Ontogenetic reactions of mesophyll of leaves of Betula nana L. on the industrial pollution of the arctic urbanized territory

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Abstract. There was determined to be concentration of heavy metals (Cu, Ni, Zn, Pb, V) in the vicinity of industrial enterprises in the soil of the city of Murmansk. High concentration of Cu, Ni, Zn was revealed. The structure of mesophyll in the ontogenesis of Betula nana leaves was studied. It has been established that under the influence of polymetallic soil contamination, thickening of leaves of B. nana on experimental sites of the city of Murmansk is higher, than in control and occurs due to increasing of palisade or spongy mesophyll during ontogenesis.

1. Introduction
In recent decades, the Russian Arctic has been increasingly exposed to anthropogenic impact due to the active development of industry, transport and logistics, hydrocarbon and gas exploration [1]. In this regard, the pollution level of the Arctic urban areas is increasing. In an urban environment, trees and shrubs are affected by gaseous toxicants, smokiness of the atmosphere, dust settling on the surface of their leaves and containing heavy metals [2]. The accumulation of toxic substances in plant tissues affects, first of all, the structure and functioning of the assimilation tissues. Studies on the effect of industrial pollution on the mesostructure of plant leaves in the urbanized territories of the Arctic are not numerous [3, 4].

The studies were carried out in Murmansk, the largest non-freezing port in Russia, located beyond the Arctic Circle (68° 58′ N; 33°05′ E). The climatic features of the city are formed under the influence of the warm North Atlantic stream of the Barents Sea. The average annual air temperature is + 1.1 °C, the average winter temperature is − 7.2 °C, summer + 11.1 °C [5]. The polar night begins on 29 of November (44 days), the polar day – from 22 of May until 22 of July (62 days). The duration of the growing season is 120 – 130 days. Sources of air pollution are the Murmansk Thermal Power Plant, the Murmansk Commercial Sea Port, a plant for the thermal treatment of municipal solid waste and automobile transport. The environmental situation in recent years has been deteriorating. Since 2010, inorganic dust emissions have more than doubled. In 2017, there was an increase in the total indicator of soil pollution in the city of Murmansk (K soil), that amounted to 5.84, which is 2.7 times higher than in 2015 – 2016 [6].

The biomonitoring object is Betula nana L. (dwarf birch), typical hypoarctic species, nanophanerophyte and chamaephyte. It is specific for temperate northern regions of the Atlantic Arctic, where it forms thickets in the southern part of the tundra zone. B. nana is a stunted shrub, its leaves differ from the other types of birches in both shape and dwarf size: round, leathery, shiny.
Studies of the mesostructure of B. nana leaves in the conditions of circumpolar climate and environmental pollution in Russia are only few [7, 8].

Objective of the research: to study the influence of heavy metals soil pollution in Murmansk on leaf mesophyll of B. nana in process of their ontogenesis.

2. Material and methods

In June 2018, four experimental sites, on which B. nana grows, differing in the level of environmental pollution, were laid in the city of Murmansk from north to south. PP1 – hillside on Lobova Street, PP2 – vicinity of Semenovsky Lake, PP3 – surroundings of Srednee Lake, PP4 – Geroev Rybach’ego Street. The control site is located 35 km of Serebryansky road, north of the city. All experimental sites are located in areas with increased technogenic load. PP1 (Lobova Street) – 400 m from the ship repair plant «Zvyozdochka», nearby «Atomflot» base, where there is reloading and temporary storage of liquid and solid radioactive waste. PP2 (vicinity of Semenovsky Lake) – 953 m from the Murmansk Commercial Sea Port, where coal is crushed and transshipped by open way. PP3 (the vicinity of Srednee Lake) is located 373 m from one of the most environmentally hazardous facilities in Murmansk – the plant for the thermal treatment of municipal solid waste. PP4 (Geroev Rybach’ego Street) is close to the road and a garage cooperative.

In all experimental sites in September 2018 soil samples were collected for analysis on the content of heavy metals. The determination of the gross content of heavy metals (Cu, Ni, Zn, Pb, V) was carried out at the Center for Laboratory Analysis and Technical Measurements of the city of Murmansk on an atomic absorption spectrophotometer MGA-915 M for low concentrations and an atomic absorption spectrophotometer Contr AA 300 for high concentrations of heavy metals. In May 2018, 10 shrubs of B. nana were marked on each experimental site. For anatomical studies at the end of June, July and August 2018, leaves were collected and then were fixed in a FAA solution. The study of mesophyll was carried out on cross sections under a light microscope with a magnification of 400 times, using an ocular micrometer. The studied indicators were: the thickness of the leaf, the thickness of the palisade and spongy mesophyll. In each sample N = 50. The experimental data were processed using generally accepted methods of variation statistics.

