

The Impact of Industrial Pollution on the Stem Anatomical Characteristics of Woody Plant Undergrowth in the City of Dnipro, Ukraine

Tetiana Iusypiva^{1,a} and Galyna Miasoid^{2,b}

¹Associate Professor, The Department of Plant Physiology and Introduction, Oles' Honchar Dnipro National University, Gagarina str., 72, Dnipro, Ukraine - 49010

²Associate Professor, The Department of International Tourism and Language Training, Alfred Nobel University, 18, Sicheslavskva Naberezhna str., Dnipro, Ukraine – 49055

^aJusypivaTatjana@i.ua, ^bgalyna.miasoid@gmail.com

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Abstract. The paper examines the influence of industrial emissions of sulphur (IV) and nitrogen (IV) oxides on the percentage of stem anatomical characteristics of the autochthonous woody plant species undergrowth of *Acer platanoides* L. and *Fraxinus excelsior* L. in the southern industrial zone of the city of Dnipro (Ukraine). It is ascertained, that the ratio of the primary cortex share to the central cylinder share does not change in the stems of annual shoots of the both studied woody plant species when they are exposed to the influence of the toxic gases. However, there are significant changes in the ratio of shares of histological elements in the undergrowth stems of *F. excelsior*. The use of both absolute and relevant values of anatomic parameters of the plant vegetative organs is needed to analyse the woody plants resistance to technogenic pollution of the environment.

1. Introduction

The current environmental situation is environmentally disastrous and dangerous to both humans and the environment in many regions of Ukraine. The Prydniprovyia region is the most polluted among them [1], as it ranks second in the country in terms of its industrial development and which provides the country with 53.0% of metallurgical products, 19.0% of the products of machine-building, chemical and petrochemical industries, and 23.5% of the country's electricity supply [2].

The state of ecosystem in the major industrial centres is largely determined by the viability and adaptation strategies of wood ecosystem engineers. Woody vegetation absorbs a significant proportion of pollutants, thus purifying ecotypes of toxins and moderating the effect of man-made growth conditions on the other components of the biological community. However, the phytocenosis can only perform its protective functions if the woody plants, which are highly resistant to certain environmental pollutants, are used in the gardening in the contaminated areas [3, 4 et al.]

It is necessary to take into account the resistance of plants at different stages of ontogeny, since the long-term existence of artificial forests demands normal course of the self-healing process of woody ecosystem engineers, the most important stages of which are growth and development of self-seeding and undergrowth [5]. It is an optimal combination of decorative and aesthetic properties of plants with their gas-, dust-, smoke- and iron-resistance at all stages of individual development, which ensures the successful operation and a long piece of forest ecosystems within the industrial cities.

To assess the state of woody plants under the influence of anthropogenic pressures and their resistance to contaminants a number of criteria are used: morphometric, physiological, biochemical, cytogenetic and anatomical ones. The latter take longer periods to obtain information, though they are reliable enough to diagnose the ability of plants to survive in conditions of anthropogenic growth [6]. In addition, the environmental-anatomical method allows to identify adaptive properties

of plants at the level of cells, tissues and organs, as the processes of laying, growth and differentiation of vegetative organs reflect physiological and biochemical mechanisms of plant growth and development in both normal conditions and under the influence of various stress environmental factors [7, 8] In the steppe zone of Ukraine, where the city of Dnipro is situated, there are also hard soil-hydrological and climatic conditions, which are extreme environmental factors for woody plants, additional to the influence of phytotoxic pollutants [9].

The analysis of previous research indicates that the issue of the impact of pollutants on the anatomical structure of the stems of self-seeding trees and the undergrowth is understudied. Most authors [4, 10-16 et al.] examine the effect of the ingredients of industrial emissions on histological structure of the shoots of adult trees at the virginal and generative stages of their development. The major part of these papers describes the research on coniferous species [10, 11, 16 et al.]. Some articles, which examine the impact of man-made pollution on changes in the anatomic parameters of angiosperm woody plants at juvenile, immaturity and virginal stages of individual development, aim at studying the leaves and roots of seedlings of forest trees [18-21]. Few research papers are devoted to the study of anthropogenic impact on the histological structure of the stem undergrowth of trees and shrub species [23, 24], but the studies they report on were completed in natural areas other than the area of our investigation.

