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Fluctuating Asymmetry in Morphological Characteristics of *Betula Pendula* Roth Leaf under Conditions of Urban Ecosystems: Evaluation of the Multi-Factor Negative Impact

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Abstract: The fluctuating asymmetry (FA) in *Betula pendula* Roth was estimated as an integrated measure of five morphometric characteristics of a lamina. Samples were collected in seven cities that differ both in climatic conditions, moderately to sharply continental. In total, 33 ecotopes were distinguished with various level of anthropogenic load. The statistical data processing involved correlation, one-way and factorial ANOVA, regression analyses, and principal component analysis (PCA). The impact of 25 climatic and anthropogenic factors on the FA value was considered. In most urban ecotopes, the integrated fluctuating asymmetry (IFA) value was higher than in natural biotopes of the same region. No significant inter-annual differences in IFA values were found. FA dependence on traffic load is noted to be statistically significant. The covariation analysis of IFA, climatic, and anthropogenic variables in various urban ecotopes revealed the impact of three groups of factors that together explain 93% of the variance in environmental parameters. The complex analysis clearly arranged the studied ecotopes by pollution gradient and climatic patterns. The primary effect of the total anthropogenic load on the developmental stability of *B. pendula* results in an IFA increase. IFA can play a key role in bioindication assessment of environmental quality. The climatic factors have no significant effect on the developmental stability of *B. pendula* in urban conditions.

Keywords: fluctuating asymmetry; developmental instability; silver birch; *Betula pendula*; traffic load; complex anthropogenic impact; urban area

1. Introduction

Trees in urban landscapes play an important role in shaping and safeguarding the local environment due to their production of oxygen and phytoncides, air ionization, shaping the microclimate, and catching pollutant particles. It is significant that the plants in urbanized territories are subject to a negative impact of chemical compounds in the soil and air. The use of fill soil and construction waste deteriorates the soil quality in cities, which is aggravated by the lack of nutrients and a decrease in the activity of geobiotic and herpetobiotic organisms. This leads to worse nutrition conditions for plants and deterioration of vegetation. Peculiar microclimatic conditions of urban ecosystems can lengthen the vegetation period of plants, precipitate some phenological stages, and induce early defoliation [1–5]. Changes in the abiotic parameters of the environment and biotic interactions, and anthropogenic impact start the mechanisms of various adaptation responses in plants on different structure organization levels [4,6–8]. The ontogeny of trees in cities is 2–3 times accelerated, and their life span is decreased correspondingly [9,10]. Since cities belong to evolutionary young ecosystems, it is of interest to study the vital functions of plants in urban conditions. Being aware of the complexity of problems facing the living organisms in the urban environment and taking into account the multiplicity of their effects, we must ask ourselves the question of whether it is possible to reduce the whole complex of the negative factors to a common denominator that provides an idea not of the content of individual pollutants but of the condition of an organism and ecosystem as a whole. We believe that for trees, one of the advisable methods is an assessment of disturbances in developmental stability by the level of fluctuating asymmetry of the leaf.

Stress-inducing stimuli cause changes in the organism developmental homeostasis that can be estimated by disturbances in morphogenetic processes. One of the indicators of these from the morphological perspective is fluctuating asymmetry (FA). FA is small undirected deviations from the ideally symmetrical state. Under normal circumstances, their level is minimal, but it increases with various negative factors [11,12]. The FA value can be used as an all-purpose indicator of the net stress-inducing impact [13–17]. It was noted that developmental instability is increased in conditions of both environmental and genetic stress [11,16–18]. In natural habitats, the destabilization of ontogeny and increase in the FA level are observed with the distance from the optimum zone, and the greatest increase in FA is observed in peripheral populations: In the north and in the mountains [19–23], a response to the global changes in environmental temperature was observed [24,25]; for plants, a response to soil condition [26,27]. The capacity of FA to react to stress-inducing factors of a various nature is a potential prospect for application in bioindication studies. In recent decades, there appeared many works devoted to the influence of separate factors and general environmental pollution on the manifestation of FA in organisms of different taxonomic groups both in aquatic and in terrestrial ecosystems. Disturbances in developmental stability on all levels, including the morphogenetic, were registered in angiosperm plants, fishes, amphibians, and small mammals in the area affected by the Chernobyl disaster [28]. In areas affected by the mining industry, in addition to an increase in FA of trees and small mammals, changes in the community composition and population structure of small mammals were noted [22,29]. An increased FA level in anurans is used as an indication of the condition of freshwater ecosystems [30–34]; the use of fish for this purpose does not always show consistent results [35]. Contamination of marine nearshore ecosystems is assessed using the condition of fishes and aquatic invertebrates by analyzing the deviations from bilateral and radial symmetry [36–38]. It is recommended to use FA in the monitoring of bird populations in anthropogenically affected areas [39].

By reason of their sessile nature and having multiple uniform morphological structures, plants are widely used in studies of the impact of pollution and other factors on developmental stability [29,40–47]. It is worth noting that a study of FA of plants and animals does not always produce unambiguous results. There are field and laboratory studies where the FA level did not show an expected increase under the influence of negative factors [48–53]. Along with the negative experimental results of assessment of the effect of individual pollutants on the FA level in plants, there are also positive and partially positive results [54,55]. Interestingly, even within the same geographic region, the assessment of the impact of the same factor of chemical pollution on the FA level of the same species may vary among different authors to the extent of directly opposite conclusions about the applicability of FA for the assessment of environmental quality [41,51]. On the other hand, in other cases, the assessment of the impact of the same factor, such as high background radiation in the area of the Chernobyl disaster, in studies of different species by different authors revealed similar trends [28,56,57]. We entirely agree with Graham et al. [58] that fluctuating asymmetry should be used with great discretion, sample sizes should be large, and replicate measurements are essential. Additionally, in order to avoid ambiguity in bioindication of anthropogenic impact, one must factor in the specifics of the biology of the species used in the study lest stress-inducing factors of natural origin interfered with the analysis [59,60].

In the case of negative results, some of the authors came to the conclusion that FA is unsuitable as an indicator of genetic and environmental stress [51–53]. However, it is important to remember that in actual practice, a researcher cannot be sure whether the negative impact is stress inducing [58]. Individuals and populations may be well adapted to the environment that seems stress inducing to us or may be able to avoid stress by virtue of behavioral or other adaptations [58,61]. Perhaps more reasonable are the conclusions of the researchers who note that negative results can be explained by the insufficient sensitivity of the method to the stress-inducing factors or by the possibility that, in the given study, the given environmental impact is not stress inducing [48,54], or who in the absence of an evident negative impact make attempts to find the sources of a hidden habitat pollution [47].

The reliability of FA's use for assessment of the environmental stress can be increased by using it in combination with other indicators of environmental quality [22,26,47,62–66]. Thus, data on FA use for the assessment of environmental stress in general and anthropogenic pollution in particular are conflicting. There is an extensive record of some connection of the FA level with environmental factors that covers more than 40 years of research history. At the same time, there is a number of field works with negative results. Experimental studies also fail to clarify the situation, and the lack of a unified approach to the assessment of the FA level further hinders data analysis. Regarding plants, we believe that the conflicting and hard to interpret data are partially explained by the fact that they are exposed to pollutants from two media: The atmosphere and the soil. Note also that analysis of the impact of soil pollution is more difficult, since not only the concentration of pollutants is varied but also the content of nutrients, environmental chemistry, and sorption capacity. Perhaps, for this reason, the works examining the impact of atmosphere pollution on the FA level in plants are more likely to reveal a positive correlation [42,44,63].

The aim of the research was to assess the combined effect of an anthropogenically transformed environment of urban ecosystems on manifestations of fluctuating asymmetry in the silver birch. For the unification of our results, we conducted a one-time parallel study of one and the same species found in all seven cities, with sampling and material processing performed in a uniform way.

2. Materials and Methods

Research object: *Betula pendula* Roth belongs to the family Betulaceae. It is a tree 25–30 m tall and 0.6–0.9 m in diameter. It has a broad egg- to cone-shaped crown, often with pendulous branchlets. The silver birch has two types of vegetative shoots: Long and short; leaf arrangement is alternate; leaves are up to 8 cm long and to 5.5 cm wide, hairless, with double-toothed serrated margins; and the leafstalk is 2–3 cm long. Long shoots bear triangularly rhomb-shaped leaves; short shoots, rhomb-shaped leaves. Short shoots, as a rule, bear 2 leaves divided by 1-mm internodes [67–69].

B. pendula has a vast geographic range covering the European part of Russia, West and East Siberia, the Altai, the Caucasus, the Far East (except its far north-east), Western Europe, Central Asia, Mongolia, China, Korea, and Japan [67,68]. Previously, it was thought that on the territory of Yakutia, two separate species are found: The silver birch and the Japanese white birch (*Betula pendula* and *B. platyphylla*), but at the moment, *B. platyphylla* is considered to be synonymous to *Betula pendula* [69–71]. Some researchers have expressed an opinion that in the specimens of *Betula pendula* encountered from Europe to the east across Siberia, specific traits become closer to ssp. *mandschurica* (former *Betula platyphylla*) [72], but this was not observed in our materials, so we consider all samples as belonging to the same species.

B. pendula is undemanding, it belongs to hemieurybiotic species, and has a wide tolerance range to climatic and soil factors [73,74]. At the same time, many authors consider this species to have low resistance to toxic gases [1,75–77]. We selected this species for its abundance in the study area, widespread use in city street plantings, and sensitivity to pollution. In addition, for this species, a method of assessment of the FA level with extrapolation to environmental health had been developed [59].

Studied regions: The material was collected in 2019 in a uniform way in 3–5 sites on the territory of 5 cities with different anthropogenic loads. Additionally, we analyzed long-term data on three of the cities and on one city, with the data collected in 2007. The geographical location of the study site is shown in Figure 1, and the geographical coordinates and the main climatic parameters are given in Tables A1 and A2 [78–85].

Figure 1. The regions of the study [86].

1. Naro-Fominsk is located in the south-west of Moscow oblast, on the Central Russian Upland, 56 km from Moscow [84]. The city has 8 large and 38 small-scale industries, including a machine building plant, plastics plant, house-building factory, textile, food, cosmetics, and processing enterprises [87]. It is an important transit hub of road and rail transport.
2. Yoshkar-Ola is the capital of the Republic of Mari El, Russian Federation. It is located in the east of the East European Plain. The city has machine building, measuring tool manufacture, wood processing, pharmaceutical, and food industries. The most significant contribution to air pollution is made by motor transport: Up to 70–85% [88,89]. The Mari El Republic is one of the regions of the Russian Federation where high levels of atmosphere pollution have not been registered; this is facilitated by the terrain and climatic conditions favorable for pollution dispersion, i.e., by the zone of low air pollution potential [89].
3. Nazarovo is located 220 km west of Krasnoyarsk, in the Prichulymskaya forest-steppe zone of Minusinsk Hollow. It belongs to the category of small cities [84]. In the immediate vicinity from it, there is a deposit of brown coal of Kansk-Achinsk coal basin. Its main industrial enterprises are Nazarovsky open-pit coal mine and several machine-building and construction plants. Through the city passes a highway of regional significance [90]. Due to a high concentration of anthropogenic contamination sources, a significant transformation of vegetation can be noted there [90].
4. Achinsk is also located in the Krasnoyarsk krai. Through the city passes the Trans-Siberian Railway, as well as federal highway "Baikal". Currently, Achinsk is in the vanguard of the industry of the Krasnoyarsk krai [91]. The city's dominant industries are non-ferrous metallurgy, oil refining, and light and food industries. The main local enterprise is the Achinsk Alumina Refinery (AAR), the greatest source of air emissions in the city [92]. In the government report "On the State of the Environment and Conservation Measures in the Russian Federation in 2018", the cities of Achinsk and Nazarovo are included in the list of cities in Russia characterized by the highest values of the parameter "emissions of air pollutants from stationary sources" [93].

