Delayed Fluorescence from Pinus Silvestris Leaves as an Indicator of Urban Environmental Quality

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Abstract. This article presents the measurements of delayed fluorescence from Pinus sylvestris leaves against the functional load on the urban territory. We compare the values obtained with the results of chemical and analytical studies of the soil quality. The sampling was carried out according to a spatial grid designed on the basis of the functional load on the territory. The bioindication method as well as chemical and analytical tests of plant and soil samples taken at each site under study allow to differentiate these locations according to their ecological conditions. We made attempts to assess the degree of influence of various factors on some indicators of plant vitality. Calculations of the correlation coefficient between the content of a number of components in the soil and bioindication data suggest that the photosynthetic activity of plants in urban conditions is more dependent on the state of the atmosphere.

1. Introduction

A very important role in biological monitoring is played by methods that allow obtaining information on the negative impact on biological objects at a very early stage, before ecosystems undergo irreversible changes.

The activity of the photosynthetic apparatus is worth to be mentioned as one of the plant characteristics that can be used to assess the physiological state of vegetable organisms. This is one of the first indicators that start varying under the influence of external factors, before the appearance of irreversible lethal or sublethal changes in plant organism. It is possible to determine with a high level of reliability the condition of a plant’s photosynthetic apparatus by making measurements of delayed chlorophyll fluorescence. The simplicity of the measurement of this indicator allows monitoring a large area and processing large samples with minimum financial and time costs.

Coniferous trees are the most suitable for conducting bioindication studies since they do not renovate the main part of their photosynthetic organs every year, and consequently their photosynthetic apparatus is exposed to external factors during more than one season, which increases the level of changes occurring in it under the influence of the environment [1].

In connection with this, the goal of this study is to identify the dependence between the delayed fluorescence values for Pinus sylvestris leaves and the level of functional load on the urban territory taking in this case as example the city of Kazan, and also to compare these values with the results of chemical and analytical measurements of soil quality.
In connection with the goal of this research, we solved the following tasks:

- designed a spatial grid for sampling based on the functional load on the territory of Kazan;
- performed bioindication as well as chemical and analytical studies of plant and soil samples collected at the chosen sites;
- classified the researched sites according to their environmental condition on the basis of the results of statistical processing;
- determined the degree of influence of various factors of the soil environment on the indicators of plant vitality.

2. Methods

2.1. Measurement of delayed chlorophyll fluorescence

The method used involves measuring and recording delayed chlorophyll fluorescence from pine leaves. This indicator makes it possible to determine with a high level of reliability the vitality of plants, since it reflects some important characteristics as the number of living cells in the studied sample and the condition of the photosynthetic apparatus of the plant.

The main steps of the study are as follows:

1. Sampling. Second-year leaves are harvested from a few trees standing near to each other. The collected leaves are transported in a sterile paper bag to the laboratory.
2. Preparation of samples for the study. We randomly select 30 of the collected leaves. A \(20 \text{ mm} \pm 1 \text{ mm}\) segment is cut out from the middle part of each leaf. The leaves are distributed over three fluorometer cuvettes (10 leaves in each one).
3. Preparation of the device for measurements. The device is connected to the COM port of a computer. The instrument is calibrated (zeroing it with an empty cuvette). The cuvettes with the samples are placed in the device.
4. Switching the device on. After placing the samples in the device and zeroing it, the instrument is reset through the computer program \(2\), the device is started in the mode needed for the measurement of the delayed fluorescence relative index (DFRI) during three (or more) turns.
5. Statistical processing of data. The output data of the PHOTON-10 device is transferred for statistical processing to Microsoft Excel. Delayed fluorescence absolute (DFAI) and relative (high-light and low-light; DFRI) indexes are used.

We chose for the study various sites of the city that are subject, on the one hand, to different levels of anthropogenic load, and require, on the other hand, additional environmental and sanitary control.

The sampling sites in the city of Kazan are shown on Fig.1.

2.2. Quantitative chemical analysis of soil cover composition

Soil sampling was carried out in accordance with GOST 17.4.4.02-84 and GOST 28168-89. The pH was determined in conformity with guidelines established by E.V. Arinushkina \[3\]. The content of nitrates was assessed according to GOST 26951-86. The assessment of copper, manganese, and iron content was carried out in accordance with GOST R-50684-94 and GOST R-50685-94. The soil composition was tested using an infrared spectrophotometer (FT-IR, Fourier-transform infrared spectrophotometer).
3. Results and discussion

3.1. Results of measurement of delayed chlorophyll fluorescence

The results obtained during the study were compared with the control results. The latter were calculated on the basis of data on delayed chlorophyll fluorescence of coniferous from places with low anthropogenic load. The DFAI for such sites is on average 2900±300. The DFRI is 5.5±1.2. Such a large deviation is explained by the fact that the delayed fluorescence index can vary depending on the temperature at which the samples were collected, the position of the branches from which the leaves were taken, and the density of plantations at a given site. It is impossible to keep an absolute uniformity of these conditions, and this increases data deviation. However, if the sampling was carried out at sites with similar climatic conditions, without discrepancies in sampling season, then the data sets obtained are compatible.