Therefore, at this stage the chronic impact of industrial emissions on anatomical structure of undergrowth of tree species in the conditions of steppe Prydniprovyia was not studied enough, and the long-term effect of pollutants on the ratio of components in the undergrowth stems, i.e. the percentage of each tissue compared to the total thickness of the primary cortex and central cylinder of the shoot, still needs thorough research. Meanwhile, qualitative changes in stems and changes in the share of its main histological components are a manifestation of deep disorders of the aboveground plant organs, leading to their death [15]. In view of the above mentioned, the purpose of our work is to identify changes in the shares of histological elements in the undergrowth stems of *Acer platanoides* L. and *Fraxinus excelsior* L. under the chronic pollution by SO₂ and NO₂ in conditions of the southern industrial zone of Dnipro (Ukraine). This work is a continuation of the study on the anatomical structure of the undergrowth stems of Norway maple and European ash when exposed to the pollution of industrial toxic gases [22].

2. Materials and Methods

Study area

The research was conducted in the city of Dnipro, which is the regional center of Dnipropetrovsk oblast, i.e. region. The zone is called Prydniprovyia because of its geographical position (the word ‘Prydniprovyia’ means ‘near the Dnipro River’). The region is situated in the northern-eastern part of Ukraine in the middle and downstream Dnipro River, the main river of Ukraine [1]. The Prydniprovyia region is also a part of the steppe physiographic zone with moderate continental climate characteristic of hot summer and dry winter [2]. Windy weather is common to the steppe Prydniprovyia during 270–298 days a year. As for the wind diagram, west and northwest winds prevail in winter, while winds from the east and northeast predominate in summer.

The major grounds are ordinary black soil; the predominant vegetation type is steppe vegetation since the most of the steppes are tilled. As the plain relief is crossed with valley of the Dnipro River and its tributaries, cutbanks and small flat-bottom valleys, there are some other vegetation types, like forest vegetation (ravine forests), meadow vegetation, and bog vegetation [9]. Recent research states that the amount of woodland in Dnipropetrovsk region makes 2.41%. However, most of them are of artificial origin and of sanitary and protective value, including those around industrial enterprises [1].

Dnipro is a powerful multi-industrial centre of Ukraine [25], which has more than 200 companies united in four major industrial regions: Western, Left-bank, Southern and Eastern, or 27 small industrial sites. The most contaminated areas are the Western and the Southern areas, the latter includes the CJSC ‘Plant Dnipropres’ (hereinafter ‘Dnipropres’) [26].

Thus, the study area is characterized by a combination of climatic, soil and hydrological conditions and man-made factors, which are extreme for growth and development of woody plants.

Monitoring points

Storage plant material was collected in three monitoring points (Fig. 1): two test areas, which are located near the Dnipropres, and a reference area. Monitoring point 1 is characterized by the highest pollution rate and is located as far as 500 m to the southwest of the source of industrial emission (48°39'03.4" N, 34°99'50.6" E.) It features average concentrations of toxic gases as indicated below: SO₂ – 0.31 mg/m³, NO₂ – 0.26 mg/m³. An average pollution rate is found at monitoring point 2 as far as 2000 m to the south of the plant (48°38'00.8" N, 35°00'17.5" E), where the concentrations of SO₂ and NO₂ are 0.29 mg/m³ and 0.24 mg/m³ accordingly. The relatively clean reference area [26–28] is located in the Botanical garden of Oles' Honchar Dnipro National University (48°43'67.6" N, 35°04'04.2" E), where the concentrations of sulphur (IV) and nitrogen (IV) oxides do not exceed the maximum permitted values (SO₂ – 0.05 mg/m³, NO₂ – 0.04 mg/m³) according to the City Sanitation Committee report.