5. Krasnoyarsk is the administrative, scientific, and industrial center of the region; it is located in the valley of the Yenisei River at the border between the West Siberian Plain and the Central Siberian Plateau. Its key industries are non-ferrous metallurgy, space industry, hydropower, and education. The city is an important transport hub. Industrial enterprises are located within the main city territory, along with residential and recreational areas. Street tree plantings on the territory of Krasnoyarsk are fragmentary and their area is shrinking [92]. The city air contains approximately 200 kinds of pollutants, which come, among other sources, from three large power plants operating exclusively on brown coal [91,92].
6. Mirny is situated in the west of Yakutia, in the Irelyakh River valley (Vilyuy River basin). It is the center of the diamond mining industry of Yakutia [94]. Until 2001, diamond mining was conducted with the open-pit method; the city is situated on the edge of a giant pit, which at the time of its closure was over 500 m deep and had a diameter of 1200 m. The city has a processing plant; its waste rocks form dumps surrounding not only the industrial zone but the entire territory of the city, including residential areas.
7. Yakutsk is the administrative, scientific, and academic center of the Sakha Republic (Yakutia); it is located in the Lena River valley. It has a house building factory and food industry enterprises. The air flow over the territory of the city is rather weak due to the specifics of the terrain and wind conditions (average annual wind speed of only 2.4 m/s, often without wind [80], so air pollutants linger over the city, and in winter, fogs are frequent. Due to the difficult climatic conditions and permafrost, there is no subway system or electric public transport; because of this, motor vehicles are numerous: In 2016–2018, for 300,000 inhabitants, there were more than 119,000 motor vehicles [94]. The level of air pollution is characterized as elevated. The largest contribution to the total atmospheric pollution is made by particulate matter and benzpyrene; heavy air pollution is observed in the Central district [94].

Each of these cities is characterized by certain climatic conditions and specifics of the economy. Climate varies from moderately to sharply continental; average temperature and precipitation decrease eastward (Table A1) [78–85]. The climate grows more distinctly continental in the eastward direction from Naro-Fominsk to Yakutsk (Table A1). A combination of the mildest climatic conditions with a safe environmental situation is observed in Yoshkar-Ola. Krasnoyarsk is characterized by the largest population, high traffic load, and a big industry. Naro-Fominsk, Achinsk, Nazarovo, and Mirny are cities with a small population and high industrial load. Yakutsk is comparable in population size to Yoshkar-Ola but is characterized by higher levels of pollution due to motor transport and terrain parameters. Table A4 presents the characteristics of the studied sites on the territory of seven cities with a gradient of increasing industrial and transport loads [88,89,91–96]. Henceforward, we shall use the numeration of cities and sites used in Table A4. The city numbers increase eastward, and the numbers of sites within each city increase from the control ecotopes to the sites with higher industrial and traffic load.

Material collection: The studies were carried out in accordance with the method proposed by Zakharov et al. [59], whereby to characterize one site, 100 leaves (10 leaves from 10 trees) are necessary. We followed this method strictly, except when there were fewer than 10 trees of the suitable age in the studied site. Leaves were collected after their growth process was completed (starting in July), from short shoots of the previous year (one leaf per shoot) from different branches along the perimeter of the lower part of the crown, at a height of about 120–150 cm. the morphogenesis of the leaf from a short shoot, as compared with the leaf from the long shoot is fairly stable in young, middle-aged, and old individuals of *B. pendula* of the reproductive period [47]. For the analysis, we used the leaves of the trees from approximately the same conditions of light intensity and moisture. We took into consideration that the birch is a light-demanding tree, and in shading, it can manifest higher FA levels [59,97], so we selected trees standing in open areas. In addition, we used only the trees of reproductive age, since in prereproductive and postreproductive trees, an increase in FA levels was recorded even when compared to the neighboring reproductive individuals [93]. According to the method, only medium-sized leaves are suitable for the analysis, because very small and very large laminae are likely to be characterized by an increased FA level [59,97]. The leaves damaged by pests or diseases were not analyzed either, as they also can skew the final outcome in the direction of overestimated FA [27,97–99]. Additionally, the leaves were collected only from the trees with distinct species traits, because in interspecific hybrids, an increase in FA is possible, as a consequence of development destabilization [19,59,100].

Material amount: For the assessment of the combined anthropogenic load in 33 ecotopes on the territory of 7 cities, a total of 9000 leaves were collected. In 14 of these sites, changes in the FA level over the course of 3–4 years were analyzed, and in 19 sites one-season studies were conducted. To assess the effect of the traffic load, a count of motor vehicles in 30 sites and an assessment of FA of 3000 leaves on the territory of two cities was performed. A total of more than 12,000 birch leaves were collected, and 120,000 measurements of the right and left leaf sides were taken.

Material processing: For the analysis, we used fresh or herbarized leaves. Leaves collected in the territory of the city 1 and 3–7 were scanned with a 300–400 dpi resolution. Our preliminary studies showed that a resolution of over 400 dpi is meaningless, since it does not affect the accuracy of measurements. Prior to specimen preservation and scanning, the information about the site where the leaves were collected was encrypted, and the measurements were taken for code-labelled images. Measurements were taken using software ImageJ (cities 3–5) [101] or Bio (cities 1, 6–7) with the following accuracy: Linear, up to 0.1 mm; angle, up to 0.1°. Leaves collected in the city 2 were preserved and then measured with a metal ruler and a protractor with an accuracy of 1 mm and 1°; the correctness of the measurements obtained with this procedure was ascertained by the authors of this method in 2000 [59]. We consider it possible to compare the measurements obtained in these two manners, because, as we demonstrated earlier, the measurement error depends mainly not on the manner in which the measurement was taken but on the (lack of) experience of the researcher [60], while in the present study, all the researchers had experience with the method of over 5–10 years. FA was assessed by five characteristics of the lamina structure and venation (Figure 2). For each of the characteristics, its FA value was calculated as the absolute value of the ratio of the difference between the measurements of the left and right halves of the leaf to their sum (Formula 1):

$$FA = \left|\frac{(L-R)}{(L+R)}\right|, \tag{1}$$

where FA, fluctuating asymmetry absolute value; L and R, measurements of the left and right halves of the leaf. Integrated fluctuating asymmetry (IFA) was calculated as the five characteristics' mean. We calculated the average IFA for each of the examined trees and for the site as a whole.

Traffic load (TL) in the territories of Yakutsk and Naro-Fominsk (cities 1 and 7) was estimated at 30 sites triply during 15 min of peak-activity hours (8.30 through 9.00 and 17.30 through 18.30). The results were averaged and converted to the number of vehicles per hour [102]. For the rest of the cities, we used the official data from the committees of ecology and nature conservation [83,86–90]. Industrial load (IL) at different sites of the cities was estimated based on the official data from the committees of ecology and nature conservation considering the distances to various industrial enterprises and the types of industrial production [89,92–96]. A 4-grade scale was used for both indices. For each site, an average score was calculated: Transport and industrial load (TIL).

The total anthropogenic load on a city was scored based on three parameters: 1. Population size [84]: Up to 100,000, 250–350,000, and over 1 million; 2. Functional role: Administrative, administrative-industrial, or industrial center; 3. Environmental pollution as averaged by five parameters: Annual emissions by industrial enterprises in the territory of a city, atmospheric and soil pollution as related to the average over Russia, gaseous pollutant content in the atmosphere, solid particle content in the atmosphere [89,92–96]. To estimate the total anthropogenic load on the site, we considered the total anthropogenic load on the city and the transport-industrial load on the site.

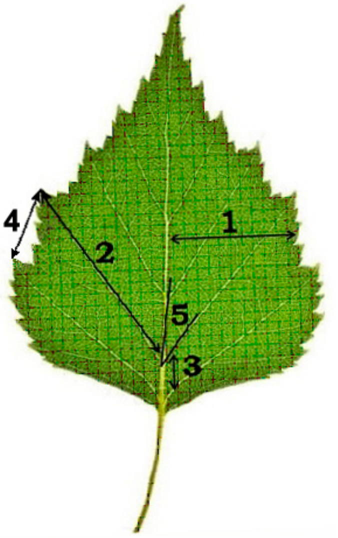


Figure 2. Scheme of the birch leaf characteristics used for the assessment of fluctuating asymmetry. Measurement key: 1. Leaf half-width; 2. Length of the second from the leaf base secondary vein; 3. Distance between the bases of the first and second secondary veins; 4. Distance between the ends of these veins; 5. Angle between the primary vein and the second from the leaf base secondary vein.

For assessment of the possible climate influence, standard parameters were analyzed: The average annual temperature, average temperatures of the warmest and coldest months (July and January), and average precipitation: Annual, that of the warm and cold periods of the year (with daily mean temperatures above and below 0° C). Additionally, we considered the duration of the warm period (with daily mean temperature above 0° C), frost-free period, growing season (with mean daily temperatures above +5° C), and the sum of active temperatures (the sum of positive temperatures during the growing season). The last two factors are of great significance for plants, including *Betula*. In addition, we used two calculated parameters: Hydrothermal coefficient (HTC) and climate inclemency (CI). The hydrothermal coefficient indicates whether the territory has sufficient rainfall during the growing season, which is of great importance for plant life (Formula 2):

$$\mathrm{HTC} = \frac{\mathrm{WPP}\cdot 10}{\mathrm{SAT}}, \tag{2}$$

where HTC, Hydrothermal coefficient for the sum of active temperatures; WPP, warm period precipitation; SAT, sum of active temperatures of the growing season. Climate inclemency values (in general, in summer, and in winter) were obtained by ranking the considered climatic parameters and calculating the arithmetic mean (Formula (3)):

$$\mathrm{GCI} = \frac{\sum \mathrm{R_n}}{\mathrm{n}}, \tag{3}$$

where GCI, general climate inclemency; R, ranks of particular climatic parameters; and n, the number of parameters. The general climate inclemency was evaluated by eight climatic parameters (Table A2). SCI, summer climate inclemency, and WCI, winter climate inclemency, were calculated using the same formula, respectively, by five and three parameters (Table A2).

In the discussion, we made a comparison of the received data to the scale proposed by Zakharov et al. [59] for the assessment of environmental health by the developmental stability of the silver birch (Table 1).

Table 1. Numerical scale for the assessment of environment quality by the developmental instability of the silver birch [59].

FA Level	Conditions	Rank	Score
Below 0.040	Undisturbed, minimal deviations from the normal state	I	1
0.040–0.044	Minor deviations	II	2
0.045–0.049	Moderate deviations	III	3
0.050–0.054	Significant deviations	IV	4
Above 0.055	Considerable deviations, critical state	V	5

The statistical data processing was carried out in *Statistica 10* software package using nonparametric statistics, Spearman's coefficient, one-way ANOVA, factorial ANOVA, Scheffe test, multiple regression, clustering method, and principal component analysis (PCA) [103].

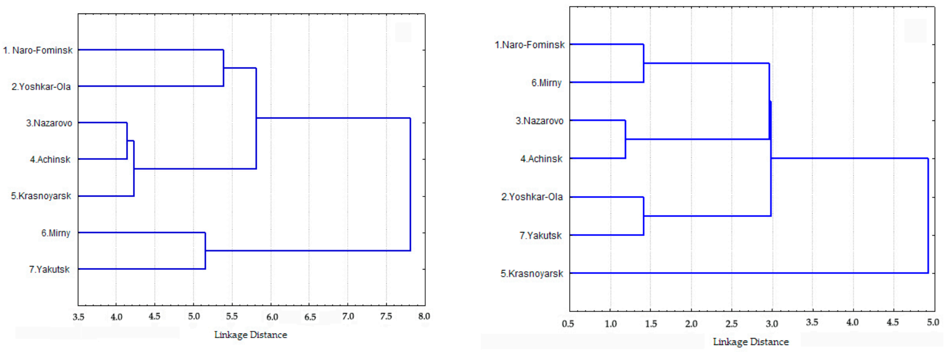
3. Results and Discussion

3.1. Characteristics of Climatic and Anthropogenic Load

Since the studied cities are situated at a significant distance from each other, they differ in climatic conditions. Thus, we correlated climatic variables among themselves and with geographical confinement as well. For the seven studied cities, most of the climatic variables showed a significant correlation with geographical longitude, while only the cold period precipitations are correlated with geographical latitude (Table A3). We suppose this is explained by rather insignificant differences in latitude with considerable variation of longitude.