The results of delayed chlorophyll fluorescence measurement are shown in Table 1. Thus, in the territory of Kazan urban agglomeration, the level of pollution determined through the DFAI and DFRI varies from average to critical. This is due to the heterogeneity of the anthropogenic load and distribution of pollutants in the city and suburbs. The presence of multiple sources of pollution does not allow us to talk about a specific site having the highest concentration of pollutants in a certain area of the city: pollution has a mosaic structure. However, in a general spatial dynamics, we may say that pollution decreases as we move away from the city center, where the highest level of motorized transport load is found. The indicators of ecological condition obtained by bioindication methods on the territory of the ecosystems depend on both the general background of pollution in the region and the proximity of local sources of pollution.

The Orgsintez site is located near the main building of Kazanorgsintez (approximately 500 meters from it), a factory whose production cycle involves the use of substances from the 3rd and 4th hazard classes, including phenolic compounds. This company is equipped with modern systems for filtering air emissions and liquid wastes, namely:

- dust and gas collecting systems;
- systems for collection and disposal of hydrocarbon blowoffs with partial return to the technological process;
- circulating water system;
- local facilities for the treatment of contaminated sewage,
- biological treatment facilities;
- thermal treatment plant for neutralization of liquid wastes from production.

**Table 1.** The results of delayed chlorophyll fluorescence measurement.

| Sampling sites                                      | DFAI  | DFRI | Pollution level      |
|-----------------------------------------------------|-------|------|----------------------|
| 2th Republican Clinical Hospital                    | 825.33| 1.42 | Heavy pollution      |
| Vasilievo settlement, Sosnovy Bor sanatorium        | 1050.77| 1.93 | Medium pollution     |
| Yudino settlement, nursery school                   | 1102.55| 2.22 | Medium pollution     |
| Yudino settlement, outpatient hospital              | 843.44| 1.97 | Heavy pollution      |
| 7th city hospital                                   | 480   | 1.26 | Critical level of pollution |
| Lebyazhye lake                                      | 967.78| 1.71 | Medium pollution     |
| Livadia sanatorium                                  | 968   | 1.65 | Medium pollution     |
| 1st maternity hospital                              | 1141.88| 1.66 | Medium pollution     |
| 11th city hospital                                  | 691.89| 1.23 | Critical level of pollution |
| Children’s Republican Clinical hospital             | 1098.11| 1.92 | Medium pollution     |
| 4th maternity hospital                              | 902.11| 1.82 | Medium pollution     |
| 18th city hospital                                  | 862.77| 1.84 | Heavy pollution      |
| Kazanorgsintez chemical plant                       | 564.11| 1.22 | Critical level of pollution |

Despite this fact, the complete retention, return to the production cycle or disposal of waste is impossible at this technological level. The emissions by this enterprise have a pronounced negative impact on the nearby ecosystems, one of whose edifier species is *Pinus sylvestris*.

The lowest results were obtained at the 7th City Hospital site. This may be due to the fact that at the time of the study this area was exposed to a high anthropogenic load connected with construction works using heavy equipment. After an almost complete destruction of the soil cover at this site, it underwent a reclamation soil plan, which included the delivery of new soil. These activities allowed to develop a landscape with species that are traditional for cities. The results of the study, however, show that the reclamation works carried out could not fully compensate for the consequences of the massive anthropogenic impact.

The Yudino site at 9 Krasikova st. (nursery school), the Sosnovy Bor Sanatorium and the Children’s Republican Clinical Hospital are located at a considerable distance from the main center of transport activity in the city, large industrial enterprises, landfills for disposal of solid domestic waste, and other focal sources of pollution. There have been no large-scale construction activities on these territories for a long time; one- and two-storey buildings prevail, with low population density. All this contributes to minimizing the environmental impact of the city pollution background and reduces the stressful action on biological objects.

The result obtained at 1th Maternity Hospital is rather interesting. Despite the proximity of the junction of two major highways (Adoratski street, and Marshal Chuykov street), this site may be considered as relatively safe from an environmental point of view. This may be due to the fact that the level of gardening activities on this territory is much higher than the average for the city. A combination of both deciduous and coniferous plantations contributes to a high level of dust retention.
The relative remoteness from large residential quarters and the proximity of natural water bodies also reduces the stress effect on vegetation.