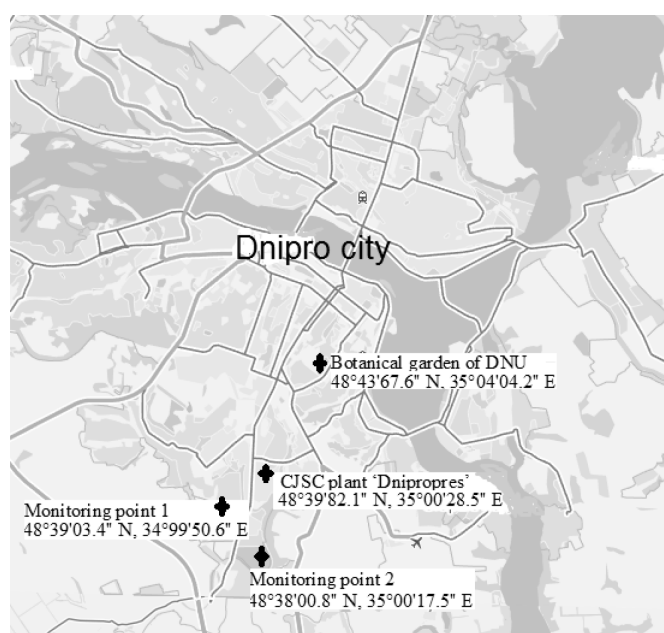


Figure 1. The map of Dnipro city and the sites of monitoring points [29].

Plant material

The objects of study were undergrowth of the autochthonous species of Norway maple *Acer platanoides* L. (*Aceraceae* Juss.) and European ash *Fraxinus excelsior* L. (*Oleaceae* Hoffmgg. et Link.). In each of the multiple monitoring points 30–50 annual shoots of undergrowth from each of the woody species were selected. The samples were fixed in 96.0% ethanol. The stem cross sections were made at a distance of 2 cm from the base of the shoot. To identify the lignified cells the cross sections were stained with 1.0% solution of phloroglucinol [8]. BRESSER Biolux LCD 40x-1600x light microscope was used to measure the tissue thickness with an amplification of 100 times. The replication was 30–50 stem cross sections for each monitoring point.

Statistical Analysis

The results of the study were handled using a multifunctional application software package *STATGRAFICS* Centurion XV. Mean absolute error was calculated. To compare the anatomical parameters in woody plant undergrowth stems of reference and test samples we used Student's t-test ($p < 0.05$). Normality allocation of the sample had been preliminary assessed.

3. Results and Discussion

In our previous paper, we examined the impact of toxic gases on the anatomical characteristics of woody plant undergrowth stems in absolute values [22]. However, to analyse the changes in the shares of histological elements of undergrowth stems of woody plant species in anthropogenic areas, we need to know the indices of anatomical parameters in relative values, i.e. the percentage of each tissue compared to the total thickness of the primary cortex and central cylinder of the shoot.

The impact of industrial pollution on ratio of the primary cortex share to the central cylinder share of woody plant undergrowth stems

Our research indicates that chronic effect of toxic gases of SO₂ and NO₂ does not affect the share of the primary cortex and the central cylinder in the total annual stems volume in the undergrowth shoots of the both studied species (differences between reference and test samples are insignificant at $p < 0.05$) (Table 1). Thus, although the thickness of both parts of the stem undergoes quantitative changes when exposed to the influence of industrial emissions of sulfur (IV) and nitrogen (IV) oxides [22], the same ratio between the shares of these parts of the stem is inherent in plants in the relatively clean zone. A. Lugovskyy (1992) came to a similar conclusion, demonstrating that qualitative structural features of the stem and the shares of components in it are independent of the plant nature and extent of contamination in the plants of *Pinus sylvestris* L. and *Quercus robur* L. at different age stages. The presence of qualitative changes in the stem and the shift of shares of its main histological components are the evidence of deep faults in above-ground organs, leading to the plant death [16].

Table 1. Impact of SO₂ and NO₂ on the share of the primary cortex and central cylinder in the stems of woody plant undergrowth annual shoot, % (M ± m).

Indicator	Control (n=50)	Monitoring point 1 (n=50)	Monitoring point 2 (n=30)
<i>Acer platanoides</i>			
Primary Cortex Thickness	7.3 ± 0.14	7.45 ± 0.31	9.0 ± 0.32
Stem Diameter	92.7 ± 0.45	92.6 ± 0.35	91.0 ± 0.60
<i>Fraxinus excelsior</i>			
Primary Cortex Thickness	14.1 ± 0.11	13.7 ± 0.07	12.9 ± 0.20
Stem Diameter	85.9 ± 0.80	86.3 ± 0.23	87.1 ± 0.34

The impact of industrial pollution shares of anatomical characteristics in primary cortex of woody plant undergrowth stems