The correlation analysis of climatic variables showed the strongest relations between the annual temperature, annual and cold period precipitations, as well as free-frost, warm, and growing periods' length ($p < 0.05$) (Table A3). Summer precipitations and the sum of active temperatures of the growing season are weakly related. Such conventional variables as winter and summer precipitation amounts calculated for winter (November, December, January) and summer (June, July, August) months appeared to be ineffective. The average temperatures of July and January do not correlate with longitude and latitude and are weakly related with other climatic variables. No statistically significant correlation was revealed for IFA and climatic variables ($p > 0.05$).

However, for the climate effect consideration in the further analysis, we estimated all the listed variables, as well as the conventional score coefficients calculated on their basis. The analysis of all the variables yielded three clusters of the cities: The European part of Russia (cities 1–2) with the mildest moderately continental climate; Central Siberia (cities 3–5) with a continental climate; and the most distinct cluster of East Siberia (cities 6–7) with a sharply continental climate (Figure 3a). This classification coincides with the geographical confinement of the studied region.



(**a**) (**b**)

Figure 3. Similarity dendrograms of the studied cities by climatic characteristics (**a**) and complex anthropogenic load (**b**), (Euclidean distances).

To evaluate the anthropogenic impact, we ranged both traffic, industrial, and anthropogenic loads based on the data on the functional role of the studied cities, their population size, and ecological situation (Table A5). Correlation analysis of IFA and all the types of anthropogenic loads using the Spearman's rank correlation coefficient showed rather a strong relation for most variables (Table A5). Cities' classification based on anthropogenic load differed markedly from the above-mentioned classification based on the climate (Figure 3b). Thus, the functional significance of a city and environmental pollution is most likely to play a major role here. As a result, Krasnoyarsk, a city with a population size of over one million and a strongly polluted environment, stands apart from the other studied cities. Among the rest of six cities, two administrative centers are clustered as well as two pairs of industrial centers: 1 + 6 with a small population size and the absence of hazardous industry and 3 + 4 featuring a high amount of heavy industry enterprises and use of brown coal in the power industry.

3.2. FA of Betula Pendula in Urban Territories

City 1: On the territory of Naro-Fominsk, a total of 2800 leaves of *B. pendula* from 28 sites were analyzed. These sites can be divided into five locations with respect to the nature of anthropogenic impact. The lowest IFA of 0.038 was recorded in the recreation area of the city (location 1.1); a somewhat higher IFA was noted in the old center of the city at some distance from the roadway (location 1.2). Significantly higher IFA figures were noted at locations 1.3–1.4, in proximity of industrial enterprises (Figure 4, Table 2). The least satisfactory condition was observed in the south-eastern part of the city, where the main industrial facilities are concentrated and there is a high traffic load; the average IFA there was 0.050, varying within the location in the range of 0.049–0.054. The highest figures were observed in *B. pendula* trees growing along the federal highway (location 1.5). One-way ANOVA showed significance of the ecotope factor, F = 4.22, $p < 0.001$, with the Scheffe test giving significant differences between the studied areas of the city (Table 2). Thus, *B. pendula* growing in the center of the city had relatively low IFA figures, while the greatest degree of ontogeny destabilization is observed in the south-eastern part of the city, due to a high traffic load and location of the most of industrial enterprises; also, it can be associated with the predominance of north-westerly winds.

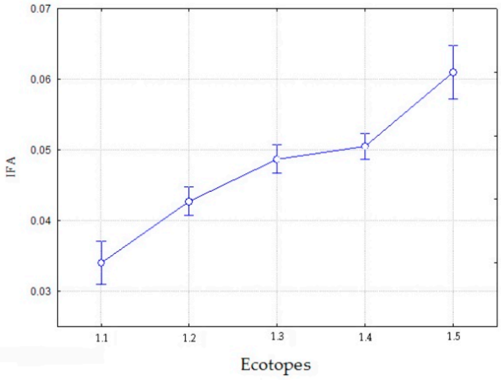


Figure 4. IFA of *Betula pendula* in different ecotopes of Naro-Fominsk (2007) (vertical bars, confidence interval, 95%).

Table 2. IFA of *Betula pendula* and the rank of environmental health [59] on the territory of 7 cities.

City	Ecotope	Year	IFA		Rank
			n	M ± m	
Naro-Fominsk	1.1	2007	300	0.034 ± 0.001	I
	1.2	2007	600	0.042 ± 0.001	II
	1.3	2007	900	0.049 ± 0.001	III (IV) *
	1.4	2007	800	0.050 ± 0.001	IV (III) *
	1.5	2007	200	0.061 ± 0.002	V
	One-Way ANOVA. Intercept F = 6853,00, $p < 0.001$. Ecotope Current effect F = 43.5, $p < 0.001$				
Yoshkar-Ola	2.1	2000. 2001. 2016. 2019	400	0.037 ± 0.001	I
	2.2	2000. 2001. 2016. 2019	400	0.040 ± 0.001	II (I) *
	2.3	2019	100	0.045 ± 0.001	III (II) *
	2.4	2000. 2001. 2016. 2019	400	0.047 ± 0.001	III
	2.5	2000. 2001. 2016. 2019	400	0.050 ± 0.001	IV (III) *
	One-Way ANOVA. Intercept F = 2475.84, $p < 0.001$. Ecotope Current effect F = 4.42 $p < 0.01$ **Factorial ANOVA** Intercept F = 6257.18, $p < 0.001$. Ecotope F = 25.17, $p < 0.001$. Year F = 1.54 $p = 0.20$ Tree F = 0.52 $p = 0.86$				
Nazarovo	3.1	2019	100	0.044 ± 0.001	II (III) *
	3.2	2019	90	0.055 ± 0.001	V (IV) *
	3.3	2019	80	0.064 ± 0.002	V
	One-Way ANOVA. Intercept F = 6379.6, $p < 0.001$. Ecotope Current effect F = 2.24 $p < 0.001$				
Achinsk	4.1	2019	100	0.033 ± 0.002	I
	4.2	2019	100	0.044 ± 0.001	II (III) *
	4.3	2019	100	0.049 ± 0.001	III (IV) *
	4.4	2019	100	0.057 ± 0.001	V
	4.5	2019	100	0.068 ± 0.002	V
	One-Way ANOVA. Intercept F = 1689.9, $p < 0.001$. Ecotope Current effect F = 4.45, $p < 0.001$				
Krasnoyarsk	5.1	2019	100	0.055 ± 0.001	V (IV) *
	5.2	2019	100	0.068 ± 0.001	V
	5.3	2019	100	0.067 ± 0.002	V
	5.4	2019	100	0.072 ± 0.001	V
	One-Way ANOVA. Intercept F = 2620.09, $p < 0.001$. Ecotope Current effect F = 7.43, $p < 0.001$				
Mirny	6.1	2003. 2011. 2014	300	0.043 ± 0.001	II
	6.2	2003. 2011. 2014	300	0.045 ± 0.001	III (II) *
	6.3	2003. 2011. 2014	300	0.047 ± 0.001	III
	6.4	2003. 2011. 2014	270	0.050 ± 0.001	IV (III) *
	6.5	2003. 2011. 2014	300	0.052 ± 0.002	IV
	One-Way ANOVA. Intercept F = 6720.6, $p < 0.001$. Ecotope Current effect F = 4.14, $p < 0.001$ **Factorial ANOVA** Intercept F = 5905.05, $p < 0.001$. Ecotope F = 5.88, $p < 0.001$. Year F = 0.12 $p = 0.89$				
Yakutsk	7.1	2019	100	0.038 ± 0.002	I (II) *
	7.2	2016. 2017. 2018. 2019	400	0.042 ± 0.001	II
	7.3	2016. 2017. 2018. 2019	400	0.044 ± 0.001	II (III) *
	7.4	2016. 2017. 2018. 2019	400	0.048 ± 0.001	III
	7.5	2016. 2017. 2018. 2019	400	0.053 ± 0.001	IV
	7.6	2018. 2019	200	0.058 ± 0.002	V
	One-Way ANOVA. Intercept F = 1396.4, $p < 0.001$. Ecotope Current effect F = 7.72, $p < 0.001$ **Factorial ANOVA** Intercept: F = 7817.6, $p < 0.001$. Ecotope F = 19.65, $p < 0.001$. Year: F = 2.19, $p = 0.087$				

Note: *, the ranks corrected for error; M, the arithmetic mean; m, error.

City 2: The analysis of IFA of *B. pendula* in Yoshkar-Ola in 2019 revealed a dependence of its increase on the increase in anthropogenic pressure (F = 4.42, $p < 0.01$) (Tables 2 and 3). During the four years of the study, IFA of *B. pendula* in the site 2.1 varied within 0.036–0.039. These trees grow in a protected recreational forest within the city limits, but the level of pollution from motor vehicles and industrial plants is minor [88]. IFA of the trees found in a residential area in conditions of a weak traffic load and at a distance of 1 km from an enterprise in construction materials manufacture (site 2.2) varies within 0.037–0.041. IFA of the trees found in recreation areas (sites 2.3 and 2.4) indicate moderate developmental abnormalities in *B. pendula*. These ecotopes are in the area moderately polluted with industrial and transport emissions. Despite being located in the area affected by an industrial enterprise and surrounding roads, the level of air pollution in the site 2.3 remains low, which was revealed by direct monitoring of the atmosphere [89]. Judging by IFA, *B. pendula* in the site 2.5 has moderate to significant developmental abnormalities. The registered maximal IFA most likely indicates a continuous impact of emissions not only of a chemical plant but also of motor vehicles.

Table 3. Average values of IFA of *Betula pendula* on the territory of Yoshkar-Ola in different years of research.

Site	Years of Research, M ± m			
	2000	**2001**	**2016**	**2019**
2.1	0.036 ± 0.001	0.036 ± 0.001	0.039 ± 0.004	0.038 ± 0.002
2.2	0.039 ± 0.001	0.037 ± 0.003	0.041 ± 0.002	0.041 ± 0.002
2.3	–	–	–	0.045 ± 0.001
2.4	0.046 ± 0.002	0.048 ± 0.003	0.047 ± 0.002	0.047 ± 0.002
2.5	0.050 ± 0.001	0.048 ± 0.002	0.053 ± 0.002	0.048 ± 0.001

Note: "–", absence of data; M, the arithmetic mean; m, error.

The performed three-factor ANOVA revealed that the influence of environmental parameters on the developmental stability of the marked trees of *B. pendula* in different years of the research is significant ($p < 0.001$). Despite the weather fluctuations, we did not find a statistically significant effect of such a factor as the year of research on IFA ($p > 0.05$). Note that the "tree" factor was not found to be significant in the marked trees, i.e., the FA level is not an individual characteristic but varies over years. Therefore, in 20 years, the environmental quality as determined by the value of the integrated index of the developmental stability of *B. pendula* remained at the same level. The Scheffe test revealed significant differences between the groups in IFA of *B. pendula* of the sites 2.1, 2.2, and 2.4, 2.5 ($p < 0.001$). The analyses of atmosphere pollution by stationary posts (sites 2.1–2.4) also indicated minor fluctuations in toxicant emissions by year [84,85].

The assessment of the developmental stability of *B. pendula* by its IFA in different districts of Yoshkar-Ola agrees with the data of the chemical analysis of the atmosphere [88,89] and other bioindication parameters of *B. pendula* [47].