3.2. Results of chemical and analytical studies

The pH of the aqueous soil extracts at the sites studied varied from slightly acidic to alkaline. Such differences are explained by the pronounced heterogeneity of the soil environment, and the diverse nature of pollutants. The correlation between the bioindicative index and the pH of the soil extract is very weak. This is explained by the life strategy of the test object: just like most stress-tolerant plants, the pine is quite indifferent to the acidity of the soils on which it grows.

The results of pH measurement are shown in Table 2.

| Sampling sites                                      | pH   |
|-----------------------------------------------------|------|
| 2th Republican Clinical Hospital                    | 7,66 |
| Vasilievo settlement, Sosnoviy Bor sanatorium        | 5,81 |
| Yudino settlement, nursery school                    | 5,8  |
| Yudino settlement, outpatient hospital               | 5,9  |
| 7th city hospital                                    | 7,33 |
| Lebyazhie lake                                       | 5,7  |
| Livadia sanatorium                                   | 6,27 |
| 1th maternity hospital                               | 8,3  |
| 11th city hospital                                   | 7,4  |
| Children’s Republican Clinical hospital              | 8,15 |
| 4th maternity hospital                               | 7,7  |
| 18th city hospital                                   | 7,66 |
| Kazanorgsintez chemical plant                        | 6,38 |

A graph was constructed (Fig.2) to display the relationship between DFAI and soil pH. Here these indicators are presented on a comparable scale.

![Graph showing DFAI and soil pH dynamics in sampling sites.](image)

**Figure 2.** DFAI and soil pH dynamics in sampling sites.

No statistically significant correlation was found. The parameter of the parametric correlation is 0,12; the Spearman coefficient is 0,14.
The content of nitrates in soil samples from all sites does not exceed the MPC and does not approach critical values. MPC at 130.0 mg/ml, is established by hygienic standard GN 2.1.7.2041-06 (Table 3).

| Sampling sites                                      | mg/kg NO₃ |
|----------------------------------------------------|------------|
| 2th Republican Clinical Hospital                   | 11.98      |
| Vasilievo settlement, Sosnovyi Bor sanatorium       | 6.98       |
| Yudino settlement, nursery school                   | 6.35       |
| Yudino settlement, outpatient hospital              | 8.86       |
| 7th city hospital                                   | 11.11      |
| Lebyazhye lake                                      | 6.10       |
| Livadia sanatorium                                  | 10.36      |
| 1th maternity hospital                              | 3.60       |
| 11th city hospital                                  | 9.86       |
| Children’s Republican Clinical hospital             | 4.97       |
| 4th maternity hospital                              | 8.36       |
| 18th city hospital                                  | 9.11       |
| Kazanorgsintez chemical plant                       | 1.34       |

When processing the results of the study, we found an inverse correlation between the content of nitrates and delayed chlorophyll fluorescence from Pinus sylvestris leaves. The lowest content of nitrates is found at the sites that were characterized as relatively safe by the results of bioindication studies.

At the City Hospital № 7 site, where the results of biotesting gave the highest level of pollution, we noted a high content of nitrates in the soil.

The results obtained at the Orgsintez site are quite interesting. Despite the fact that the bioindication study shows a critical level of pollution at this site, the content of nitrates in the soil there is extremely small. This is due to a number of factors, the main of which is that the greatest influence on the condition of plants at this site is produced by atmospheric pollution rather than soil pollution. It is also important that soils at this site are sandy, with both a low content of humus and a weak accumulative capacity (these data are based on the results of infrared spectroscopy). Most of the soluble substances, including nitrates, are washed out of sandy soils.

The diagram (Fig. 3) presents a comparison of data on delayed chlorophyll fluorescence from leaves and the content of nitrates in the soil at a comparable scale, for a visual representation of the inverse correlation. It shows that, in the general case, a low content of nitrates is characteristic for sites regarded as relatively safe according to the results of the bioindication study.
Figure 3. DFAI and content of nitrates in soil dynamics.

The results of heavy metals mobile forms content in soil measurement are shown in table 4. The data was received by spectroscopic investigation of acetate-ammonium soil extract.

