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USING BIRCH LEAVES TO INDICATE AIR POLLUTION

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ABSTRACT: The present study investigated the utility of *Bétula pubéscens L.* leaves as a bioindicator of air pollution within an industrial Russian city. The study focused on the effects of air pollution on fluctuating asymmetry (FA) of leaves. *B. pubéscens L.* leaves (n=3800) were sampled from 190 trees growing at 38 different sites in Chelyabinsk. The relationship between the data was determined using Pearson's test with a significance level of 0.05. Correlations were observed between the integral index of fluctuating asymmetry (IIFA) and air pollutants such as suspended solids (P=0.527), nitrogen dioxide (P=0.313), sulfur dioxide (P=0.355), inorganic dust SiO_2 (P=0.790), and the air pollution index (P=0.607). The correlation was the highest for inorganic dust since dust clogs the stomata on the leaves of plants, affecting their photosynthesis and respiration. *B. pubéscens L.* could be considered as a bioindicator of air pollution of Chelyabinsk, Russia.

Keywords: Fluctuating Asymmetry (FA), Bioindicator, Air Pollution, Leaves

1. INTRODUCTION

Fluctuating asymmetry (FA) consists of small, non-directional deviations from perfect symmetry in morphological characters. FA can be used as a handy indicator of stress experienced by organisms.

FA has been considered a marker of development instability for over half a century. FA is a nonspecific indicator of the conditions of development and a measure of random deviations in development. This gives us the opportunity to use it to assess the conditions of existence, both natural and artificial populations. It is believed that the stronger the external influence, than higher the FA [1]–[10].

Despite the fact that this method is widely used, including for monitoring the state of atmospheric air, it has been repeatedly criticized in the literature [11]–[14]. Recent works [15], [16] recommend this method under the following conditions: (1) using the blind method, where the person conducting measurements is not aware of the origin of samples being measured; (2) pay utmost attention to adequate and detailed description of data acquisition protocols, because all characteristics of instruments and methods need to be controlled to increase the quality and reproducibility of the data; (3) the measurements should be conducted with high accuracy from images of fresh or press-dried leaves.

Bétula pubéscens L. is most widely used as the object of FA studies [15].

The aim of the present work is to explore the nature of FA *B. pubéscens L.* leaves in different districts of Chelyabinsk and to explore the possibility of using this parameter to assess air pollution.

2. METHODS

2.1 Study Area

38 sampling sites were selected for researching in different districts of Chelyabinsk (Fig. 1).

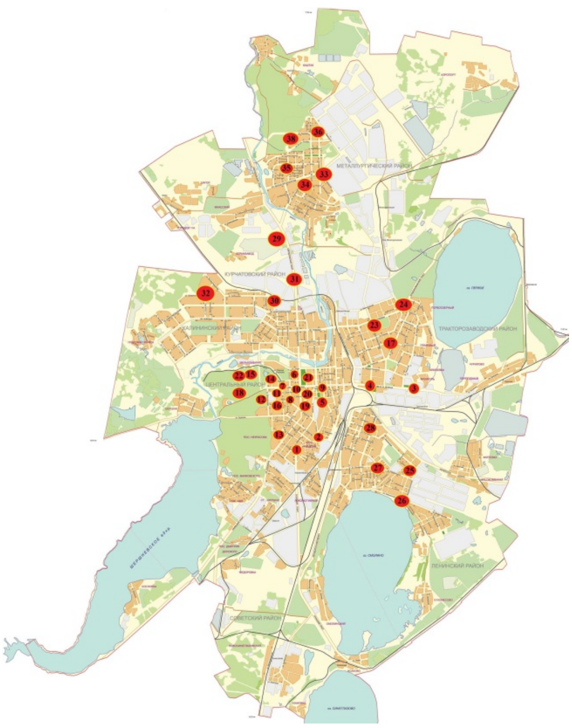

Fig. 1 The locations of sampling and air quality monitoring sites.

Chelyabinsk is a large industrial city. The air in Chelyabinsk is polluted by traffic, heavy industry (ferrous and nonferrous metallurgy, pulp production) and thermal power stations. The most dangerous pollutants are benzo(a)pyrene, formaldehyde, and nitrogen dioxide. Air quality is monitored at 24 monitoring sites.

In 2012 [16] the average concentration of benzo(a)pyrene, formaldehyde, and dioxide nitrogen exceeded the maximum allowable level of 3.9, 3.3, and 1.1 times, respectively. In addition, in 2012, disposable excess concentration of substances in the air was observed for Pb, Mn, Cd, suspended solids, H_2S, NO_2, phenol and formaldehyde [17].

