Mechanism of destruction of *Quercus robur* and *Betula pendula* leaves by exposure of organic pollutants

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Abstract. Exposure of industrial pollutants including styrene, formaldehyde, acetone in the concentration range of maximal permissible concentration of working zone on leaves of silver birch (*Betula pendula*) and English oak (*Quercus robur*) chosen as model objects were studied and a mechanism of degradation was proposed. At a macroscopic level an indicator role of the leaf pattern for industrial organic pollutants with various functional groups was established. The differentiated effect of biotoxins revealed selectivity of chemical reactions and appearance of various products of these reactions on the surfaces of the leaf blade. For all studied objects, systemic IR spectroscopic studies of cuts from oak and birch leaves were carried out. The characteristic frequencies testifying to interaction of pollutants with leaf tissue have been identified. The criteria for the control of tree plantations have been proposed, manifesting in a combination of two methods: at the macroscopic level, by establishing the indicator role of the pattern, and at the molecular level, by IR spectroscopic determination of chemical interaction in the system "functional groups of biotoxins - leaf blade". The studies carried out make it possible to create a database of industrial pollutants and propose interrelated criteria for monitoring tree plantations.

1. Introduction

Plants, as the main producers of organic matter biomass on the planet owing to autotrophic nature of their metabolism, are highly sensitive to organic pollutants [1, 2]. The studies [3-5] examined the impact of gaseous air pollutants (SO2, NO, NO2, O3) on forest ecosystems, leading to forest degradation. The ability of potted plants to reduce the content of formaldehyde in living quarters was proved in [6, 7]. At the same time, the authors found out a change in the physiological characteristics of plants under the influence of formaldehyde. Other volatile organic pollutants, toluene and 2-ethylhexanol, caused phytotoxic effects on *Dieffenbachia maculata*, *Spathiphyllum wallisii* and *Asparagus densiflorus*, as shown in [8]. Change of the physiological activity of wheat under the exposure of styrene vapor was discussed in [9] and manifested in the stress state of the plant.

The study of the influence of industrial organic pollution on vegetation is one of the important scientific directions, which includes comprehensive monitoring of emission sources, environmental pollution and damage to vegetation. Plants have specific biological mechanisms that respond to the
intake of chemicals. The regulation of gas uptake is mainly determined by the sensitivity of the stomata, retaining the ionic balance and buffer properties of the cytoplasm [10].

Within the industrial zone of woodworking enterprises, the vital activity of woody plants undergoes certain changes in the presence of organic gas emissions of II - IV hazard classes (formaldehyde, styrene, acetone, etc.). The physiological approach to determining the damageability of plants and the selection of plants resistant to biotoxicants presented in studies [11, 12], which made it possible to form a new direction in plant physiology - gas resistance [13]. Under exposure of pollution, plants develop various physiological, morphological and anatomical changes [14]. As a result of exposure of various toxic gaseous substances degradation of woody plants occur, manifesting a decrease in gas exchange, photosynthesis activity, a decrease in chlorophyll, disruption of stomatal movement, leading to increased transpiration and disruption of the water regime, inhibition of enzymatic activity, as well as in the appearance of various types of necrosis and depressions [15-17]. Leaf characters including cuticle, stomata, epidermal cells, and guard cells get affected due to stress induced by the air pollutants. Disturbance of the gaseous exchange as well as respiration in plants serves as an indicator of environmental stress. The effects of individual pollutants are quite variable because they vary from species to species. Changes in leaf in characters induced due to the effect of air pollutants seem to be small, but during the survival of the plant in stress, they can be of great consequence [18]. To record degradation of the tree layer at an initial stage in order to prevent irreversible changes, various methods have been used [19-22]. The most effective of them are diagnostic indicators, determining, for instance, the effect of toxic components on the physiological state of plant leaves. The main functions of leaf are photosynthesis, gas exchange and transpiration. When diagnosing and classifying the damaged forest ecosystems, it was found out [23] that the effect of atmospheric toxicants on plants is a biological phenomenon at the level of metabolic and physiological processes with a change in the ultrastructure of leaf cells. In this case, external, visually distinguishable damage appears [24, 25]. The plant response depends on the nature of the pollutants. For example, the pollutants induce oxidative stress in the plant with the production of free radicals (hydroxyl radicals) and reactive oxygen derivatives (ROD) that cause damage in the leaf. Air pollution, by modifying plant physiology and biochemistry, has a decisive effect on ecosystems, including plant-insect interactions [26]. The level of plant damage is determined by the possibility of industrial pollutants entering the inner tissues of leaves and the completeness of their detoxification or inclusion in metabolism without disrupting the functions and structure of assimilation organs [27]. The predominance of one or the other in plant depends on the anatomical and morphological structure of leaves, i.e. from the presence of a certain form of gas resistance in the plant.