The structure of the primary cortex includes cork (basic part of tectorial tissue of the periderm), strengthening tissue of collenchyme and cortex parenchyma. Its cells are suberized, do not pass gas and water, which prevents the entry of pathogens and depredators, providing comprehensive stem protection from adverse environmental factors. These features of the anatomical structure of the primary cortex are the pre-adaptations to the impact of pollutants, so the reaction phellem, i.e. the decrease or increase its share in percentage in the primary cortex stems can illustrate the type of resistance to certain toxic compounds of anthropogenic origin. In young stems, under the phellem there is a layer of mechanical tissue of collenchyma. The reinforcing function is performed by thick walls of cells, which protect deep rows of parenchyma living cells against compression [7]. The additional functions, which collenchyma cells perform, are the photosynthesizing function, as they contains chloroplasts, and the storing function, since the cells of this tissue contain a significant amounts of pectic substances and hemicellulose, which can be used by the plant as spare polysaccharides. Moreover, the ability of collenchyma cells to restore its mitotic activity when its tissue is damaged can lead to its transformation into a wound meristem.

Cortex parenchyma performs mainly the storing function. The development of these components of the primary stem cortex is essential for the normal functioning of the shoots of

woody plants. A change in the share of a particular histological element of the primary cortex, i.e. its percentage in the whole volume, is an evidence of rebuilding livelihoods of shoot tissues under the influence of negative environmental factors in a particular direction.

In our previous research [6] we found that under the chronic coke production emissions (SO_2 , NO_x , H_2S , NH_3 , phenols, suspended particles) in the stems of self-seeding of *A. platanoides* and *Robinia pseudoacacia* L., the cork share in the primary cortex of the stem is similar to the value of the phellem diameter in the reference samples, and the structural adjustment of cork are mainly related to the change of shares of the mechanical tissue and parenchyma.

The results of this study (Fig. 2–3) show that when exposed to sulfur (IV) and nitrogen (IV) oxides, the shares of the primary cortex elements changes in the both species. Therefore, the proportion of the protective stem tissue, i.e. the cork, to the total volume of the cork in the plants genus *F. excelsior* reduces significantly (by 16.0%) compared to the reference plants in the area of average concentrations of toxic gases in the atmosphere. The mechanical tissue is known to perform protective functions as well, and when its cell walls become thick, it reduces the entry of toxic compounds deep into the stem [7, 8]. We found, that the share of the mechanical tissue of the primary cortex increases by 12.0% and 31.1% in areas of heavy and medium pollution, respectively, which to some extent neutralizes the decrease of the phellem share. The share of cortex parenchyma in the *F. excelsior* undergrowth stems diminishes in volume only at the site with average concentrations of toxic gases. As for *A. platanoides*, the phellem share drops in the stems of this species in point 1 by 13.0% compared to the reference value indicator, whereas the collenchyme and cortex parenchyma shares remain unchanged, with the difference between the test and reference values are insignificant at $p < 0.05$.

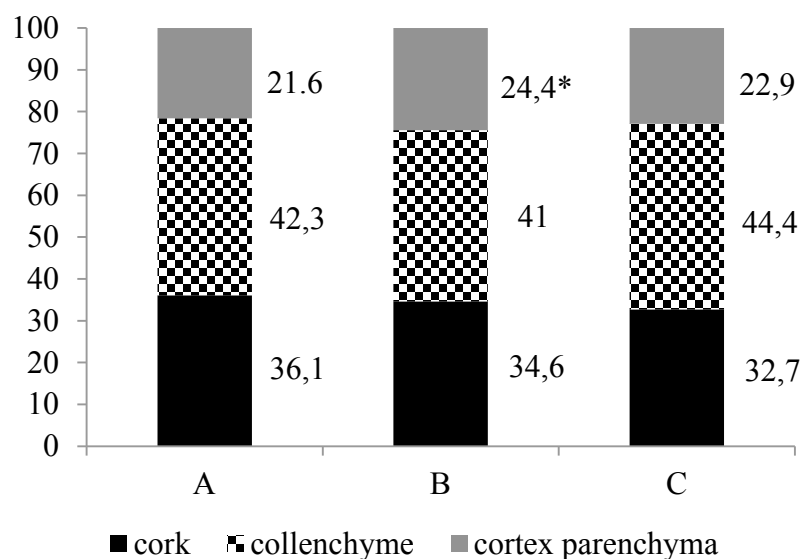


Figure 2. Impact of SO_2 and NO_2 on the share of histological elements of primary cortex of annual shoot of *Acer platanoides* undergrowth stem, %: A – Control (reference area) (n=50), B – Monitoring point 1 (n=50), C – Monitoring point 2 (n=30).
* – differ significantly from control ($p < 0.05$)