2.2 Leaf Collection

We collected birch leaves in July 2012. We chose July because leaf growth stops at this time. We collected leaves from 5 trees at each site, 20 leaves from each tree. The total number of leaves collected at each site was 100. A total of 190 *B. pubéscens L.* trees were studied. We considered a few factors when we were selecting trees according to the recommendations of the literature [1]. First, it is important to determine that the plant belongs to the investigated species. *B. pubéscens L.* is able to interbreed with other species of birches, and it forms interspecific hybrids which have characteristics of both species. To avoid errors, we chose trees with distinct characteristics *B. pubéscens L.* Second, leaves should be collected from plants located in similar environmental conditions (it is necessary to consider the light level, moisture, etc.). We chose trees growing in open areas, because shading conditions are stressful for birch and significantly reduce the stability of plant development. Thirdly, the age of trees should be considered. We chose trees which had a generative age state.

We collected leaves from the maximum number of branches available evenly around the tree from the lower part of the crown. The leaves were collected only from short shoots. Leaf size was similar - the average for this plant. Damaged leaves were not used for the analysis [1].

56 students and teachers of South Ural State University participated in collecting and measuring leaves.

2.3 Preparation and Storage of the Leaves

We made measurements of leaf immediately on the day of collection or kept short leaves 2–3 days in the refrigerator on the bottom shelf.

In addition, all leaves were dried according to standard Botanical methods for further measurements of the air-dried leaves blind. For this purpose the leaves were dried under standard Botanical press to air-dry state. Further, the leaves from each plant were stored in separate envelope. We carefully marked envelope, indicating the place and date of leaf collection and has a number of the tree.

2.4 Measurements of the Leaves

We made measurements of each birch leaf twice. The first time, we measured fresh leaves on the day of collection or within 2–3 days after collection. The second time, we measured the press-dried leaves. The second measurement was performed blindly with respect to the results of the first measurement.

For each leaf, we measured five parameters on the left and right sides (Fig. 2, 3):

(1) the width of the halves of the leaf;

(2) the length of the second order nerve that is the second from the base of the leaf;

(3) the distance between the bases of the first and second nerves of the second order;

(4) the distance between the ends of these nerves;

(5) the angle between the main vein and the second from bottom nerve of the second order.

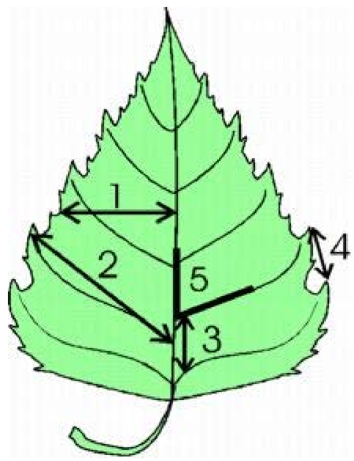

Fig. 2 Measurements of five parameters.

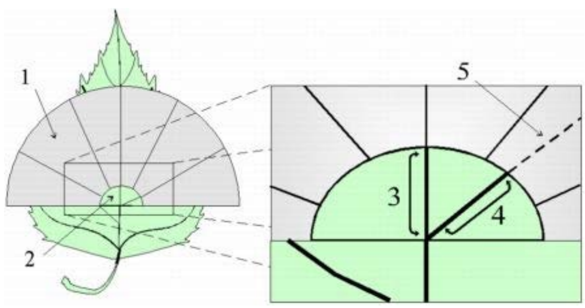

Fig. 3 Measurement methodology.

These measurements were performed with the use of a slide caliper, a ruler, and a protractor.

We used a scale (Table 1) to assess the degree of violation and consequently to assess disturbances in the ecosystem.

Table 1 Rating scale of comfort for living environment fluctuating asymmetry [1]

IIFA	Characteristics of the environmental condition of the territory
< 0,040	Conditionally clean
0,040–0,044	Disturbed
0,045–0,049	Considerably disturbed
0,050–0,054	Adverse
> 0,054	Extremely adverse

We calculated the integral index of fluctuating asymmetry (IIFA) using normalized difference algorithm [1]:

$$\bar{A}_1 = \frac{1}{m \cdot n}\sum_{i=1}^{m}\sum_{j=1}^{n}\frac{L_{ij} - R_{ij}}{L_{ij} + R_{ij}}, \quad (1)$$

where L_{ij} and R_{ij} are the values of the j-th character in the i-th leaf to the left and right side of the plane of symmetry and convolution of the functions, respectively, that in the form of finite sums can be represented by the following formula [18]:

$$\bar{A}_2 = 1 - \frac{1}{m}\sum_{i=1}^{m}\frac{2\sum_{j=1}^{n} L_{ij} \cdot R_{ij}}{\sum_{j=1}^{n}(L_{ij}^2 + R_{ij}^2)}. \quad (2)$$

Statistical processing of the experimental data was performed with N.A. Plohinskiy methods [19] using the Microsoft Excel 2013 software suite.

