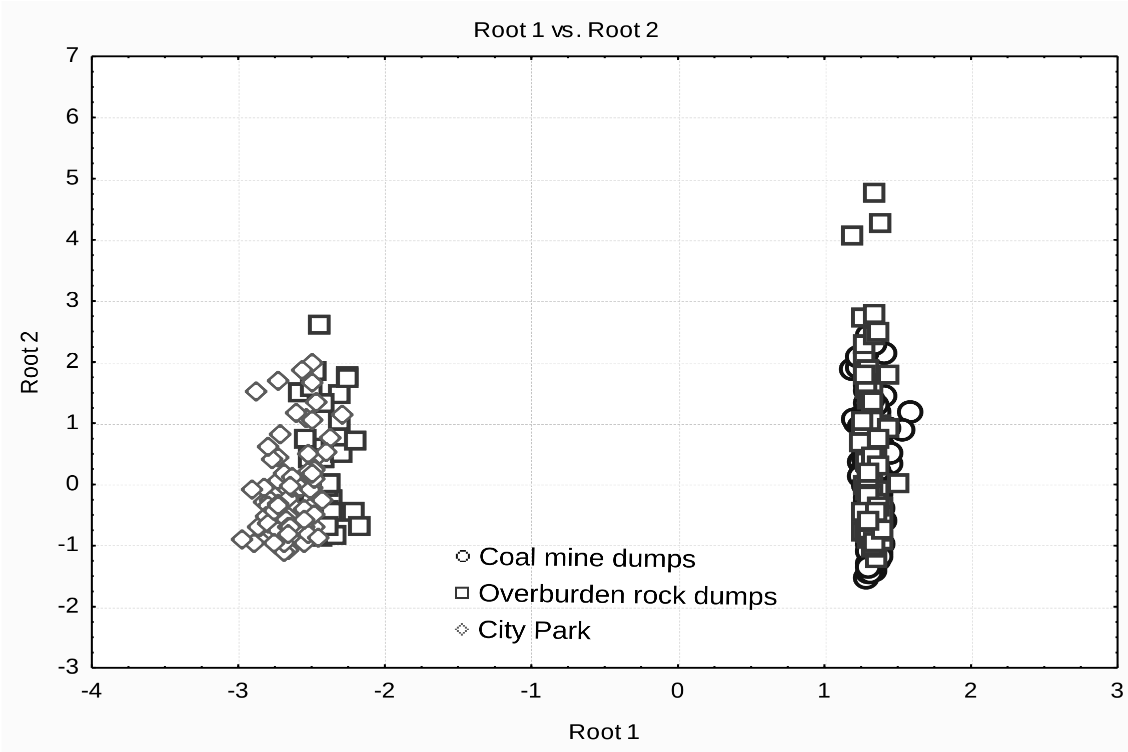

Figure 6. *Populus nigra* L. leaf blades from different ecosystems allocation in canonical space

So, from three analyzed indices of *P. nigra* leaf blade asymmetry (pseudosymmetry index of leaf general shape, shape asymmetry coefficient of leaf blade tip, and shape asymmetry coefficient of leaf blade base) the most informative indicator of environment state is shape asymmetry coefficient of leaf blade base. This parameter separates in the best way samples of leaf blades from less transformed ecosystems and ecosystems that are under significant anthropogenic pressure.

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