Bioindication methods in environmental engineering

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Abstract. One of the activities in the field of environmental engineering is the monitoring of environmental pollution, in which a preliminary stage is carried out – bioindication studies. The Siberian spruce assimilation apparatus is a very reliable tool for the air environment estimation because of high sensitivity to pollutants and the possibility to perform an annual observation. The system of aerogenic pollution estimation in terms of biochemical parameters deviations is considered to be rather reliable. The anthropogenic air pollution leads to the change of the plant metabolism. The increase of the terpenoid compounds content in the needles is due to their biosynthesis intensification as the response of the plant to an unfavorable environment. Under similar conditions other secondary metabolites – phenol substances – are accumulated in tissues. The phenol compounds content in the Siberian spruce assimilation apparatus is increased by more than 60% in the polluted areas compared with the background one. The pigment system of the assimilation apparatus is influenced as well. This study suggests the Siberian spruce assimilation apparatus to be an indicator of air pollution. Results of its composition research and morphometric parameters give a full picture about environment condition.

In the context of modern globalization of industrial production, it becomes clear that the existing regulatory environmental oversight measures to address the issue of reducing the negative impact of anthropogenic factors are no longer sufficient.

According to the global online air monitoring map AirVisual, Krasnoyarsk ranks first in the world in terms of air pollution. The air quality index (AQI) for 18.02.2020 was 226, which means a very unhealthy environment for all its inhabiting organisms. In this regard, a new segment of engineering is becoming more and more popular – environmental, whose main task is to modernize the industrial and civil sectors that have a negative impact on the environment from the point of view on environmental safety.

One of the main directions of ecoengineering is environmental monitoring, which assesses the level of pollution of environmental components, which makes it possible to offer timely implementation of innovative environmental equipment in those industries that make the main contribution to environmental pollution. As part of the monitoring, it is recommended to perform an initial assessment of the level of environmental pollution based on the response of biological agents to emissions.

Conditions for growing woody plants in the city differ significantly from natural ones. Their development is affected by the influence of many negative atmospheric factors, such as: unusual vapor and gaseous impurities, the structure of plantings, other physical and microbiological support. In polluted areas, the concentrations of background components, insolation, and temperature change
significantly. Serious differences in growing conditions are reflected in the metabolism of plants, primarily in the assimilation apparatus, which is manifested in its morphometric indicators and component composition.

Atmospheric pollution in some areas of Krasnoyarsk was studied by the variability of some chemical components of Siberian spruce needles – one of the most sensitive woody plants to emissions. It is known that under the influence of pollutants, in addition to reducing the life span of needles, changes in morphometric parameters and humidity, affect its chemical composition [1,2].

High sensitivity to pollutants and the possibility of year-round monitoring makes the Siberian spruce assimilation device a convenient indicator for assessing the state of the air environment in urban areas. 82 test sites of Siberian spruce plantations in different parts of Krasnoyarsk were identified (figure 1). The most polluted urban areas and forest stands at a distance of 30-70 km from the city were selected for the study.

![Figure 1](image.png)

**Figure 1.** Arrangement of spruce places in Krasnoyarsk with various pollution intensity.

The assessment of air pollution by deviation from the norm of biochemical parameters is considered to be sufficiently reliable [3,4]. Convenient for this purpose are terpenoid compounds of the assimilation apparatus characterized by sufficient temporary stability – essential oils. During the research, the yields and component composition of the oils of the background and highly polluted areas of spruce were compared during the year. The results of quantitative determination of its content in the coniferous young growth of one of the most polluted plantings and background phytocenosis are shown in table 1.
Table 1. Seasonal dynamics in the content pine needles essential oil, %.

| Selection time, month | Forestry | Urban area | Deviation, % from background |
|-----------------------|----------|------------|-----------------------------|
| January               | 0.79 ± 0.03 | 0.62 ± 0.03 | - 0.17                      |
| February              | 0.82 ± 0.04 | 0.59 ± 0.02 | - 0.23                      |
| March                 | 0.94 ± 0.04 | 0.74 ± 0.03 | - 0.20                      |
| April                 | 0.96 ± 0.03 | 0.80 ± 0.03 | - 0.16                      |
| May                   | 0.79 ± 0.04 | 0.71 ± 0.02 | - 0.08                      |
| June                  | 0.70 ± 0.02 | 0.72 ± 0.02 | 0.02                        |
| July                  | 0.90 ± 0.03 | 1.00 ± 0.03 | 0.10                        |
| August                | 1.00 ± 0.03 | 1.02 ± 0.03 | 0.02                        |
| September             | 1.10 ± 0.03 | 1.14 ± 0.03 | 0.04                        |
| October               | 1.04 ± 0.04 | 1.06 ± 0.03 | 0.02                        |
| November              | 0.94 ± 0.03 | 0.78 ± 0.02 | - 0.16                      |
| December              | 0.90 ± 0.03 | 0.73 ± 0.03 | - 0.17                      |
| x̄±m                  | 0.91 ± 0.05 | 0.83 ± 0.05 | 0.11 ± 0.02                 |
| σx                   | 0.130      | 0.179      | 0.077                       |
| ν, %                  | 14.2       | 21.6       | 70                          |

It is indicated that the anthropogenic air pollution changes the activity of metabolism in plants [6]. It makes adjustments both in the content of essential oil in conifers and the dynamics of its accumulation. In heavily polluted areas, as previously noted in the example of pine forests, its contribution is reduced. The same is observed in the compared case, when the content of essential oil in the coniferous forest is on average 9 % higher than in the urban area. However, starting in June and ending in October, its contribution to urban planting is the same or even slightly higher than the oil level of the forest area.