2.5 Air Pollution Index

We used data on service monitoring of urban air on 11 substances for the assessment of air pollution: suspended solids, carbon dioxide, sulfur dioxide, nitrogen dioxide, nitrogen monoxide, phenol, formaldehyde, sulfuric acid, inorganic fluorides, Mn, and inorganic dust SiO_2. We calculated the air pollution index (API) based on Chelyabinsk air monitoring by the standard method RD 52.04 186-89 [20], RD 52.04.667-2005 [21].

API shows the air pollution level characteristic of chronic, long-term air pollution [22].

API is calculated using the formula:

$$API = \sum_{i=1}^{N}\left(\frac{C_i}{MPC_i}\right)^{K_i}, \quad (3)$$

where C_i is an average i-substance concentration in mg/m^3; MPC_i – the average daily maximum permissible concentration of i-substance in mg/m^3; K_i – dimensionless constant depending on the substance hazard category, N – the number of contaminants considered.

K_i values are equal to: 0.85 for substance hazard category 4; 1.0 for substance hazard category 3; 1.3 for substance hazard category 2; 1.5 for substance hazard category 1. The five substances with the highest index are used.

With an API of ≤ 5, air pollution level is considered low. If $5 < API \leq 7$, then air pollution level is increased. If $7 < API \leq 14$, it is high, and if API is > 14 it is very high.

2.6 Data Processing

Microsoft Excel 2013, MapInfo Pro 15.0, and SPSS 24.0 software were used to organize and analyze the data. The Kolmogorov–Smirnov test was used to test data normality. The relationship between the data was determined using Pearson's test with a significance level of 0.05; the linear regression was considered significant.

3. RESULTS AND DISCUSSION

The values of IIFA for the studied sites are presented in Table 2 and Figure 4.

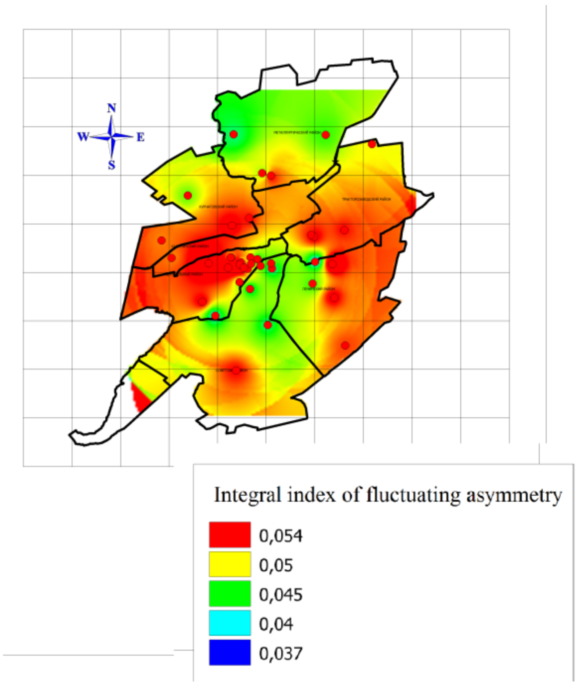

Fig. 4 Integral index of fluctuating asymmetry.

Table 2 IIFA in different sites

Site	IIFA
1	0.041±0.011
2	0.043±0.013
3	0.060±0.015
4	0.039±0.010
5	0.037±0.010
6	0.048±0.012
7	0.047±0.011
8	0.051±0.017
9	0.049±0.016
10	0.046±0.014
11	0.081±0.015
12	0.058±0.011
13	0.055±0.011
14	0.052±0.012
15	0.053±0.011
16	0.057±0.012
17	0.053±0.011
18	0.047±0.013
19	0.064±0.011
20	0.042±0.011
21	0.047±0.011
22	0.042±0.015
23	0.048±0.011
24	0.056±0.013
25	0.054±0.011
26	0.055±0.011
27	0.053±0.011
28	0.054±0.012
29	0.046±0.012
30	0.047±0.012
31	0.056±0.011
32	0.054±0.014
33	0.052±0.011
34	0.054±0.011
35	0.046±0.017
36	0.048±0.014
37	0.050±0.011
38	0.043±0.011

We observed only 2 sites with IIFA < 0.04. According to Table 1 and Table 2, there were 2 sites with a conditionally-clean environment, and 5 and 8 sites with disturbed and considerably-disturbed environment, respectively.