City 3: On the territory of Nazarovo, we registered a significant variation in IFA of *B. pendula*: 0.044–0.064 (Table 2). The lowest IFA was registered in the site 3.1, which is characterized by a relatively low anthropogenic load; it is situated in the Chulym River valley, approximately 0.5–1 km from a housing estate and a road with a low traffic load. Significant abnormalities are registered on the site 3.2, which is associated with increased exposure to motor transport: At a distance of less than 800 m, there is a highway of regional importance and a garage block; besides, approximately 1.5 km away, a GRES (regional power station) is located, which also contributes to the deterioration of environmental quality. Samples collected at the edge of the industrial zone in the site 3.3 indicate a significant anthropogenic impact on the trees. IFA of *B. pendula* in this case is 0.064, which is due not only to the impact of motor transport but also to emissions of adjacent industries. One-way ANOVA has shown that the F-test for ecotope was 74.9 with a high level of significance ($p < 0.001$); the Scheffe test revealed significant differences between all three sites ($p < 0.001$). Thus, the territory of Nazarovo in general is characterized by elevated levels of IFA. Presumably, it is connected with the long-term negative impact of anthropogenic sources of pollution and the fact that the GRES is operating on lignite.

City 4: In the natural and anthropogenically transformed ecotopes of Achinsk, significant differences in IFA of *B. pendula* leaves were found (Table 2). For instance, in the background site (in a forest near Ozero Bol'shoe village), IFA was 0.032, and in individuals, it varied within 0.022–0.046. In the recreation area of Achinsk, IFA of *B. pendula* leaves was 0.044, which indicates minor deviations in the development stability of the birch. In the residential area (site 4.3) of Achinsk, IFA is affected by the motor transport and the food industry enterprise. The IFA of *B. pendula* leaves there is 0.049, and it is significantly higher than in the natural biotope. In the ecotope 4.4, IFA was 0.057, which is probably due to the heavier traffic and a close proximity to the southern industrial zone. The highest figures of IFA (0.068) were found in the area affected by the southern industrial zone of Achinsk (ecotope 4.5), where several heavy industry enterprises are operating. One-factor ANOVA showed, the same as with Yoshkar-Ola and Nazarovo, a high significance of the ecotope factor (F = 23.2, $p < 0.001$), but the Scheffe test revealed that the differences between the sites 6.1 and 6.4 ($p < 0.05$), 6.1 and 6.5 ($p < 0.001$), and 6.2 and 6.5 ($p < 0.01$) were statistically significant. Thus, on the territory of Achinsk, the IFA of *B. pendula* in the areas of heavy traffic and industrial loads is significantly higher than in the natural biotope and recreation area. In general, in all the studied ecotopes of Achinsk, a negative effect of various environmental factors was found, mostly of the anthropogenic factor, because a large number of industrial enterprises are concentrated in a city with an area of 103 km^2.

City 5: In Krasnoyarsk, the IFA of *B. pendula* leaves in the studied areas varied within 0.054–0.071. Even in the control site, which lies within the recreation area of the city, the IFA of *B. pendula* was 0.054, which is significantly higher than in the natural habitats of the region, for example, in the vicinity of Ozero Bol'shoe village, which is located 140 km away (Table 2). Site 5.1 (Tatyshev Island) is located within the city limits and is actively used by the Krasnoyarsk residents for sporting events, dog competitions, picnics, driving lessons, etc. Thus, the flora of the island experiences a heavy anthropogenic load, and the presence of motor vehicles increases general and gas pollution [104].

Three other sites within Krasnoyarsk are arranged along the gradient of increasing traffic and industrial load, from low (site 5.2) to high (5.4). No significant differences were found between them, and all three points are characterized by the highest IFA figures among the examined cities. One-way ANOVA showed the significance of the ecotope factor ($p < 0.001$). Note that all three sites in the residential areas of the city were different from the recreation area, but with varying degrees of statistical significance, which rose from site 5.2 to site 5.4 (Scheffe test respectively: $p < 0.05$, $p < 0.01$, $p < 0.001$).

City 6: On the territory of Mirny (Western Yakutia), the leaves were collected in five sites: In the city recreation area, in the city center (in the yards and in wooden building quarters), and also in the northern and southern outskirts. The IFA ranged from 0.042 to 0.053, with differences between the sites both in separate years and in average data, in most cases, not reaching statistical significance, except for sites 6.4 and 6.5 (Table 4, Figure 5). In the northern part of the city, near the ore processing plant, IFA was significantly higher than in the recreation area ($p < 0.05$), and in the southern part of the city it differed significantly from the sites 6.1 and 6.2 ($p < 0.001$ and $p < 0.05$, respectively). Multi-factor ANOVA revealed the significance of the effect of the ecotope factor at IFA (F = 4.13, $p < 0.001$), and no significant differences between the years of research (Table 2).

Table 4. Average IFA of *Betula pendula* on the territory of Mirny in different years.

Site	Years of Research, M ± m		
	2003	2011	2014
6.1	0.043 ± 0.002	0.044 ± 0.003	0.042 ± 0.001
6.2	0.044 ± 0.002	0.045 ± 0.002	0.047 ± 0.002
6.3	0.046 ± 0.002	0.046 ± 0.002	0.049 ± 0.002
6.4	0.052 ± 0.003	0.048 ± 0.002	0.049 ± 0.003
6.5	0.053 ± 0.003	0.053 ± 0.003	0.049 ± 0.002

Note: M, the arithmetic mean; m, error.

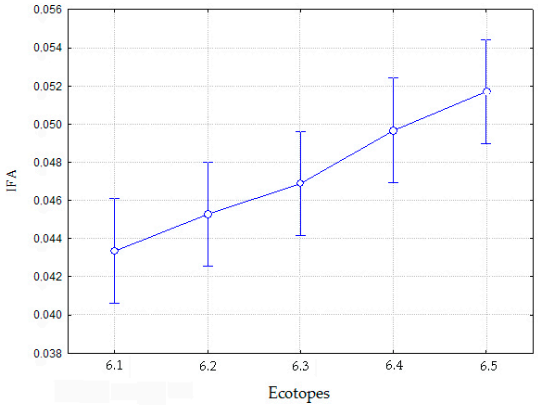


Figure 5. IFA of *Betula pendula* in different ecotopes of Mirny (2003, 2011, 2014) (vertical bars, confidence interval, 95%).

City 7: For assessment of the impact of the anthropogenic factor on the territory of Yakutsk, *B. pendula* leaves collected in 5 sites in 2019 were examined; as a control, an undisturbed birch forest was chosen, where IFA was 0.038. The condition of *B. pendula* in the recreation area of the city was studied on the territory of the Botanical Garden (site 7.2); the IFA there was somewhat higher than in the natural biotope, 0.042 (Table 5). Perhaps, this is due to the fact that in the botanical garden, the birch experienced a negative impact of pests and diseases due to the proximity of cultivated plants infected by them. Previously, we analyzed the developmental stability figures of *B. pendula* on the territory of Yakutsk and used IFA to divide the territory of the city into three zones. On this basis, in the present study, we selected four sites in the residential areas of Yakutsk from different zones of the city. Site 7.3 is in the zone of minor impact; there is a forest and some exurban plots nearby; traffic load is low; and the IFA in this zone was 0.043. Site 7.4 is in the zone of moderate impact; the IFA there was 0.047. Site 7.5 is in the central area of the city, with a high traffic load and near food industry enterprises; this is a zone of major negative impact, where the IFA was 0.056, which differs significantly from the natural habitat ($p < 0.01$). The highest level of developmental instability in *B. pendula* (0.060) was observed in an area that is being actively built up with high-rise apartment buildings. This area is characterized with a high traffic load, including trucks, and contamination with construction dust. This site significantly differs by IFA from the sites 7.1–7.3 (Scheffe test with $p < 0.05$, 0.01, and 0.001, respectively). One-factor ANOVA showed significant differences in IFA by ecotopes (F = 7.72, $p < 0.001$) (Table 2).

Table 5. Average IFA of *Betula pendula* on the territory of Yakutsk in different years.

Site	Years of Research, M ± m			
	2016	**2017**	**2018**	**2019**
7.2	0.043 ± 0.002	0.042 ± 0.002	0.042 ± 0.002	0.042 ± 0.002
7.3	0.044 ± 0.002	0.042 ± 0.002	0.045 ± 0.002	0.043 ± 0.002
7.4	0.045 ± 0.002	0.046 ± 0.002	0.053 ± 0.003	0.047 ± 0.002
7.5	0.046 ± 0.002	0.055 ± 0.002	0.053 ± 0.003	0.056 ± 0.003

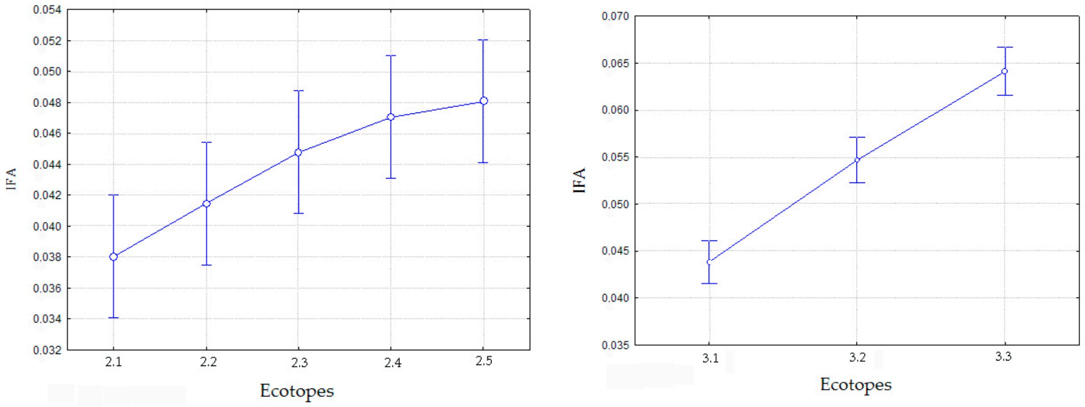
Note: M, the arithmetic mean; m, error.

To carry out a three-factor ANOVA of the long-term data, four sites from different zones of the city were selected (Table 2); a significant effect of the environmental parameters on the developmental stability of *B. pendula* in different years of the research was revealed (F = 19.6, $p < 0.001$). Note that the generalized data for 3 years showed an even higher significance of differences: Site 7.5 was significantly different in IFA from sites 7.2–7.4 ($p < 0.001$), and site 7.4 had a significantly higher IFA than the recreation area ($p < 0.001$).

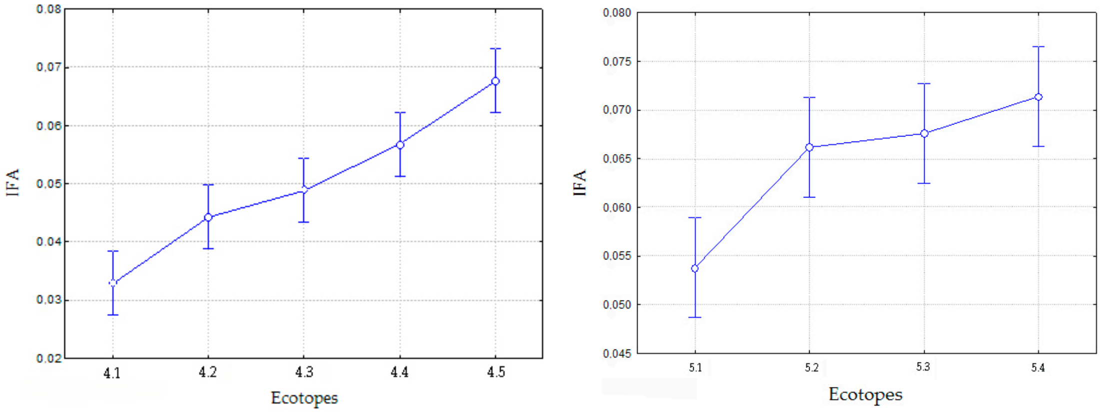
For Yakutsk, just as for Yoshkar-Ola and Mirny, no significant effect of the factor of year at IFA of *B. pendula* was found ($p > 0.05$). Data on atmosphere pollution from stationary posts also indicate minor fluctuations in the toxicant emissions by year [94]. On the whole, it should be noted that on the territory of the city, a significantly increased IFA was registered only in the areas with a high density of traffic, but even the recreation area was characterized by a higher IFA than the natural habitat. Multi-factor ANOVA revealed no significant effect of the factor of the year at IFA of *B. pendula* for the 4-year study period in two cities, so we decided to involve in the analysis the data from the two cities on the territory of which the material was collected not in 2019 but in other years.