It is assumed that the increase in the content of terpenoid compounds in conifers is due to the intensification of their biosynthesis as a response of the plant organism to the adverse effects of the environment. It is also possible that their enrichment of needles is associated with changes in some processes that lead to the formation of unrelated terpenoids, under the influence of adsorbed by the organism of technogenic pollutants.

We tested this idea on Siberian spruce. During the research, we compared the content of phenolic compounds in its April conifer, which was used in areas with background (in the array), low, medium and high air pollution in the conditions of Krasnoyarsk. The results of the analyses confirm the observed regularity – their contribution increases as the technogenic load increases. In the coniferous forest area, it is found to be equal to 1.57±0.14 %, urban areas increase from 1.57±0.23 % (Akademgorodok) and 1.86±0.21 % (Central Park) to 2.12±0.23 % (in the district of the Thermal Power station). The data obtained indicate that the content of phenolic substances in the assimilation apparatus of Siberian spruce increases by more than 60% in comparison with the background one when the air is polluted. This type of variability is consistent with their known role as inhibitors, including biochemical processes [7]. Thus, it seems that phenolic compounds, like terpenoids, play an important role in ensuring the stability of spruce in adverse conditions.

Air pollution is also reflected in the composition of the essential oil of Siberian spruce needles. It is based on monoterpenic hydrocarbons, which account for 60-65 % of the total amount of volatile terpenoids. Their predominant component is camphene. A significant proportion of the oil is accounted for by α-pinene, 3-Karen, and limonene (with β-fellandren). The total amount of these hydrocarbons is up to 80 % of the fraction. Taking this into account, changes in their content in the oil composition can serve as a basis for concluding about the type and specifics of the development of woody plants, in particular, under the influence of adverse factors. The essential oil of spruce needles...
includes other components that are typical for coniferous woody plants: centene, tricycline, β-pinene, myrcene, fellandrenes, terpinolene, terpinenes.

The largest contribution to the oxygen-containing fraction is made by bornylacetate – 25-35% of the total of all oil components. Together with another representative of the camphor group (camphor), their contribution to spruce oil is about 40%. The oxygen-containing fraction also includes other derivatives of monoterpenes: terpene, borneol, terpinyl acetate, fenchone, fenchol, etc.

The representation of sesquiterpenoids in the essential oil of Siberian spruce needles is insignificant (2-5%). Their predominant component is caryophyllene and longifolene, the total contribution of which is about 70% of the fraction. In addition, the composition of the oil was found longicollis, isolongifolene, muurolene, cadinene, etc.

Despite significant differences in the conditions of existence of forest and urban plantations, the qualitative composition of the essential oil of pine needles differs in an insignificant way. Although additional compounds appear in the last of them, however, all the components peculiar to spruce oil of unpolluted phytocenoses are noted in their composition. However, the contribution of many of them in the compared samples differs markedly (table 2).

Table 2. Change in the component composition of spruce essential oil in case of anthropogenic pollution, % of the amount.

| Basic components       | Wood file | Urban area |
|------------------------|-----------|------------|
|                        | $\bar{x} \pm \sigma$ | $v_{%,}$ | $\bar{x} \pm \sigma$ | $v_{%,}$ |
| Triciklene             | 1.4 ± 0.3 | 62.9 | 0.8 ± 0.1 | 36.3 |
| α-Pinene               | 13.4 ± 1.6 | 28.8 | 8.6 ± 1.7 | 52.6 |
| Camphene               | 16.4 ± 0.9 | 11.4 | 10.8 ± 1.3 | 34.4 |
| β-Pinene               | 6.2 ± 0.9 | 39.7 | 5.9 ± 0.8 | 23.4 |
| 3-Carene               | 9.8 ± 0.9 | 25.3 | 8.8 ± 1.0 | 28.8 |
| Limonene+              | 13.3 ± 2.0 | 5.3 | 9.7 ± 1.7 | 29.1 |
| β-phellandrene         | 1.6 ± 2.0 | 25.6 | 1.0 ± 0.1 | 37.1 |
| Terpinolene            | 6.2 ± 0.9 | 2.46 | 5.9 ± 0.8 | 22.4 |
| In total monoterpens   | 62.1       | 45.6 |
| Camphor                | 3.1 ± 0.5 | 41.3 | 2.1 ± 0.4 | 48.1 |
| Izoborneol             | 2.7 ± 0.2 | 21.1 | 6.4 ± 0.9 | 24.2 |
| Bornylacetate          | 25.8 ± 2.0 | 20.5 | 40.3 ± 2.6 | 17.0 |
| Caryophilene           | 1.6 ± 0.3 | 48.8 | 2.6 ± 0.3 | 29.9 |
| Others                 | 4.9 ± 0.9 | 3.2 ± 0.7 |
| In total oxygencontaining and sesquiterpenoid compounds | 37.7 | 54.6 |

