Influence of Technogenic Pollution on the Pigments Concentration in the Black Poplar Leaves (*Populus nigra* L.) in Biysk

G G Sokolova*, L P Khlebova, M V Kalgina
Altai State University, Department of biology, 656049, Barnaul, Russia

E-mail: *sokolova-gg@mail.ru*

Abstract. The article presents the results of a study of the content of pigments in the leaves of black poplar growing along the auto roads in Biysk. The increase in technogenic pollution contributes to the change in the amount and ratio of basic and additional pigments. Compared with the control, a significant decrease in the content of chlorophyll a and the ratio of chlorophyll a/b, a significant increase in the content of chlorophyll b and carotenoids were revealed.

1. Introduction
Photosynthesis is an integral process of plant life. The main photosynthetic pigments of plants are chlorophyll *a*, chlorophyll *b* and carotenoids. The predominant pigment is chlorophyll *a*, which performs the following important functions: selective absorbs the energy of light, storing the resulting energy and converting it photochemically into the chemical energy of primary photoreduced and photooxidized compounds [1, 2]. Chlorophyll *b* and carotenoids are auxiliary photosynthetic pigments, they perform a protective function. Normally, the ratio of chlorophylls *a*, *b* and carotenoids is approximately 5: 3: 2, which is considered the most optimal for the effective course of photosynthesis [3, 4].

Under conditions of technogenic environmental pollution, the content and ratio of pigments change: the amount of chlorophyll *a* decreases, the total content of pigments and the content of auxiliary pigments (carotenoids and anthocyanins) increase. Such adaptation of plants is considered as adaptation of the assimilation apparatus to stress [5–9]. The content of photosynthetic pigments and their ratio serve as criteria for the functional state of woody plants under conditions of technogenic pollution and indicators of the ecological state of the environment [10, 11, 12, 13, 14, 15, 16].

The main sources of atmospheric air pollution in Biysk are factories, a thermal power plant, and automobile transport. Dust, soot, nitrogen dioxide, dioxide, formaldehyde, dust, benzpyrene and other substances prevail in the atmospheric air of Biysk. These compounds significantly affect pigments, destroy chlorophyll *a*, and change the ratio of basic and additional photosynthetic pigments, reduce the accumulation of chlorophyll *b*, contribute to the violation of plant metabolism.

Black poplar (*Populus nigra* L.) is the most common type of woody plant used for landscaping settlements. Black poplar is ecologically plastic. This is a photophilous plant species with a high competitive ability. Black poplar belongs to cold-resistant plants and is adapted to exist in the conditions of a long harsh winter. It grows well in fairly moist soils with a groundwater level of 1–1.5 m, tolerates long-term excess or temporarily insufficient moisture. Poplar plants grow well on humus-rich, well-aerated soils with pH = 5–7. Salinity of the soil is one of the main factors limiting the success of growth and the durability of poplar stands. Poplars negatively react to soil compaction by pedestrians or immoderate grazing.

In cities, it shows high resistance to air pollution by dust, smoke and gaseous substances. During the growing season, one poplar absorbs 20–30 kg of dust or soot from the atmosphere. According to the intensity of oxygen evolution, one tree can replace 7 spruce, 4 pine or 3 linden trees.

The purpose of our work was to study the dynamics of the content of photosynthetic pigments in the leaves of black poplar (*Populus nigra* L.), growing near the factories and car refuelings in Biysk.
2. Materials and methods
To test the pigment content in poplar leaves, 6 trial sites were laid: control, oleum factory, glass factory, thermal power station, car refueling №1, car refueling №2. At each test site, 10 trees were selected, from which intact leaves were collected in the lower part of the crown in the amount of 100–200 pieces. The leaves were harvested in the summer of 2018–2019. For quantitative and qualitative analysis of photosynthetic pigments, such biological repetition is considered sufficient for reliable characterization of the biochemical composition of leaf organs [17].