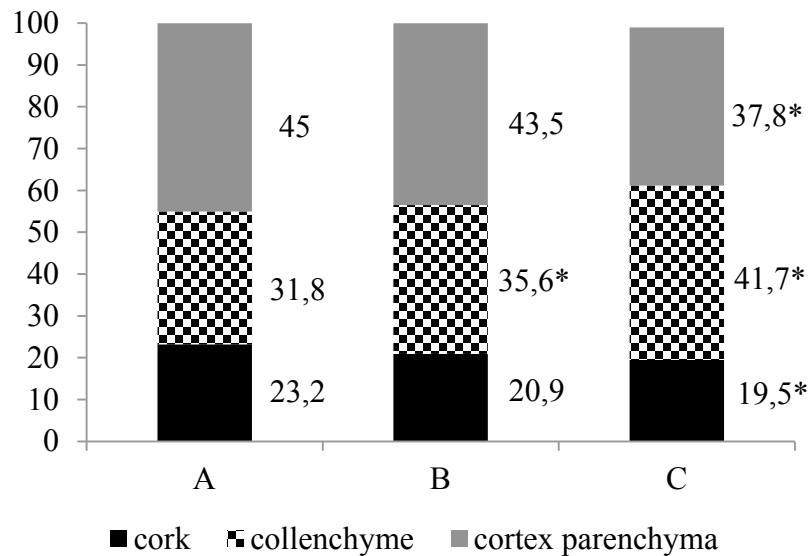


Figure 3. Impact of SO₂ and NO₂ on share of histological elements of primary cortex of annual shoot of *Fraxinus excelsior* undergrowth stem, %: A – Control (reference area) (n=50), B – Monitoring point 1 (n=50), C – Monitoring point 2 (n=30).
* – differ significantly from control (p < 0.05)

The impact of industrial pollution on shares of anatomical characteristics in central cylinder of woody plant undergrowth stems

The main components of the central cylinder of the stem are a complex of vascular tissues, the phloem and xylem, and the pith, which is the largest by volume and has medullary sheath in its peripheral part, which consists of small-sized well-lignified cells that perform a storage function. The scholars distinguish the following histological elements in the secondary cortex: soft bast, formed by conducting elements of the phloem (sieve tube and companion cells) and bast parenchyma and hard bast as its strengthening tissue (bast fibers), which cells have lignified cell walls [30]. Phloem ensures that the products of photosynthesis transport from the assimilatory organs of plants to places of their consumption or supply [7]. Therefore, reducing the share of soft bast in the total secondary cortex may affect both the supply of other organs with organic substances [18] and the intensity of photosynthesis due to inhibition of outflow photosynthesis products to attracting centres of plants and their further accumulation in leaves.

The structure of the histological component of xylem includes its major elements such as vessels and tracheids, wood fibres (fibriform) and wood parenchyma, each of which performs mainly some function of this tissue, namely the mechanical, transport, storage of nutrients and other ones. Therefore, increasing the share of one or another of the histological elements of wood demonstrates that this part of xylem mainly forms and indicates the direction of changes of the stem, either adaptive or destructive, under the influence of certain environmental stressors.

As components of the transport system of plant, both phloem and wood are exposed to abiotic, biotic and anthropogenic environmental factors that affect their functioning [3, 5, 6, 12-13, et al.]. The changes that occur in the phloem and xylem, make it possible to detect the result of the reaction of trees to anthropogenic stress, either forming adaptation or dramatic damaging and destructing its tissues and organs. In this sense, it is the steady shares of the histological elements of the stem central cylinder, i.e. the percentage of shares of hard bast, soft bast, wood, medullary sheath and pith in the total volume of the central cylinder of the stem, that is an informative indicator of plant resistance to man-made growth conditions [16].