When moving from an uncontaminated to a contaminated site, the content of α-pinene, limonene, camphor, and especially camphene decreases in the essential oil of spruce needles, and, on the contrary, the proportion of oxygen-containing compounds, primarily bornylacetate, increases. The observed changes are most likely related to chemical transformations occurring inside the body under the influence of atmospheric pollution. Their largest-tonnage components in cities are considered to be oxides of sulfur and nitrogen, emitted mainly by heat stations and vehicles. From the air, they penetrate into plants, creating an acidic environment inside them. It is known that under such conditions, camphene reacts with organic acids (formic, acetic, etc.) to form borneol esters.

Acetic acid belongs to the known metabolites of coniferous woody plants. Therefore, its interaction with camphene with the participation of sulfuric acid as a catalyst is quite real. For the same reason, due to the acidity of the medium, the transfer of these esters to camphor is inhibited. Direct oxidation of camphene to camphor occurs only when the process is catalyzed with chromic acid, which is
unlikely in the conditions of Krasnoyarsk [8]. This may explain the decrease in the content of camphor and camphor and the increase in the concentration of bornylacetate in atropogenic air pollution. The presence of an acidic environment can also be explained by the decrease in the essential oil of α-pinene and monocyclic terpenes (limonene and β-fellandren).

The pigment system of the assimilation apparatus also reacts significantly to air pollution. A special role in this case played by chlorophyll, the content of which is determined by the photosynthetic efficiency.

When studying the issue, samples of spruce needles of 1, 2, 3 and 4 years of life were studied, selected in forest and urban areas with different aerogenic loads (table 3).

| Age of needles, years | Chlorophyll a | Chlorophyll b | Relation a/b | Carotenoid |
|----------------------|---------------|---------------|--------------|------------|
| **Wood site**        |               |               |              |            |
| 1                    | 1396 ± 9      | 543 ± 11      | 2.57         | 270 ± 15   |
| 2                    | 1419 ± 12     | 554 ± 10      | 2.56         | 295 ± 10   |
| 3                    | 1428 ± 12     | 556 ± 11      | 2.57         | 308 ± 13   |
| 4                    | 1410 ± 10     | 538 ± 12      | 2.62         | 258 ± 16   |
| Whole                | 1411 ± 12     | 540 ± 10      | 2.61         | 294 ± 15   |
| **Academgorodok**    |               |               |              |            |
| 1                    | 1608 ± 13     | 607 ± 8       | 2.65         | 258 ± 14   |
| 2                    | 1627 ± 10     | 615 ± 12      | 2.65         | 277 ± 12   |
| 3                    | 1635 ± 11     | 620 ± 10      | 2.64         | 308 ± 9    |
| 4                    | 1614 ± 13     | 609 ± 10      | 2.65         | 264 ± 13   |
| Whole                | 1620 ± 11     | 612 ± 12      | 2.65         | 275 ± 14   |
| **Central park**     |               |               |              |            |
| 1                    | 1819 ± 11     | 672 ± 11      | 2.71         | 212 ± 13   |
| 2                    | 1846 ± 13     | 681 ± 9       | 2.71         | 234 ± 14   |
| 3                    | 1855 ± 9      | 686 ± 8       | 2.7          | 238 ± 14   |
| 4                    | 1822 ± 12     | 672 ± 11      | 2.71         | 225 ± 10   |
| Whole                | 1828 ± 13     | 677 ± 12      | 2.7          | 232 ± 13   |
| **Thermal power plant** |               |               |              |            |
| 1                    | 1665 ± 14     | 608 ± 13      | 2.74         | 182 ± 12   |
| 2                    | 1683 ± 15     | 612 ± 11      | 2.75         | 198 ± 9    |
| 3                    | 1688 ± 13     | 616 ± 10      | 2.74         | 202 ± 12   |
| 4                    | 1674 ± 12     | 614 ± 11      | 2.77         | 188 ± 13   |
| Whole                | 1677 ± 13     | 613 ± 11      | 2.74         | 197 ± 11   |

If there is little anthropogenic pollution (Academgorodok, Central Park), the total chlorophyll content increases by 15-30 %, which can be explained by using the plant’s reserve capacity due to adverse conditions. In this case, the ratio of chlorophylls a and b increases slightly as the environment deteriorates. The observed changes indicate that the contribution of the latter is more likely to decrease under the influence of pollutants. Similar information was noted in the study of the impact of industrial emissions on forests [4,9]. A serious increase in the load, as it occurs in the area of the thermal power plant, leads to a decrease in the contribution of chlorophylls in conifers, which, apparently, is caused by a partial resolution of the pigment system. The nature of the accumulation of carotenoids in the assimilation apparatus is even more regulated by the intensity of pollution: with its increase, their content decreases, and with its weakening, it increases.
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