The content of photosynthetic pigments in poplar leaves was evaluated using a SHIMADZU UV-1800 spectrophotometer. Pigment absorption maxima were measured in triplicate at the wavelength of photosynthetic pigments: 662 nm – chlorophyll a, 644 nm – chlorophyll b, 440.5 nm – carotenoids. The pigment concentrations in poplar leaves were calculated in two stages according to the formulas [18]:

Stage 1. Calculation of the concentration of leaf pigments in an alcohol solution (mg/l):

\[ C_a = 9.784D_{662} - 0.99D_{644}, \] (1)

\[ C_b = 21.426 D_{644} - 4.650D_{662}, \] (2)

\[ C_a + C_b = 5.134D_{662} + 20.436D_{644}, \] (3)

\[ C_k = 4.695D_{440.5} - 9.268(C_a - C_b), \] (4)

where \( D_{662}, D_{644} \) and \( D_{440.5} \) are the optical density at wavelengths of 662, 644, 440.5 nm, respectively; \( C_a, C_b, C_k \) – the concentration of chlorophyll \( a \), chlorophyll \( b \) and carotenoids in the leaves of the objects of study (mg/l).

Stage 2. Calculation of the number of pigments in the sample (mg /100 g):

\[ C_o = C \cdot V \cdot V_2 / m \cdot V_1 \cdot 10, \] (5)

where \( C \) – the pigment concentration, mg/l; \( V \) – the volume of the original extract, ml; \( V_1 \) – the volume of the extract taken for dilution, ml; \( V_2 \) – volume of diluted extract, ml; \( m \) – the mass of the sample.

The data obtained were statistically processed using Student’s t-test.

3. Results and discussion
Analysis of the content of pigments in the leaves of black poplar growing in Biysk revealed the following patterns.

3.1. Chlorophyll a. In the control, the content of chlorophyll \( a \) in the leaves of black poplar ranged from 4.81 to 5.57 mg/100 g. A slight decrease in the content of chlorophyll \( a \) was noted at the end of the growing season. The content of chlorophyll \( a \) in poplar leaves growing near the factories and car refuelings was significantly lower by 47–67% compared with the control (Table 1). In addition, a large number of leaves with necrosis were noted in these areas.

| Sampling points          | Content of chlorophyll \( a \), mg/100 g |
|--------------------------|---------------------------------------|
|                          | june       | july       | august     |
| Control                  | 5.22±0.05  | 5.48±0.09  | 4.89±0.08  |
| Car refueling №1         | 2.75±0.08  | 2.87±0.06  | 2.00±0.08  |
| Car refueling №2         | 2.58±0.09  | 2.71±0.06  | 1.73±0.02  |
| Glass factory            | 2.46±0.12  | 2.55±0.07  | 1.87±0.04  |
| Oleum factory            | 2.33±0.04  | 2.51±0.05  | 1.92±0.10  |
| Thermal power station    | 2.28±0.12  | 2.40±0.08  | 1.61±0.07  |

Note: \( 2.75 \pm 0.08 \) – the value is reliable at \( p \leq 0.05 \)

3.2. Chlorophyll b. The content of chlorophyll \( b \) in poplar leaves growing in the control plot varied from 4.89 to 5.48 mg/100 g. During the growing season, a slight decrease in the content of chlorophyll \( b \) was observed in August (Table 2).
Table 2. Dynamics of the content of chlorophyll $b$ in poplar leaves growing near the factories and car refuelings

| Sampling points               | Content of chlorophyll $b$, mg/100 g |
|------------------------------|--------------------------------------|
|                              | june  | july  | august |
| Control                      | 1.48±0.08 | 1.61±0.06 | 1.30±0.08 |
| Thermal power station        | 2.53±0.06 | 2.16±0.03 | 1.75±0.09 |
| Oleum factory                | 2.46±0.04 | 2.39±0.09 | 1.68±0.05 |
| Glass factory                | 2.41±0.09 | 2.13±0.08 | 1.51±0.05 |
| Car refueling №1             | 2.37±0.07 | 2.15±0.06 | 2.00±0.09 |
| Car refueling №2             | 2.32±0.04 | 2.29±0.04 | 1.83±0.06 |

Note: $2.53 \pm 0.06$ – the value is reliable at $p \leq 0.05$

The content of chlorophyll $b$ in the leaves of poplars growing near the factories and car refuelings varied from 1.51 to 2.53 mg/100 g, which is significantly higher by 14–41% compared to the control (Table 2). The concentration of the studied pigment significantly decreased during the summer period.