Thus, multi-factor ANOVA revealed no significant effect of the factor of the year on IFA of *B. pendula* in the 3–4-year study period in the three cities. Erofeeva [42], on the basis of a 5-year study, also came to the conclusion that in the urban territory, in conditions of intensive impact, the main factor affecting the birch FA is not weather conditions but the anthropogenic factor. We believe that it makes it possible to use IFA as a universal criterion of developmental stability and of urban environment health when data collected in different seasons are analyzed.

Comparison of the IFA levels of the birch within one growing season on the territory of five cities has shown that there is a trend of FA increasing with the anthropogenic load, but the degree of manifestation of these differences varies: On the territory of Yoshkar-Ola and Yakutsk, a gradual increase is observed, and some of the urban sites are comparable in FA to undisturbed biotopes. However, the cities of the Central Siberia are characterized by a sharp increase in FA and significant differences with the natural biotopes and the recreation areas (Figure 6).

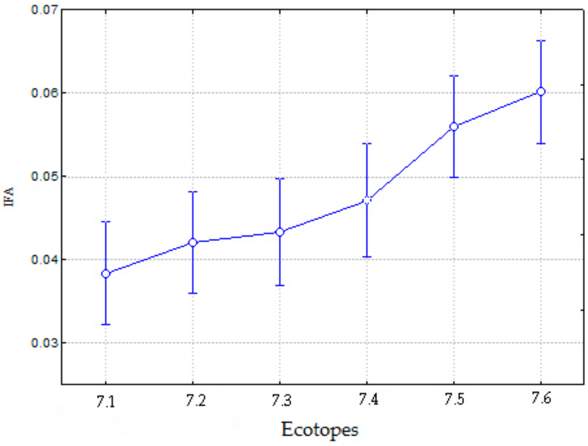


(**a**), **Yoshkar-Ola** (**b**), **Nazarovo**



(**c**), **Achinsk** (**d**), **Krasnoyarsk**

Figure 6. *Cont.*



(**e**), **Yakutsk**

Figure 6. IFA of *Betula pendula* on the territory of 5 cities in 2019 (vertical bars, confidence interval, 95%).

3.3. Ranking Environmental Quality Based on FA Level of Betula Pendula

In most case studies of urban territories, we deal with a complex of factors affecting the environment and developmental stability of plants [5,10,20,46,47,63]. In Section 3.2, we already mentioned that the *B. pendula* IFA ranged widely from 0.033 to 0.072 (Table 2). Low IFA values (0.033-0.038) were recorded at the provisional control sites: Natural biotopes or ecotopes with a slight recreational load in the cities 1-2, 4, and 7 (Naro-Fominsk, Yoshkar-Ola, Achinsk, and Yakutsk). The control sites in the cities 3 and 6 (Nazarovo and Mirny) showed some higher IFA values (0.043-0.044). Only city 5 (Krasnoyarsk) stands apart for its unfavorable conditions even at the provisional control site (recreation area) due to significant background pollution, yielding an FA increase up to 0.054 (Figure 6, Table 2). All the studied cities tend to an FA increase from the natural biotopes towards the most polluted areas. This was also noted before by Zakharov et al. [46,62]. This gives rise to the task of environmental assessment and ranking of the studied cities and ecotopes.

Environmental assessment using the FA rank coefficient (IFAR) implies that IFA of less than 0.040 indicates high developmental stability, being indirect proof of the well-being of both internal and ambient environments, i.e., characterizing this environment as favorable for living. Further increments of 0.05 indicate increasing ontogenesis destabilization and may serve as a measure of environmental disturbance. Correlation between the absolute values of IFA and IFAR made up 0.98 (Spearman's $t = 25.8$, $p < 0.001$), and score averaging for various biotopes showed that the developmental stability of trees in natural biotopes was ranked as I-II, with the average score of 1.2 (Table 6). For recreational areas, including parks, gardens, and city outskirts, developmental stability could vary within ranks II-IV, while for residential areas beyond the industrial and traffic impact within II-V. The most significant deviations at the level of IV-V were recorded in trees growing in the territories affected by traffic and industrial enterprises. The dispersion analysis showed significant FA differences among the ecotopes (Scheffe test $p < 0.001$). As a whole, IFAR mirrors the results of IFA analysis, though it more clearly demonstrates an increase in tree ontogenesis destabilization in deteriorated urban ecosystems.

As judged by the IFA values and IFAR rank scale, the most ecologically unharmed city is Yoshkar-Ola. Its background index falls under rank I of environmental health, while even the most troubled ecotopes fall under rank IV. In Mirny, the IFA values also do not exceed rank IV, while the background ecotope is ranked II, and the residential area is within ranks III-IV. This may prove the general ill-being of the environment. Yakutsk, Naro-Fominsk, and Achinsk display the whole range of the rank scale from safe natural biotopes through to a critical situation in separate urban ecotopes. Nazarovo features an even worse environment as assessed within ranks II-V. Extremely high IFA values for Krasnoyarsk state the worst environmental quality there: The recreational area is ranked IV, while the remaining three sites are ranked V.

Table 6. Assessment of *Betula pendula* development instability in different ecotopes (average data for seven cities).

Ecotopes	**IFA**		**Rank**	**Score**
	n *	**M ± m**		
Natural biotopes	5	0.037 ± 0.001	I	1.2
Recreation zones	6	0.046 ± 0.001	III	2.7
Residential area beyond the industrial and traffic influence	9	0.050 ± 0.001	IV	3.1
Industrial zones and highways	12	0.058 ± 0.001	V	4.5

Note: M, the arithmetic mean; m, error; n *, number of ecotopes (leaves = n × 100).

3.4. Influence of the Traffic Load and Petroleum-Based Emissions on FA in the Silver Birch

We analyzed *B. pendula*'s developmental stability in terms of traffic density in direct proximity to the road at 20 sites in Yakutsk and 10 sites in Naro-Fominsk. The vehicles were counted during the peak-activity hours. In Yakutsk, traffic density ranged from 463 to 2570, while in Naro-Fominsk, from 100 to 3000 vehicles per hour. In both cases, the correlation between IFA and traffic load (TL) appeared to be statistically significant. Spearman's test made up 3.14 ($p < 0.01$) for Yakutsk and 2.96 ($p < 0.05$) for Naro-Fominsk. The regression analysis of the combined data showed insignificant differences between the cities and the significant impact of TL on IFA ($t = 37.4$, $p < 0.001$) (Figure 7). As a rule, increased FA in the areas with a high traffic density comes from the aerotechnogenic pollution. It is well known that exhaust gases carry into the atmosphere of the city particulate matter of elements, such as Cr, Mn, Ni, Cu, Zn, Cd, and Pb [105]. Erofeeva [42] noted that the traffic load has a strong positive correlation ($r = 0.80$–0.90; $p < 0.05$) with the peak emissions of the major pollutants contained in vehicle exhaust gases (oxides of sulphur, nitrogen, and carbon; hydrocarbons, formaldehyde, soot). Thus, the road-side dust in Yakutsk is stated to have a 3.4 times increase in Mo, 2.7 times increase in Zn, and 2 times in Pb as compared to the control dust sample [106]. There are records of heavy metals, such as Pb, Zn, and Cu, in the soil of the traffic zone. The 5 m-wide roadside zone is exposed to severe dusting: Up to 184 g dust per 1 m^2 contain 6.13 mg Pb, 37.47 mg Zn, 8.12 mg Cu, and no less than 0.02 mg Cd. At the distance of 20–50 m from the road, the dust and heavy metal load are decreased approximately 10 times; and at the distance of 100–250 m, 100 times [107]. The dependence of the FA level of the birch on the traffic load and technogenic air pollution was noted before by us and other authors [29,42,44,45,63].

The data obtained by studying *Betula pendula* are confirmed by data on other tree species. The factor analysis we performed earlier showed that developmental instability in the Scots pine *Pinus sylvestris* on the territory of Krasnoyarsk is also associated with traffic emissions. As a result of the accumulation of heavy metals in the soil cover and in plant tissues, a change in the morphometric characteristics (IFA) of the needles of the Scots pine takes place in the ecotopes of Krasnoyarsk with a high traffic load [108].

Some sites within the urban territories differed from the background by the developmental instability of plants. This could be the result of some specific pollution types. It is well known that gas stations are one of the sources of air pollution in city limits [109]. Within Naro-Fominsk, an increase of *B. pendula* IFA was recorded at the sites exposed to oil-product pollution (Figure 8). Differences between the natural biotope and the ecotopes confined to fuel filling stations appeared to be statistically significant (Scheffe test $p < 0.05$). Earlier, we already recorded an FA increase in birch at the sites severely exposed to oil hydrocarbons. The results were proved by the bioassay and chemical analyses data [64].

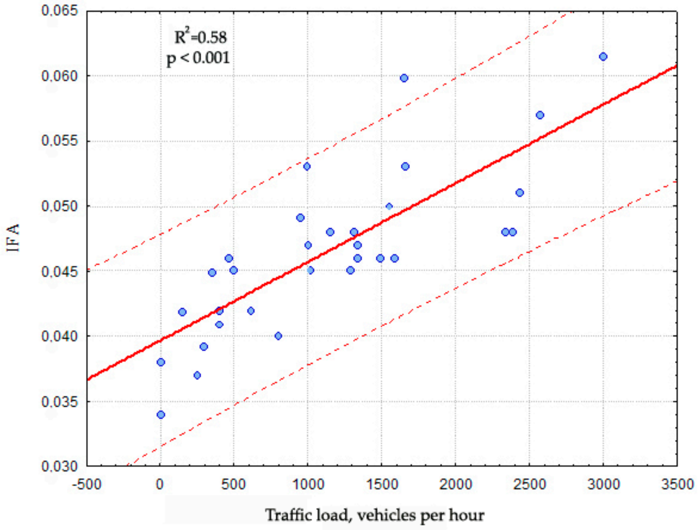


Figure 7. *B. pendula* FA dependence on traffic load (the dotted lines represent 95% confidence interval).

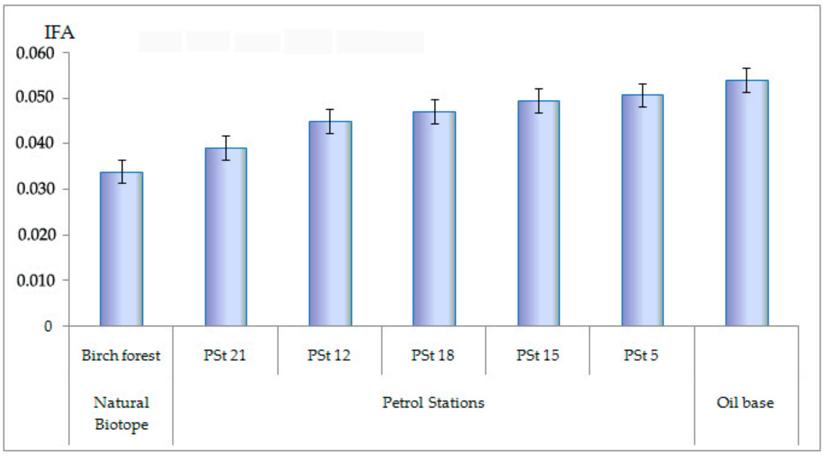


Figure 8. *B. pendula* IFA values at fuel filling stations of Naro-Fominsk (vertical bars, standart error).