Having studied the anatomical structure of the *P. sylvestris* L. stem, A. Lugovskyy (2004) notes that the most common and typical pattern that reflect the reaction of complex vascular tissues, manifested at all stages of ontogeny, is independence of their structural changes of the complex natural and anthropogenic environmental factors. The author focuses on the fact that the shares of

the tissues and structures in the stem under the influence of anthropogenic pressure are evident until a certain stage of converse changes. If there are significant changes in the shares of histological elements of the conductive part of the stem, it leads to the plant degradation and death.

In our study (Table 2), the shares of hard bast, wood and medullary sheath change significantly in total stele volume, while there are slight differences between the shares of other histological elements in the reference and test samples. Thus, the share of hard bast rises in the axial cylinder stem volume in the undergrowth stems of *A. platanoides* in both monitoring points 1 and 2 compared with plants of the clean area by 24.2% and 30.3% respectively; the share of the medullary sheath goes up by 13.0% in monitoring point 1. The shares of the soft bast, wood and the pith in the total stele volume are similar to the reference values.

Table 2. Impact of SO₂ and NO₂ on the share of histological elements of central cylinder in the stems of woody plant undergrowth annual shoot, % (M ± m).

Indicator	Control (n=50)	Monitoring point 1 (n=50)	Monitoring point 2 (n=30)
<i>Acer platanoides</i>			
Hard Bast Thickness	3.3 ± 0.18	4.3 ± 0.12*	4.1 ± 0.11*
Soft Bast Thickness	4.6 ± 0.21	4.6 ± 0.25	5.2 ± 0.19*
Wood Thickness	29.9 ± 1.04	29.9 ± 0.87	28.5 ± 0.35
Medullary sheath Thickness	3.0 ± 0.14	3.9 ± 0.23*	3.6 ± 0.30
Pith Diameter	59.2 ± 1.15	57.3 ± 0.95	58.6 ± 1.28
<i>Fraxinus excelsior</i>			
Hard Bast Thickness	3.5 ± 0.13	5.6 ± 0.20*	5.2 ± 0.39*
Soft Bast Thickness	5.4 ± 0.34	6.6 ± 0.15*	5.9 ± 0.45
Wood Thickness	21.0 ± 0.88	20.7 ± 1.39	15.3 ± 1.52*
Medullary sheath Thickness	3.3 ± 0.22	4.5 ± 0.40*	5.1 ± 0.33*
Pith Diameter	66.8 ± 3.12	62.4 ± 4.43	68.5 ± 2.17

* – differ significantly from control ($p < 0.05$)

As for significant changes of the shares of histological elements of the central cylinder in the stems of *F. excelsior* undergrowth in the both monitoring points, there is an increase of shares of the hard bast and medullary sheath, although the wood share reduces in the zone of average pollution with SO₂ and NO₂. The soft bast share in the volume of axial cylinder rises only under high concentrations of pollutants.

Thus, the common anatomical stem changes in technogenesis for undergrowth of the studied woody plant species are the increase of the hard bast share and medullary sheath share in the total stele volume, which are observed in the both monitoring points. There is also a significant reduction of the wood share in the central cylinder of *F. excelsior* undergrowth stem in the area of average pollution, which accounts for 25% compared to the reference value. Therefore, complex vascular tissues undergo the major changes in the stems of the undergrowth.

5. Conclusion

The results obtained indicate that the long-term effect of SO₂ and NO₂ industrial emissions on the plants does not change the ratio of the primary cortex share to the central cylinder share in the stems of *A. platanoides* and *F. excelsior* undergrowth. Significant share changes of some histological elements are found in the stems of *F. excelsior* species, in particular, the shares of the primary cortex cork and parenchyma reduce, while collenchyma share increases in the total volume of the stem primary cortex, whereas the wood share goes down significantly in the stele volume.

Comparing the research findings on the changes of anatomical parameters of the undergrowth annual shoot stems in absolute and relative values showed, that to determine the resistance of woody plants to anthropogenic environmental factors we should consider both the tissue thickness

in micrometers, and the shares of the stem components, i.e. the percentage of each histological element in the total organ volume. Although in our previous research, based on the set of anatomical indicators of annual shoots, we found greater resistance of *F. excelsior* undergrowth to industrial emissions of SO₂ and NO₂ compared to *A. platanoides*, the former species shows a more significant change in the shares of histological elements of its stem, that can lead to profound distortions in the organ structure and the undergrowth viability reduction in technogenic growth conditions.

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