3.3. The ratio of chlorophyll $a/b$. The ratio of chlorophyll $a/b$ in poplar leaves growing in the control plot was 3.40–3.76. The chlorophyll $a/b$ ratio in poplar leaves growing near plants and gas stations was significantly lower compared to the control at all test sites. The highest values of the chlorophyll $a/b$ ratio were observed in poplar leaves growing near the car refuelings, the smallest – in poplar leaves growing near the factories and thermal power station (Table 3).

Table 3. Chlorophyll ratio $a/b$ in poplar leaves growing near the factories and car refuelings

| Sampling points               | Chlorophyll ratio $a/b$ |
|------------------------------|-------------------------|
|                              | june  | july  | august |
| Control                      | 3.53  | 3.40  | 3.76   |
| Car refueling №1             | 1.16  | 1.33  | 1.00   |
| Car refueling №2             | 1.11  | 1.18  | 0.94   |
| Glass factory                | 1.02  | 1.20  | 1.24   |
| Oleum factory                | 0.95  | 1.05  | 1.14   |
| Thermal power station        | 0.90  | 1.11  | 0.92   |

3.4. Carotenoids. The content of carotenoids in the leaves of poplars growing in the control plot varied from 0.83 to 0.94 mg / 100 g. During the growing season, no significant changes in the content of carotenoids were detected (Table 4).

The content of carotenoids in the leaves of poplars growing near the factories and car refuelings varied from 1.47 to 1.93 mg/100 g, which is significantly higher by 43–51% compared with the control. During the growing season, a slight increase in the content of carotenoids was noted at all test sites. The highest content of carotenoids was found in poplar leaves growing near the factories, the smallest content – in poplar leaves growing near car refuelings (Table 4).

Thus, the content of photosynthetic pigments in poplar leaves in Biysk depends on the location of growth and the level of pollution. Under the influence of pollutants, chlorophyll $a$ is destroyed or its transition to chlorophyll $b$ is accelerated. In this case, the content of chlorophyll $b$ increases. This process is confirmed by data of other authors [7, 8, 9].

An increase in the content of chlorophyll $b$ is an adaptation process. The content of chlorophyll $b$ varies less compared to the content of chlorophyll $a$, which indicates a greater resistance of the chlorophyll $b$ molecule to pollutants [19].

Carotenoids perform protective functions, with increasing stress on the body in plant plants, the content of carotenoids increases, which is also an adaptive reaction.
Table 4. Dynamics of the content of carotenoids in poplar leaves growing near the factories and car refuelings

| Sampling points            | Content of carotenoids, mg/100 g |
|---------------------------|-----------------------------------|
|                           | june    | july    | august  |
| Control                   | 0.83±0.08 | 0.90±0.08 | 0.94±0.03 |
| Thermal power station     | 1.74±0.09 | 1.80±0.10 | 1.93±0.02 |
| Oleum factory             | 1.69±0.07 | 1.73±0.07 | 1.84±0.06 |
| Glass factory             | 1.62±0.03 | 1.69±0.07 | 1.72±0.09 |
| Car refueling №1          | 1.57±0.03 | 1.64±0.05 | 1.69±0.07 |
| Car refueling №2          | 1.47±0.05 | 1.52±0.08 | 1.61±0.03 |

Note: 1.74 ± 0.99 – the value is reliable at p≤0.05

4. Conclusions
1. The content of chlorophyll a in poplar leaves growing near the factories and car refuelings is significantly lower by 47–67% compared with the control.
2. The content of chlorophyll b in poplar leaves growing near the factories and car refuelings is significantly lower by 14–41% compared with the control.
3. The content of carotenoids in the leaves of poplars growing near the factories and car refuelings is significantly higher compared with the control by 43–51%.
4. Over the months, there were no significant changes in the content of chlorophylls and carotenoids at all test sites.

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