3.5. Assessment of the Combined Influence and Contribution of Different Environmental Factors to FA Variability

As we mentioned before, the ability of FA to respond to stress factors of various nature is of great interest for bioindication studies since it can be used for an estimation of the total negative impact. However, this method suffers from one flaw: Under conditions of multiple impacts, it is hard to distinguish the leading factor. In some cases, it is possible to trace FA's change along the gradient of some environmental factor, like the radiation background or dust pollution at open coal mining [22,28,29]. FA of *B. pendula* is also known to be dependent on atmospheric pollution; among other kinds, on pollution caused by traffic load and mining industry [42,44,63]. However, in most cases, on the territory of a contemporary city, with multiple factors affecting the picture, it is difficult or impossible to isolate the leading factor that has the most negative effect on developmental stability. This is why we attempted to assess the combined anthropogenic impact.

The complex anthropogenic impact on birch growing in various ecotopes of seven cities was estimated based on the variables described in Table A5. A statistically significant correlation was revealed between IFA, TL, and industrial load (IL), as well as between complex parameters: Traffic and industrial load (TIL), environmental pollution (EP), city anthropogenic load (CAL), site anthropogenic load (SAL) (Spearmen's rank correlation coefficient = 0.46–0.75, significance from $p < 0.05$ to $p < 0.001$). IFAR displayed a higher correlation with TL and IL, and some weaker relations with the complex parameters CAL and SAL. The correlation with EP was not statistically significant. The closest relationships were revealed of IFA with SAL (Figure 9) and IFAR with TIL, though general variance trends and similar significance levels remained.

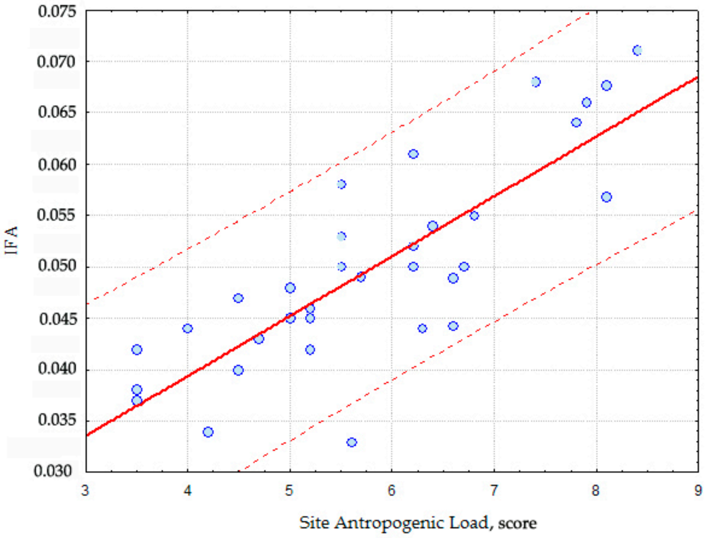


Figure 9. *Betula pendula* FA's dependence on site anthropogenic load (the dotted lines represent 95% confidence interval).

Subsequent regression analysis justified the correlation results. Of the six anthropogenic variables involved, the most satisfactory appeared to be the model of the IFA and SAL relationship ($R^2 = 0.647$, $p < 0.001$). The anthropogenic load increase yielded a 0.0046 times growth of IFA: $y = 0.0141 + 0.0046x$. This regression analysis model proves 65% of the variance caused by SAL.

The multivariate dispersion analysis was conducted, involving various climatic and anthropogenic factors and ecotopes as variables. A statistically significant variability of *B. pendula* IFA was revealed in terms of the climatic characteristics of the cities ($p < 0.001$), except for one variable, the average July temperature ($p > 0.05$). As to the anthropogenic factors, IFA was proved to increase with the growth of TL, IL, TIL, EP, CAL, and SAL ($p < 0.001$). As it was stated in Section 4, a statistically significant positive correlation was revealed between IFA and all the listed variables of anthropogenic load ($p < 0.05$), while no significant correlation was recorded between IFA and climatic variables ($p > 0.05$).

To understand the contribution and estimate the significance of the studied anthropogenic and climatic variables for different 33 ecotopes of seven cities, a principal component analysis (PCA) was applied. First, all 27 variables were analyzed: IFA, IFAR, and 10 anthropogenic and 15 climatic variables. However, this produced a degenerate matrix, so residual correlations were removed to leave nine variables for further analysis. Hereunder, the results are given without factor rotation.

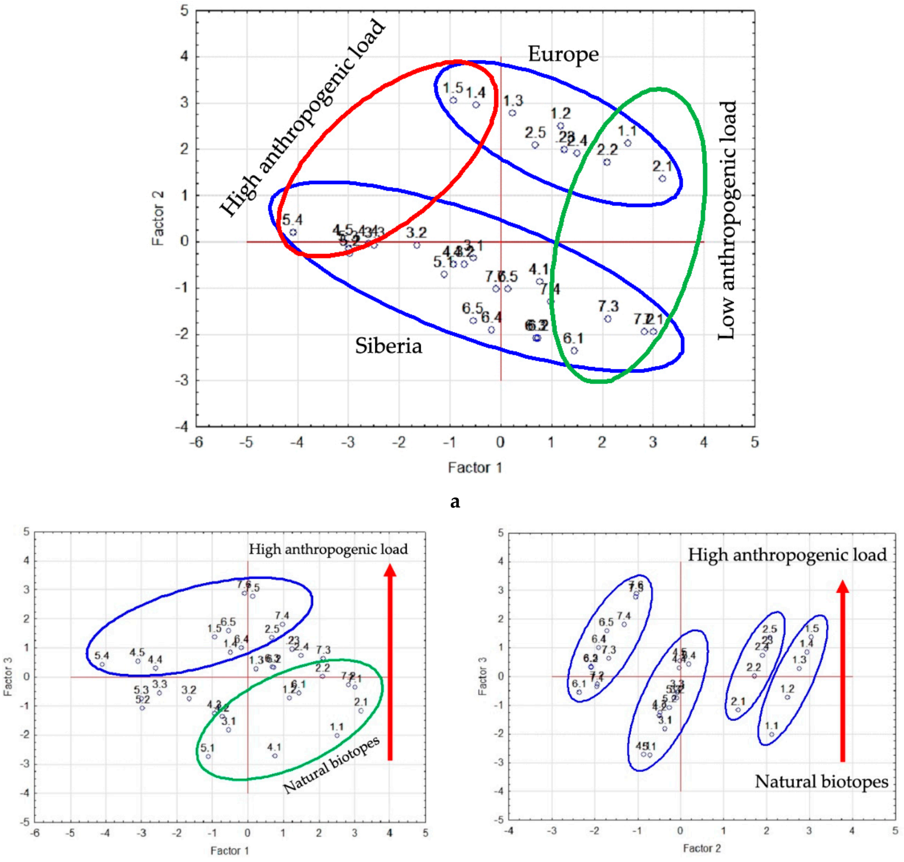
The covariation analysis of IFA, climatic, and anthropogenic variables for various urban ecotopes revealed the influence of three groups of factors that together explained 93% of the variance in the studied 33 ecotopes of 7 cities. PC I has the highest weight and reflects anthropogenic factors. It accounts for 41% of the variance and has a negative correlation with *B. pendula* IFA, EP, CAL, and SAL. PC II accounts for 31% of the dispersion reflecting climatic characteristics. It is correlated positively with the sum of active temperatures, duration of the growing season, and average annual precipitation. These variables are considered when the seasonal development of plants and their productivity are characterized [110]. PC III has a positive correlation with TL, explaining 21% of the variance (Table 7).

The scatterplots on Figure 10 depict the ordination of the studied ecotopes along the significant factor gradients. Now, it is possible to estimate visually how similar or distinct the ecotopes are with respect to *B. pendula*'s IFA, anthropogenic, and climatic factors. For PC I, the ecotopes of seven cities are situated along the decreasing values of IFA and anthropogenic load, from left to right: From urban ecotopes to natural biotopes (Figure 10a). A distinct group is formed by the most polluted urban ecotopes of Krasnoyarsk Krai. They are situated on the left along the factor 1 (F1) axis. In terms of factor 2 (F2), the ecotopes are clustered by climatic characteristics (Figure 10a). The ecotopes of a moderately continental climate of the European part of Russia (cities 1-2, Naro-Fominsk, Yoshkar-Ola) are grouped in the upper part of the F2 axis. The ecotopes of the continental climate of Central Siberia (cities 3-5, Krasnoyarsk Krai) are in the middle of the axis, while the ecotopes of the sharply continental climate of East Siberia (cities 6-7, Mirny, Yakutsk) are scattered down the axis.

Table 7. Factor loading for ecotope variables.

Variable	Principal Components		
	I	II	III
IFA	−0.88 **	0.03	0.29
Traffic load	−0.49	0.29	0.74 **
Industrial and traffic load	−0.59	0.23	0.68
Environmental pollution	−0.74 **	−0.11	−0.55
City anthropogenic load	−0.81 **	−0.08	−0.54
Site anthropogenic load	−0.98 **	0.04	−0.14
Sum of active temperatures	0.25	0.94 **	−0.02
Duration of the growing season	0.11	0.97 **	−0.16
Average annual precipitation	−0.16	0.90 **	−0.34
Dispersion	*0.41*	*0.31*	*0.21*

Note: ** $p < 0.01$.



a

b **c**

Figure 10. Ecotopes' ordination along the significant factors: (**a**), Factor 1 and Factor 2; (**b**), Factor 1 and Factor 3; (**c**), Factor 2 and Factor 3.

F1 and factor 3 (F3) represent the variables of anthropogenic load. In this scatterplot, the ecotopes are also clustered into two separate groups. From right to left along the F1 axis, the pollution level and anthropogenic load on the city and ecotope are increased. Bottom-upwards along the F3 axis, the traffic load is increased (Figure 10b).

The studied ecotopes also form three groups along the F2 and F3 axes (Figure 10c). From right to left along the F2 axis, the ecotopes of the cities of the European part of Russia, and Central and East Siberia are located sequentially with increasing continentality. Bottom-upwards along the F3 axis, the ecotopes are located with the increase of the traffic load.

Thus, the complex analysis clearly grouped the studied ecotopes with *B. pendula* according to various ecological factors. Anthropogenic factors play the primary role in FA variability, whereas climatic characteristics have no significant impact on the studied measure of developmental stability of plants in the urban environment.

3.6. Analysis of the Possible Causes of the Increased FA in Plants in Urbanized Territories

The data we obtained indicate significant differences in the developmental stability of *B. pendula*, both between regions and within each city, which can be explained by the geographic location and population and local industry of these cities.

First of all, the cities in question differ significantly in the air pollution level. In Krasnoyarsk city, it was estimated as very high, with an atmospheric pollution index (API) of over 14.0, and high in Achinsk and Nazarovo (API over 7). Besides, sulphur oxides and heavy metals were recorded in the air of Krasnoyarsk Krai cities, whereas no records of these pollutants were made in other cities [92,93]. Only Mirny had a comparable pollution level (API 7.0) due to its hydrogen sulphate content [111]. In Yakutsk, API was about 5–6 [94], while in Yoshkar-Ola, it was less than 1.0 [88,89].