Due to the current urgency of the problem of ecological control of the stress state of woody plants under the influence of industrial pollutants, the aim of the present study was to determine the mechanism of leaf degradation and to propose the criteria for monitoring of woody plants.

2. Methods and materials

The objects of the study included leaves of English oak (Quercus robur, 55 years old) and silver birch (Betula pendula, 30 years old), growing in the “Nagornaya Dubrava” afforestation of Voronezh region of the Central Black Earth Region of Russian Federation. The collection of oak leaves and birch leaves for investigation carried out in vitro, were done in June 2018. For this, 5 formed leaves were taken from the shoot in the middle part of the crown of each species and placed in three desiccators without taps (210x320 mm in size and 5 L in volume) for fumigation. Each desiccator had porcelain inserts, holding glass cups with corresponding volatile pollutants: i) liquid acetone (analytical grade), maximal permissible concentration of working zone MPCw.z. – 200 mg/m³, ii) liquid styrene (analytical grade), MPCw.z. – 5.0 mg/m³, iii) crystalline paraformaldehyde, product of formaldehyde polymerization with a degree of polymerization from 8 to 100 units, and a degree of purity in the range from 80 to 90 wt.% [28], MPCw.z. – 0.5 mg/m³. All reagents were supplied by Ltd “Khimlab”, Russia. Surface concentration of these pollutants at a distance of 100 m from the emission source was calculated to be 42, 0.4 and 0.04 mg/m² for acetone, styrene, and formaldehyde, respectively [29].
In view of the low concentration value, the calculations were carried out using the MPCw.z. for each pollutant, in order to establish more reliable results of IR spectroscopic studies.

During fumigation experiments, the concentration of pollutants in the desiccators was equal to 17 MPC that corresponded to pollutant concentration at the outlet of the funnel of the furniture enterprise (Mebel Chernozemiya, Voronezh, Russia). For this, calculated volume of liquid acetone and styrene was placed in cups in the first and second desiccator, respectively, taking into account the pressure of saturated vapor. The third desiccator contained certain mass of paraformaldehyde placed in glass cup and heated to 80°C to allow depolymerizing of paraformaldehyde and formation of gaseous formaldehyde [28]. The samples of leaves were kept in the corresponding desiccators for 72 h.

To determine the effect of styrene, acetone, and formaldehyde vapors on the morphology of the leaf plate and possible chemical interactions with functional groups of pollutants and leaf tissue infrared spectroscopy method was used. The studies were carried out on a Bruker Vertex 70 FT-IR spectrometer, which provides registration of IR spectra in the range of 400-4000 cm⁻¹ equipped with ATR attachment with a diamond prism, which makes it possible to obtain spectra in the mid-IR range, including biological samples. Prior the IR-analysis a 50 µm thick film was cut from birch and oak leaves with an MC-2 sled microtome (Tochmedpribor, Kharkov, Ukraine). With a resolution of 1 cm⁻¹, the reproducibility of wavenumber values was ± 4÷8 cm⁻¹ in the range from 2000 to 4000 cm⁻¹ and ±1÷3 cm⁻¹ in the range from 400 to 2000 cm⁻¹.

3. Results and discussion

A visual assessment of the state of the leaf blades of oak and birch after 72 hours of fumigation by vapors of organic solvents was carried out in comparison with control (non-fumigated) samples. Not the same type of the response of the woody plants towards exposure of organic pollutants of different nature was visually found out. By fumigation of oak leaves with acetone vapors, the surface of the leaf blade was covered with a shiny film. The effects of formaldehyde vapors exposure to oak leaves included appearance of the well-defined dark spots, indicating penetration of formaldehyde into the intercellular space. By processing the oak leaves with styrene vapor, a dense oily coating on the smooth surface of the leaf was visually detected. Similar to the case of oak leaves, birch leaves were covered with the shiny film by fumigation with acetone vapors. The film on the leaves became shinier when treated with formaldehyde vapor. The effect of styrene was the most significant causing the leaves become dark, tough and sticky.