The remaining sites corresponded to the adverse (20 Sites) and the extremely adverse (5 Sites) environment level. Very high values are observed along highways that cross the entire central part of the city. In the central part of the area, the values of asymmetry were often higher than the values in the other areas. The maximum achieved value IIFA was 0.081 in Site 11.

A map of the air pollution of the city was built according to the calculation of API (Fig. 5).

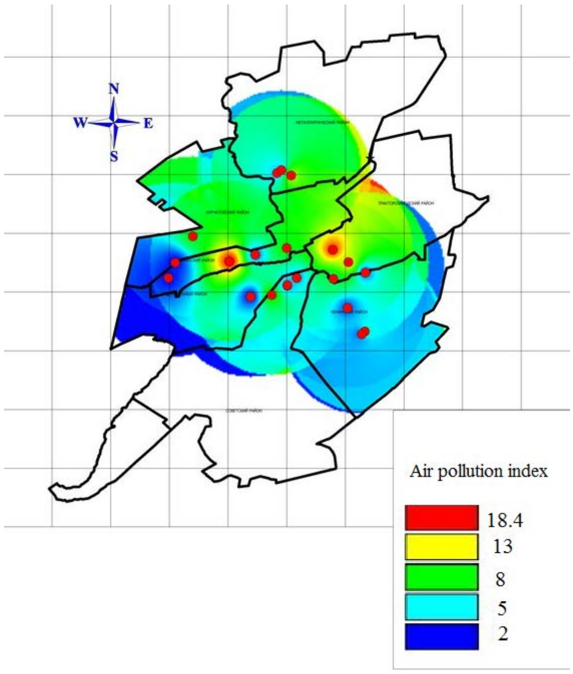

Fig. 5 Air pollution index.

Figure 5 shows that the sites with the greatest air pollution (maximum API) coincide with the areas where the highest IIFA was obtained (Fig. 4).

Coefficients of correlation between IIFA and air pollutant were calculated to more accurate estimates (Table 3).

Table 3 Pearson's tests for different air pollutants

Pollutant	Pearson's test
Suspended solids	**0.527**
Carbon dioxide	0.242
Sulfur dioxide	**0.355**
Nitrogen dioxide	**0.313**
Nitrogen monoxide	–
Phenol	0.0006
Formaldehyde	0.203
Inorganic fluorides	–
Sulfuric acid	–
Mn	0.155
Inorganic dust sio_2	**0.790**
API	**0.607**

Blank is insufficient data to calculate
Bold is significant value of correlation

The average value of API in July 2012 was 6.51. This corresponds to increased air pollution. The low degree of air pollution was observed only in the outskirts of the city away from the plants.

The values exceed the critical value of Pearson's tests 0.300 (n=38, p=0.05) for suspended solids (P=0.527) nitrogen dioxide (P=0.313), sulfur dioxide (P=0.355), inorganic dust SiO_2 (P=0.790) and air pollution index (P=0.607).

Correlation with sulfur and nitrogen dioxides was significant. Sulfur dioxide penetrates into leaves primarily in gaseous form through the stomata, although there is evidence for a limited pathway via the cuticle. Chronic damage results from long-term exposure to much lower concentrations of the gas and is essentially cumulative in nature, taking the form of reduced growth and yield and increased senescence, often with no clear visible symptoms or with some degree of chlorosis. Sulfur dioxide can also modify the response of plants to other environmental stresses, both biotic and abiotic, often exacerbating their adverse impacts. Nitrogen dioxide is the damaging component of photochemical smog. In the environment, excessive levels of nitrogen dioxide can rapidly inhibit photosynthesis per unit leaf area [22].

The highest correlation values were observed for suspended solids - inorganic dust SiO_2 and API. It was found that dust accumulation on leaf surfaces induces water stress-like conditions, such as a reduction of stomata conductance, photosynthesis and transpiration, and increased leaf temperature [23].

4. CONCLUSION

Analysis of fluctuating asymmetry of *Bétula pubéscens L.* leaves collected in July 2012 in the industrial city, Chelyabinsk, showed that:

- most sites have index deviations from its conditional standard;
- correlation coefficients between the fluctuating asymmetry and the concentration of pollutants in urban air are greater than the critical value.

In conclusion, it should be noted that, despite criticism of the method, fluctuating asymmetry of *B. pubéscens L.* leaves can be used as a bioindicator of air pollution

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