The increased *B. pendula* FA values in the three cities of Central Siberia are due to the high anthropogenic load over the region. Krasnoyarsk Krai is one of the oldest industrial regions of Siberia. Major ecosystem polluters are the enterprises of non-ferrous metallurgy, the regional power station (RPS), and the combined heat and power plant (CHP). The strong negative impact of RPS and CHP on the cities of Krasnoyarsk Krai is explained by the wide use of brown coal, while other studied cities use natural gas. Brown coal is characterized by a high ash and sulphur content. Bottom ash and boiler slag produced by the combustion of brown coal from the Kansk-Achinsk basin have high concentrations of Mn, Cu, Ni, Co, Cr, Pb, Zn, and polycyclic aromatic hydrocarbons [112], and the content of alcaline metals leads to a pH increase to 12.0 [108]. The ash and slag of brown coal are toxic for warm-blooded animals, and have a mutagenic effect on assay microorganisms, toxic effect on hydrobionts, and phytotoxic action on crop plants [113]. Nazarovo Power Plant belongs to hazardous enterprises that produce large amounts of emissions. In Achinsk, the main contribution to air pollution from stationary sources comes from the Achinsk Alumina Refinery, whose emissions contain Al_2O_3, CaO, Na_2CO_3, SiO_2, NaOH, and benzpyrene [87]. High values of IFA of *B. pendula* in Krasnoyarsk are caused by multicomponent anthropogenic pressure created by motor transport, the metallurgy, wood chemical industry, and heat-and-power plants. In Krasnoyarsk, the highest total concentration of heavy metals is observed on the territories adjacent to the heat station [114]. The studies conducted earlier determined a heterogeneous content of heavy metals (Pb, Cd, Cu, Ni, Mn, Zn, Co) in the soil cover of Krasnoyarsk, which is associated with local sources of their emission [114].

Mirny is an industrial city, but it has its specifics associated with open pit mining: The main sources of pollution, i.e., open pits, waste rock dumps, and tailings ponds, are located on the outskirts of the city [111,115]. On the posttechnogenic territories, significant deviations in the developmental stability of the birch and other trees can be observed [63,115], while on the territory of the city, the main negative technogenic impact, i.e., dust from disturbed territories, is most noticeable in the outskirts. For a long time, diamond mining was conducted with open pits, and waste rock was deposited in dumps in the immediate vicinity of the city. Mirny is on three sides surrounded by these rock dumps of up to 100 m in height, which despite the reclamation efforts continue to be a source of dust. Immediately to the south of the city lies the giant pit "Mir"; it has not been functioning since 2001 but was not reclaimed (due to its size), and due to prevailing winds (mostly easterly), the south of the city is exposed to dust to a greater extent. This is why on the territory of Mirny, pollution spreads from technogenically disturbed lands to residential areas, with the least disturbed areas being the center with high-rise buildings, and most disturbed the northern and southern outskirts [63,115]. Naro-Fominsk is also an industrial city, though it has reduced industrial production over the past decades, gradually turning into a satellite city and striving to improved living standards. Naro-Fominsk is also an industrial city, but in recent decades, it has seen a gradual decline in industrial activity and is turning into a satellite town with an increasing life quality [81]; the industrial impact there is concentrated mainly in the southeastern part of the city, and motor transport is associated with the federal highway [81,84]. Rather high FA values in Yakutsk are explained first by heavy traffic together with the specific landscape and city planning: Insufficient ventilation of the city, a large number of vehicles per capita along with old planning, narrow roadways, and scanty greenery [29,63,65,66]. Yoshkar-Ola, as it was mentioned before, features a more favorable ecological situation due to the specific location, while small industrial enterprises do not emit significant amounts of toxic substances [47,88,89].

Similar results were obtained by other authors assessing the condition of urban territories with the same methods. For example, the high IFA figures in *Betula pendula* on the territory of Krasnoyarsk, even in recreational areas, are comparable with those obtained in another city with a population exceeding one million people: Moscow [46]. On the territory of Tolyatti (industrial center, Middle Volga region), an increase in IFA from 0.035 in a natural biotope to 0.056 in urbanized ecotopes was registered [116]. Similar trends were observed in other cities where FA of *Betula pendula* was studied [42,117–123]. An increase in FA of vegetative organs on the territory of a city and along the roads is characteristic of many species of trees and shrubs, including: *Populus nigra* [124,125], *Tilia cordata* [126], *Armeniaca vulgaris* [127], *Pinus sylvestris* [108,128,129], *Pinus eldarica* [130], *Olea europea*, *Quercus ilex*, *Eucalyptus cameldulensis*, *Platanus orientalis* [125] *Aser pseudoplatanus* [45,131], *Ficus elastica Syzygium jambolanum* and *Guarea Guidonia* [132], *Cecropia pachystachya* [44], *Ligustrum japonicum* и*Olea europea* [133], *Syringa josikaea* [134], *Ulmus pumila* [135], *Sorbus aucuparia* [136], and *Fragaria vesca* [118].

The correlation between an increasing FA in trees and shrubs and indicators of environmental pollution in industrial zones and roadside areas in cities has been proven both experimentally and by parallel studies of plants themselves and geochemical parameters of their environment. Ivanov et al. [43] showed that an excess background content in birch leaves of Ni (18.5 times), lead (16.0 times), Mn (5.8 times), and Cu (3.0 times) was accompanied by an increase in the integrated FA index by 20.0%, and chronic exposure of the leaves to ionizing radiation caused an increase in IFA by 29.8% and in the content of chlorophyll a (by 50.3%), and chlorophyll b (by 82.9%). Environmental pollution by sulfur compounds causes deviations in the growth and development of the silver birch [122]. A direct dependence of the FA level of the leaf on air pollution, especially by benzpyrene, has been found [120]. The FA level is affected by the distance from the plant to the source of pollution, e.g., with the increase in the distance from the plant to the roadway, a decrease in the FA level is observed [117,121]. For the Scots pine, a distinct pattern of needle asymmetry increasing depending on the dust load has been found [129]. Skripal'shchikova et al. [137] registered a correlation between the FA level of the pine and the content of Al, F, and heavy metals in the pine needles.

The FA level increase is not the only indicator of a plant's negative response to environmental pollution. Parallel changes in pine FA and such parameters as the length of physiologically active needles, needle cross-section, and the cross-section of the central cylinder and conducting bundles have been recorded, as well as negative relations between all anatomical and morphological parameters of needles and the content of Pb and F in them [137]. Air pollution leads to an increase in the metal content in the leaves of *Tilia cordata*, displacement in the ion balance, and changes in the size and asymmetry of the leaves [138]. Hassan et al. [139] noted in plants in industrial and urban areas, a decrease in the length of shoots, but it is possible that changes in the dimensional characteristics of the organs, anatomy, and morphology depend on the intensity of the impact. On the territory of Yoshkar-Ola, an opposite trend has been noted in *Betula pendula* under conditions of increasing anthropogenic load: The shoot length as well as the leaf area increased [47]. At the same time, some parameters of the lamina anatomical structure decreased: Lamina thickness, height of the upper and lower epidermis, and height of the spongy and palisade parenchyma. We attribute this to the development of an adaptive compensatory response to moderate-force impacts, while strong stress-inducing effects may cause a transition from adaptive to destructive changes as a result of deadaptation.

Hassan et al. [139] reported a decrease in chlorophyll content; changes in other physiological and biochemical parameters correlated with the concentrations of air pollutants. In urban conditions, *Ligustrum japonicum* and *Olea europea* demonstrate not only an increase in the leaf FA but changes in the morphological structure, as well as an inhibition of catalase and superoxide dismutase activity as compared with the control [133]. Changes in such important biochemical parameters as the content of chlorophyll and ascorbic acid, activity of nitrate reductase, superoxide dismutase, and peroxidase in the leaves of *Mangifera indica*, *Cassia fistula*, and a *Eucalyptus* hybrid depend on air pollution [140].

An intensive negative impact can lead to serious abnormalities and cell death. For example, in three species of plants found close to an iron ore pelletizing plant in Brazil, necrotic areas on the leaves, cuticle erosion, stomata obliteration, breakage, and plasmolysis of trichomes were observed, and anatomical cell collapse and hypertrophy and the formation of wound tissue were found [141]. Decreases in the leaf area, petiole length, thickness of the palisade and spongy parenchyma layers, dry weight of the leaves, stomatal density, cuticle thickness, and size of the epidermis cells were observed in the leaves of the castor bean *Ricinus communis* from heavily polluted stretches of Kathmandu roadsides [142]. In Turkey, in the area affected by highway, concentrations of Zn, Fe, Pb, and Cd, as well as soluble sugar and proline in the leaves of *Eucalyptus camaldulensis* affected by pollution were significantly increased as compared with the plants cultivated in the control conditions [143]. A study of a transect of a busy road within the London city limits and analysis of exhaust gases' impact on 12 plant species from different functional groups showed a wide range of effects, including stimulation and inhibition of growth, change in gas exchange, and premature aging of leaves. The authors of the study believe that the key phytotoxic component of the exhaust gases was NO(x) [144]. Khalid et al. [145] found a significant impact of vehicle emissions on four species of herbaceous plants; a manifold increase in the concentrations of Cd, Ni, Pb, and Zn in the leaves in vicinity of roads, as well as a change in C:N ratio were registered.

Salt stress in the area affected by roads has a negative impact on *Tilia x euchlora*, leading to changes in the chemical composition of the cell wall and increased synthesis of prenyl lipids [146]. A study of the response of *Robinia pseudoacacia* to air pollutants SO_2, NO_2, and O_3 in Tehran showed that in response to urban air pollution, the spongy mesophyll layer becomes thinner, the upper cuticle of the leaf thicker, and the stomatal density and the ratio of the palisade to spongy parenchyma increase [147].

These dependencies are not always direct and unambiguous. For example, Erofeeva et al. [148] noted a two-phase dependence (a biphasic pattern) of the photosynthetic pigment (PP) content, lipid peroxidation (LPO) rate, and fluctuating asymmetry (FA) of the leaf plate on the level of motor traffic pollution, which was studied in the *Betula pendula*. At the first phase, an increase in pollution pressure leads to disturbances in the homeostasis of trees, manifested as a reduction of the PP content, intensification of LPO, and increase in the FA index. At the second phase, a further increase in pollution pressure has an opposite effect, normalizing homeostasis (LPO rate and FA index decrease, while PP content increases). However, in any case, plants on the territory of the city respond to environmental pollution with changes in morphological, anatomical, and biochemical parameters.

Along with the changes in the morphological, anatomical, physiological, and biochemical parameters of plants, in conditions of anthropogenic pollution, abnormalities in the reproductive sphere can be observed. In *Pinus sylvestris*, an increase in the percentage of cytogenetic abnormalities in pollen tubes and seedlings [149], germination capacity of the seeds, and survival rate of seedlings [150] was observed. We noted that in urban conditions, not only an increase in deviations from the developmental stability of the birch but also a decrease in the germination capacity, as well as an increase in the occurrence of abnormalities in pollen grains can be observed [65,66,151].

Thus, the observed increase in FA of the birch leaf is just one of the manifestations of the plant's response to less suitable environmental conditions in cities. In practical terms, FA has a certain advantage in assessment of the environmental quality since it makes it possible to carry out a reasonably accurate assessment of the state of the environment without expensive equipment.

4. Conclusions

We estimated the fluctuating asymmetry in *Betula pendula* in terms of five morphometric characteristics of a leaf blade. Sampling was made in the territory of seven cities of the Russian Federation that varied in climatic conditions and anthropogenic load. The studied cities represent industrial, administrative-industrial, or administrative centers and are situated in the zones of moderately-continental, continental, and sharply continental climate. In total, 33 ecotopes were distinguished with various levels of anthropogenic load, from natural biotopes and recreational sites to industrial areas and highways with heavy traffic. In most urban ecotopes, IFA values were significantly higher as compared to natural biotopes of the same region. For three cities, where sampling was conducted for 3–4 years, no significant influence of the climate and differences in inter-annual IFA values were revealed. The correlation between the FA values and traffic load appeared to be statistically significant. The highest FA values were recorded in the territory of the industrial centers of West Siberia, featuring the strongest anthropogenic load.