Thus, fumigating the tissues of oak and birch leaves with organic pollutants of various nature demonstrated their clear, externally manifested, specific reaction, which, according to outer signs, indicated the degradation of biological objects.

The study of the nature of the structure-property relationship was carried out by IR spectroscopy. In the case of treatment of oak leaves with pairs of toxicants, the absorption pattern in the IR spectra is different (figure 1a-d).

From the analytical point of view, there are two areas of 3500-3200 cm⁻¹ and 1800-1600 cm⁻¹ that represent the greatest interest. Absorption bands appearing in the high-frequency region are associated with stretching vibrations of hydroxyl groups, free and participating in intermolecular interactions. For the test sample (figure 1a), two peaks with frequencies of 3530 and 3430 cm⁻¹ are identified in the considered area. In this case, the absorption bands are blurred and show the medium level of intensity. After acetone treatment the intensity of these absorption bands in oak leaves increases significantly.

In the low-frequency area of 1800-1600 cm⁻¹, absorption bands appear due to stretching vibrations of the of free and participating in the formation of a hydrogen bond carbonyl groups. For the initial sample, a wide blurred band appears in the IR spectrum with hard-to-detect maxima in the frequency range 1720-1770 cm⁻¹ (figure 1b). The effect of acetone on oak plant tissues leads to a sharp increase in the absorption band of 1750 cm⁻¹ and the appearance of a clear band at 1845 cm⁻¹ (figure 1b). The intensity of the absorption bands slightly resulting from the superposition of stretching vibrations of C=C and N-bonds in the region of 1650-1570 cm⁻¹ somewhat increases. Based on these data, it can be assumed that the adsorption of acetone on the surface of the oak leaf is accompanied by a process of
interaction with the structural elements of plant tissue due to the formation of a hydrogen bond between the carbonyl group of the solvent molecules \((\text{CH}_3)_2\text{C}=\text{O}\) and the OH and NH groups. The significant intensity of the \(\text{C}=\text{O}\) absorption bands and their shift to the low-frequency area of the spectrum confirms that acetone molecules accumulate in the plant tissue.

Since the structures of acetone and formaldehyde \((\text{CH}_2=\text{O})\) are similar, in the case of the second solvent (figure 1c), the spectral pattern is similar to the methodology of treating oak leaves with acetone. A characteristic difference is the best resolution of the absorption bands considered: 1840, 1770, 1710, 1650 and 1605 cm\(^{-1}\) (figure 1d), which belong to stretching vibrations of the groups \(\text{C}=\text{O}, \text{NH}, \text{C}=\text{C}\). It can be assumed that formaldehyde, having a smaller effective radius and possessing functional groups with a significant polarity, is absorbed on the surface of the sheet with less energy consumption and penetrates more easily into the plant tissue of the oak leaf. The structure of formaldehyde molecules allows interaction with the structural elements of a plant cell due to the formation of a hydrogen bond of the \(\text{C}=\text{O} \ldots \text{H-R}\) type.

**Figure 1.** IR spectra of oak leaves: control sample (a) and sample after treatment with acetone (b), styrene (c), and formaldehyde (d).

The IR spectrum of an oak leaf aged in styrene vapor is characterized by two features. First, in the area of stretching vibrations of hydroxyl groups of various types, a broadened band with practically no clear separation of three maxima is relieved. Secondly, in the low-frequency area there is no absorption band of 1840 cm\(^{-1}\), and the intensity of the absorption band of 1710 cm\(^{-1}\), arising from stretching vibrations of the \(\text{C}=\text{C}\) group, significantly exceeds all other bands in this frequency range.