Table 4. Heavy metals content dynamics in sampling sites.

| Sampling sites                          | Cu(mg/kg) | Mn(mg/kg) | Fe(mg/kg) |
|----------------------------------------|-----------|-----------|-----------|
| 2th Republican Clinical Hospital       | 1.06      | 75.45     | 5.82      |
| Vasilievo settlement, Kosnoviy Bor sanatorium | 0.32  | 9.61      | 9.96      |
| Yudino settlement, nursery school      | 0.35      | 4.34      | 22.12     |
| Yudino settlement, outpatient hospital | 0.51      | 3.39      | 12.35     |
| 7th city hospital                      | 0.43      | 17.06     | 3.96      |
| Lebyazhye lake                         | 0.48      | 18.57     | 9.3       |
| Livadia sanatorium                     | 0.47      | 8.1       | 5.24      |
| 1th maternity hospital                 | 0.44      | 23.93     | 18.43     |
| 11th city hospital                     | 0.7       | 20.87     | 6.47      |
| Children’s Republican Clinical Hospital| 0.82      | 23.33     | 14.19     |
| 4th maternity hospital                 | 0.39      | 13.46     | 4.58      |
| 18th city hospital                     | 0.25      | 25.89     | 19.05     |
| Kazanorgsintez chemical plant          | 0.46      | 8.12      | 13.95     |

The maximum permissible concentration (MPC) for heavy metals (3.0 mg/kg for Cu, and 60 mg/kg, 80 mg/kg or 100 mg/kg, depending on the pH, for Mn) was not exceeded at any site. Only at 2th Republican Clinical Hospital, the MPC for Mn was found to approach the critical value.

The statistical analysis of the data allowed us to detect a significant correlation coefficient between metal content in soil and delayed chlorophyll fluorescence index only for Fe (0.465). Spearman’s rank correlation coefficient for Fe was estimated at 0.5 with a 95% confidence interval. Since the correlation coefficient is positive, the correlation must be direct. Many heavy metals, just like most trace elements, are needed in a certain amount for normal growth and development of plants. However, their excessive content in the soil can contribute to distress. Fe is one of the most important trace elements for plants, it appears in the composition of enzymes that contribute to the accumulation and recovery of NADP during the light stage of photosynthesis and represent the so-called chlorophyll-synthesizing complex.
It is quite interesting that the maximum Mn and Cu content in the soil was recorded at the 2th Republican Clinical Hospital site. The Mn content at this site approaches the MPC. This is due to an increase in pollution caused by the Elektropribor factory, situated at a distance of approximately 500 meters from the research site. Both Mn and Cu are used in the production cycle of this factory. The deficiencies of modern systems for cleaning emissions lead to the introduction of a certain amount of these metals and their compounds into the surrounding environment.

We have developed a number of methodological recommendations based on the data obtained from the research.

We found a conspicuous positive correlation (more than 80%) between the delayed chlorophyll fluorescence indexes from *Pinus sylvestris* leaves, including low-light delayed fluorescence, high-light delayed fluorescence, and DFRI (delayed fluorescence relative index). This was disclosed by using two methods of correlation: parametric correlation and Spearman rank correlation. This suggests that these indicators are sufficient to characterize the results of the study as a whole. When performing the statistical analysis of the results, this significantly reduces the amount of data sets that are to be processed as part of monitoring works using this method. This makes it possible to use larger samples in the analysis, thereby reducing the error in the results of the study. Also, this allows to increase the number of sites monitored during the same amount of time, which permits to describe more accurately the state of a macroregion as a whole.

4. Conclusions
- By comparing the results from the study of delayed chlorophyll fluorescence from *Pinus sylvestris* leaves and chemical-analytical studies of soil, we have been able to detect a direct correlation between bioindicative data and Fe content in soil, as well as an inverse correlation between bioindicative data and content of nitrates in soil.
- The value of the correlation coefficient between the content of various components in the soil and the condition of test organisms is not greater than 50%; this suggests that delayed fluorescence indices are more dependent on atmospheric pollution.
- We detected a critical level of pollution on the territory of the Orgsintez chemical plant and City Hospital № 7. This indicates the necessity for a more detailed analysis of the sources of stressful effects on the environment at these sites.
- A high content of nitrates and Cu in the soil was detected at Republican Clinical Hospital № 2. At this site, the content of mobile forms of Mn is approaching the maximum permissible concentration, which is caused by the proximity of industrial objects: Nefis and Elektropribor.
- The use of bioindication methods together with methods of quantitative chemical analysis allowed us to obtain more reliable data on the environment, and also to determine with higher accuracy the character of pollution and its source.

The use of a variety of methods is instrumental in determining with a higher reliability the character of environmental pollution and reducing the ecological danger caused by the introduction of various pollutants. The delayed fluorescence from pine leaves, a plant frequently encountered in urban green zones, can be considered as an indicator of pollution of urban biocenoses.

5. References
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