The environmental quality estimation scale IFAR showed a strong correlation with IFA and a significant difference between the ecotopes with various ranks of environmental health. It is worth noting that the FA values in *B. pendula* in most recreation sites were higher as compared to that of natural birch forests. This may suggest deteriorated growing conditions even in relatively favorable urban biotopes, thus requiring careful selection of sites for estimation of background natural ecosystems.

From IFA, IFAR, as well as 25 anthropogenic and climatic variables, principal component analysis selected only 9 variables that were grouped into 3 factors influencing the variance of the studied ecotopes. PC I had the highest weight (41% of the variance) and showed a negative correlation with *B. pendula* IFA and general indicators of pollution of the cities or separate sites (environmental pollution, city anthropogenic load, site anthropogenic load). PC II explained 31% of the dispersion, reflecting such climatic variables as the sum of active temperatures, duration of the growing season, and average annual precipitation that influence the seasonal growth of plants. PC III explained 21% of the variance and had a positive correlation with traffic load.

The complex analysis clearly divided the studied ecotopes along the gradients of environmental pollution and climatic characteristics. The primary role of anthropogenic impact on the developmental stability of *B. pendula* is apparent. This correlates with FA values, which makes it possible to use it for bioindication assessment of environmental quality. From all anthropogenic variables, IFA and site anthropogenic load provided the most satisfactory model of the relationship. Climatic characteristics have no significant impact on the developmental stability of *B. pendula* in urban conditions.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Geographical location, population, and characteristics of the climatic conditions of the study areas.

City	Population	Geographical Coordinates	Region	Climate
1. Naro-Fominsk	62,000	55° 23′ 15″ N, 36° 43′ 59″ E	Eastern Europe	Moderately continental
2. Yoshkar-Ola	272,000	56°38′ 19″N, 47°53′26″ E		
3. Nazarovo	50,000	56°00′37″ N, 90°24′03″ E	Central Siberia	Continental
4. Achinsk	106,000	56°16′09″ N, 90°29′57″ E		
5. Krasnoyarsk	1,096,070	56°00′43″ N, 92°52′17″ E		
6. Mirny	34,000	62°32′07″ N, 113°57′39″ E	Eastern Siberia	Sharply continental
7. Yakutsk	314,000	62°02′02″ N, 129°43′59″ E		

City	The length of the periods, days			Sum of active temperatures, °C
	Warm/Cold Periods	Frost-free period	Growing season	
1. Naro-Fominsk	230/135	130	177	2400
2. Yoshkar-Ola	215/150	130	167	2350
3. Nazarovo	172/193	110	147	1840
4. Achinsk	175/190	97	144	1725
5. Krasnoyarsk	170/195	120	138	1890
6. Mirny	152/213	80	117	1502
7. Yakutsk	156/209	95	127	1782

City	Precipitation, mm		Average temperature, °C			Hydrothermal Coefficient
	Annual	Warm/Cold Periods	Annual	January	July	
1. Naro-Fominsk	670	485/185	+3.8	−9.0	+18.0	2.37
2. Yoshkar-Ola	548	367/181	+3.7	−12.4	+18.6	1.60
3. Nazarovo	468	366/102	+0.1	−17.7	+18.2	2.18
4. Achinsk	480	370/110	+0.3	−16.1	+18.8	2.26
5. Krasnoyarsk	485	334/151	+1.2	−16.0	+18.7	1.86
6. Mirny	318	260/58	−6.9	−30.4	+17.2	1.33
7. Yakutsk	233	186/47	−9.3	−41.0	+19.5	1.63

The climate characteristics: Warm Period, **WP**, a period with average daily temperature above 0 °C; Cold Period, **CP**, a period with average daily temperature below 0 °C; Frost-free period, **FFP**, no frost, i.e., the temperature does not fall below 0 °C during the day; Growing season, **GS**, a period with average daily temperature above +10 °C; Sum of active temperatures, **SAT**, a sum of positive temperatures during the growing season; **GCI**, General Climate Inclemency; **WCI**, winter Climate Inclemency; **SCI**, Summer Climate Inclemency.

Table A2. The climate characteristics for calculating climate inclemency indicators.

Indicators	Average Temperature			Precipitation			The Length of the Periods				SAT
	Ann.	Jan	Jul	Ann.	CP	WP	CP	WP	FFP	GS	
GCI	🟨	🟨	🟨	🟨				🟨		🟨	🟨
WCI		🟦			🟦		🟦				
SCI			🟩			🟩			🟩	🟩	🟩

Yellow, the characteristics for calculating GCI; Blue, the characteristics for calculating WCI; Green, the characteristics for calculating SCI.

Table A3. Correlations of climate indicators with the latitude and longitude of the investigated areas.

Climatic Characteristics	Longitude			Latitude		
	Spearman	t(N-2)	*p*-Level	Spearman	t(N-2)	*p*-Level
Warm/Cold Period duration, days	−0.93/0.93	−5.59/5.59	0.001	−0.68/0.68	−2.07/2.07	0.09/0.09
Frost-free period duration, days	−0.85	−3.56	0.01	−0.68	−2.10	0.09
Growing season duration, days	−0.96	−8.14	0.001	−0.75	−2.53	0.05
Annual precipitation, mm	−0.86	−3.72	0.01	−0.64	−1.88	0.12
Cold Period precipitation, mm	−0.93	−5.59	0.01	−0.79	−2.84	0.03
Warm Periods precipitation, mm	−0.89	−4.43	0.01	−0.68	−2.066	0.09
Average Annual temperature, °C	−0.86	−3.72	0.01	−0.64	−1.88	0.12
Average January temperature, °C	−0.11	−0.24	0.82	−0.14	−0.32	0.75
Average July temperature, °C	0.39	0.96	0.38	0.14	0.32	0.76
Sum of active temperatures, °C	−0.75	−2.53	>0.05	−0.68	−2.07	0.09
Hydrothermal Coefficient	−0.50	−1.29	0.25	−0.68	−2.07	0.09
General climate inclemency	0.82	3.22	0.05	0.68	2.07	0.09
Winter climate inclemency	0.89	4.43	0.01	0.73	2.39	0.06
Summer climate inclemency	0.85	3.56	0.05	0.72	2.32	0.07

Red font color—statistically significant differences.

Appendix B

Table A4. Characteristics of *Betula pendula* leaf sampling sites.

Site	Description
1. Naro-Fominsk	
1.1	Recreational area. Natural biotope. Culture and leisure park, recreation camp. No traffic load.
1.2	High-rise residential district in the center of the city. City gardening. Low traffic load. Close to a roadway.
1.3	Mixed forest at the city outskirts. Natural biotope. Moderate traffic load. Close to a residential district and food processing enterprise.
1.4	Industrial zone, close to a machine-building factory. City gardening. High traffic load.
1.5	Kiev highway, trees along the roadway. High traffic load. Heavy dusting.
2. Yoshkar-Ola	
2.1	Nature protected territory “Sosnovaya roscha”. Natural biotope. No traffic and industrial loads.
2.2	Residential area. City gardening. Low traffic load. As far as 1000 m away from the industrial zone (construction materials plant).
2.3	Recreational area, park. City gardening. Moderate traffic load. As far as 500 m away from the industrial zone (radio equipment producing plant).
2.4	Recreational area, a park in the center of the city. City gardening. Moderate traffic load.
2.5	City gardening. Moderate traffic load. Industrial zone, in the vicinities of a pharmaceutical plant.
3. Nazarovo	
3.1	Recreational area, mixed forest. At the distance of 0.5–1 km from the mid-rise residential area. Low traffic load.
3.2	City outskirts, garage area. Moderate traffic load. 1.5 km away from the Regional Power Station (RPS).
3.3	Managed plantation. Industrial hub. Industry: machine-building and construction structures enterprises. As far as 600 m away from RPS. High traffic load.
4. Achinsk	
4.1	Krasnoyarsk Krai, natural biotope, birch forest. Traffic and industrial loads absent.
4.2	Recreational area, Victory Park. City gardening. As far as 1.5 km away from the food processing enterprise. Low traffic load.
4.3	City center. City gardening. Low traffic load. As far as 1.5 km away from the food processing enterprise.
4.4	As far as 1 km away from the southern industrial zone. City gardening. Moderate traffic load, the federal highway “Siberia” traffic.
4.5	At the boundary of the southern industrial zone: metallurgy and construction materials plants. City gardening.
5. Krasnoyarsk	
5.1	Recreational area, island on the Yenisei River in the center of the city. Low traffic and industrial loads. High recreational load.
5.2	A residential district with low traffic load and moderate industrial load. City gardening. As far as 6 km north-east of Krasnoyarsk aluminum plant.
5.3	High-rise residential area Oktyabrsky. City gardening. Traffic load moderate.
5.4	A large transportation hub in Zheleznodorozhny district of the city. City gardening. Close to the railway station and food processing enterprises. High traffic load.
6. Mirny	
6.1	Natural biotope, birch forest with an admixture of larch and shrubs. As far as 15 km away from the city. Recreational load moderate. No traffic load. Close to post-technogenic territories.
6.2	City center. High-rise residential area. City gardening. Greenings inside the district. Low traffic load.
6.3	Old district of the city. City gardening. Two-storey wooden buildings. Low traffic load.
6.4	Southern part of the city, one of the central streets at the boundary with the industrial zone. City gardening. Moderate traffic load.
6.5	Northern part of the city. City gardening. Processing plant, heavy-duty vehicles are allowed. Close to post-technogenic territories. Moderate traffic load.

Table A4. *Cont.*

Site	Description
7. Yakutsk	
7.1	Natural biotope. Edge of the *Betula* forb forest. No traffic and industrial loads.
7.2	Recreational area. Natural biotope, *Betula—Rosa acicularis-Salix forb* forest in the territory of the Botanical garden. No traffic and industrial loads.
7.3	Low-rise residential area at the city outskirts near the forest. City gardening. Low traffic load. No industrial load.
7.4	High-rise residential area. City gardening. Industry, food-processing and manufacturing enterprises as far as 1 km away from RPS. Moderate traffic load.
7.5	High-rise apartment and administration buildings in the center of the city. City gardening. High traffic load. Low industrial load.
7.6	Intensively constructed high-rise residential district. City gardening. Heavy dusting, dense heavy-duty traffic due to the construction process. High traffic load.

Appendix C

Table A5. Score of the anthropogenic impact on the territory of the studied cities.

Anthropogenic Impact	Characteristic	Degree	Score
	City description		
Population [84]	Population 35–100,000 Population 250–350,000 Population 500–1,000,000 More than 1 million	Small Medium Large Worth millions	1 2 3 4
Functional role	Center of a federal subject, most of the adult population is employed in the sphere of education, culture, general government sector	Administrative and cultural center	1
	Center of a federal subject, a combination of administrative and industrial role	Administrative, cultural, and industrial center	2
	There is 1–2 main industries, most of the adult population is employed in the sphere of industry	Industrial center	3
Environmental pollution. Average score for five indicators [89,91,93–96]	Annual emissions of industrial enterprises per capita, thousand tons	Less than 0.05 Up to 0.2 Up to 0.4 Up to 1.0	1 2 3 4
	Air pollution compared to the average level in the Russian Federation [96]	At the general level Excess of 1.1−1.4 times Excess of 1.5 times	1 2 3
	Soil pollution compared to the average level in the Russian Federation [96]	At the general level Excess of 1.1–1.4 times Excess of 1.5 times	1 2 3
	Data from the national air pollution monitoring system. Air pollution index	Low Elevated High	1 2 3
	Gaseous pollutants. Sulfur compounds [89,91,93–95].	+ -	1 0
	Site description		
Traffic load	Natural biotopes and parks, no roads Up to 499 vehicles/hour 500–1499 vehicles/hour 1500 and more vehicles/hour	Negligible Low Medium High	1 2 3 4
Industrial load	Natural biotopes and parks, no roads Residential area, light industry Light industry, pharmaceutical enterprises Open-pit mining, heavy industry (metallurgy), power industry	Negligible Low Medium High	1 2 3 4

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