It should be noted that there is a clear appearance of a maximum at 1602 cm\(^{-1}\), which arises as a result of stretching vibrations of \(\text{C}=\text{C}\) bonds in the benzene ring. All the above features of the spectral pattern (figure 1d) may be indicative of a significant adsorption of styrene molecules mainly on the surface and in a small amount within the inner volume of the oak leaf due to the interaction of unsaturated bonds of styrene molecules with the CH, OH, NH groups.
Examining of the IR spectra (figure 2) of birch leaves sections soaked in pairs of the same solvents that the nature of the changes caused by the toxicants is somewhat different for oak leaves. As it was the case of an oak leaf, the tested sample of the untreated birch leaf shows an infrared spectrum (figure 2a) with poor resolution of the absorption bands. The area of stretching vibrations of OH groups in this category is characterized by a broadened band of medium intensity with two maxima at 3420 and 3330 cm\(^{-1}\). The low-frequency area has vague absorption bands with maxima of 1740, 1710, 1650 cm\(^{-1}\), which can be attributed to the stretching vibrations of free and intermolecular C=O groups. Slices of birch leaves treated with acetone vapor have an absorption pattern in the area of 3600-3000 cm\(^{-1}\) that is almost identical to the spectrum of the test sample. The low-frequency 1800-1600 cm\(^{-1}\) optical absorption area is characterized by increasing intensity of absorption bands at 1760 cm\(^{-1}\), which is justified by the increasing number of carbonyl groups on the sheet surface as well as within its volume. Comparing the IR spectrum of a birch leaf slice treated with acetone with a similar spectrum of an oak leaf (figure 1b and 2b) indicates the more substantial adsorption of acetone molecules (CH\(_3\))\(_2\)C = O on the oak plant tissue.

![Figure 2. IR spectra of birch leaves: control sample (a) and sample after treatment with acetone (b), styrene (c), and formaldehyde (d).](image)

Infrared absorption for the birch leaves sections treated with formaldehyde indicates that adsorption of molecules with a small effective radius (CH\(_2\)=O) is equally possible both on the surface of the sheet and within its volume. This fact is confirmed by the increase and clear shapes of increasing maxima of the absorption bands of the stretching vibrations of OH groups in the range of 3450-3200 cm\(^{-1}\), as well as by the sharp increase in the intensity of the bands lying at 1800 and 1740 cm\(^{-1}\) (figure 2d). The adsorption mechanism easily forms intermolecular hydrogen bonds of C=O groups of formaldehyde with H-R groups of various molecules that make up the plant tissue.

Studying the IR spectra of birch leaves sections after styrene pairs treatment also shows a change in the intensity of the characteristic absorption bands (figure 2c). In this case, maxima are clearly manifested by the 3420 and 3330 cm\(^{-1}\). As we compare it with the spectrum of the test sample, the appearance of an intense absorption band at 1880 cm\(^{-1}\), an increase in the intensity of the bands 1800, 1740, 1600, 1515 cm\(^{-1}\), which is due to the predominant surface
adsorption of organic molecules of the aromatic series (figure 2c). The nature of the interaction of styrene with molecules of cellular contents is also determined by the formation of hydrogen bonds between π-electronic systems of unsaturated fragments (C=C bonds) and H-R, H-C groups of plant sorbent. It should be noted that the specific structure of birch leaves (smooth surface of the upper and lower parts of the leaf) determines a slightly lower amount of sorption of styrene molecules compared to those in the case of oak leaves, which, firstly, have a less smooth surface, and secondly, a different structure of two parts of the sheet.

The degrading effect of styrene and formaldehyde on plant objects is described in [5-7]. The phytotoxic effect of vapors of organic compounds was also proved on Dieffenbachia maculata, Spathiphyllum wallisii, and Asparagus densiflorus [8]. However, these works did not provide the mechanism of degradation and did not show the possibility of using the obtained data in monitoring woody plants.

4. Conclusion
At the macroscopic and molecular levels, the possibility of express identification of the degradation process of birch and oak leaves in the presence of styrene, formaldehyde and acetone vapors in the atmosphere was established.

By the differentiated interaction of pollutants with leaf tissues, the characteristic features of interrelated processes were revealed: the appearance of characteristic frequencies of IR spectra and a change in the leaf patterns. The mechanism of leaf degradation was proposed, including the differentiated interaction of functional groups of pollutants and leaf tissue, identification of pollutants, and express analysis of characteristic changes in the pattern. Thus, two interrelated criteria for monitoring woody plants exposed to industrial pollutants with different functional groups were suggested (vinyl in the aromatic ring, aldehyde, and carbonyl). The volume and analysis of the results indicates that there are prerequisites for creating a database of industrial pollutants that can be used in monitoring of woody plants.

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