3. Results and discussion

As a result of chemical analysis in all soil samples of the city of Murmansk the copper content was detected to be from 18.3 (PP4) to 91.8 mg / kg (PP3), in the control 23.1 mg / kg (table 1). A significant excess of the maximum permissible concentration (MPC) (91.8 mg / kg) was found in the vicinity of the plant for heat treatment of municipal solid waste (PP3) and the ship repair plant «Zvyozdochka» (84.2 mg / kg) (PP1). The nickel concentration in the soil of the experimental sites varies from 31.6 (PP3) to 123.3 mg / kg (PP3), in the control 4.2 mg / kg. Exceeding the MPC was revealed at three sites, the maximum in the vicinity of the incinerator (123.3 mg / kg) (PP3) and the ship repair plant «Zvyozdochka» (102.8 mg / kg) (PP1).

Table 1. Gross content of heavy metals in the soils of Murmansk in 2018 (mg / kg).

| № of site | Cu   | Ni   | Zn   | Pb   | V    |
|-----------|------|------|------|------|------|
| PP1       | 84.2 | 23.1 | 520.4| 38.1 | 141.6|
| PP2       | 38.4 | 31.6 | 298.6| 2.3  | 101.1|
| PP3       | 91.8 | 123.3| 358.4| 4.5  | 120.7|
| PP4       | 18.3 | 44.2 | 34.1 | 3/1  | 67.3 |
| Control   | 23.1 | 4.2  | 22.6 | 0.6  | 79.1 |

The city's soils are heavily contaminated with zinc, the highest concentrations are in samples from the vicinity of the shipyard «Zvyozdochka» (520.4 mg /kg), an incinerator (358.4 mg /kg) and the impact zone of the Murmansk Commercial Sea Port (298.6 mg). The gross lead content is unexpectedly low: from 3.1 (PP4) to 38.1 mg / kg (PP1), in the control 0.6 mg / kg. At all sites, the
concentration of Pb is lower than the MPC, except for PP1 (1.2 MPC). All soil samples, including control, contain vanadium, its concentration is from 67.3 (PP4) to 141.6 mg/kg (PP1), in the control 79.1 mg/kg. Chemical analysis data indicates that the soils of experimental sites are most polluted with heavy metals in the vicinity of the shipyard «Zvyozdochka» and «Atomflot» (PP1), as well as the plant for heat treatment of municipal solid waste in Murmansk (PP3).

Anatomical studies showed that the thickness of B. nana leaves at the experimental sites in Murmansk from the beginning of the growing season to the end of August exceeds the control values (figure 1). Due to the thickening of the leaves, an increase in the internal photosynthetic surface is achieved. Microphylls and thickening of leaves are a characteristic feature of arctic plants, which is associated with an increase in periclinal divisions of mesophyll cells [9].

![Figure 1](image-url)  
**Figure 1.** The dynamics of changes in the thickness of the leaves of Betula nana L. (in microns).

There are various ideas regarding which particular leaf tissues are most affected by pollutants. The formation of the leaf mesostructure proceeds simultaneously with the development of its functional activity, while the structure of the mesophyll changes in ontogenesis [10]. Studies have shown that during the development of dwarf birch leaves, a modification of the palisade mesophyll occurs, at the same time, differences in experimental sites were revealed (figure 2).

On control, in the vicinity of the «Zvyozdochka» shipyard (PP1) and on the Geroyev Rybach’eego Street (PP2) in the ontogenesis of B. nana leaves a decrease of the thickness of the palisade mesophyll was revealed. In the industrial pollution zone of the Murmansk Commercial Sea Port (PP2) and the incinerator (PP3), the value of this indicator does not change in June and July, and is slightly increasing by the end of August. (figure 2). The results are reliable, p ≤ 0.002.

A study of spongy mesophyll also revealed its changes in the ontogenesis of B. nana leaves. In control and at three experimental sites: in the vicinity of the shipyard (PP1), seaport (PP2), and the incinerator (PP3), marked a significant increase in the thickness of the spongy mesophyll (figure. 3). The results are reliable, p ≤ 0.002.
Figure 2. Dynamics of changes in the thickness of the palisade mesophyll of Betula nana L. (in microns).

These results are consistent with studies on other species of birch trees. N. N. Egorova [11] found that in the ontogenesis of the Betula pendula Roth leaf, which grows in the zone of heavy metals contamination in the Cis Urals, a layer of the spongy parenchyma at some experimental sites is almost two times thicker.

Figure 3. Dynamics of changes in the thickness of the spongy mesophyll of Betula nana L. (in microns).

The palisade index, calculated as the ratio of the thickness of the palisade mesophyll to the spongy, decreases in the ontogenesis of B. nana leaf at all sites, including the control (table 2).

| Months | Control | PP₁ | PP₂ | PP₃ | PP₄ |
|--------|---------|-----|-----|-----|-----|
| June   | 0.75    | 0.84| 0.7 | 0.66| 0.6 |
| July   | 0.65    | 0.67| 0.56| 0.56| 0.64|
4. Conclusion
The data obtained indicates that in the ontogenesis of dwarf birch leaf, the proportion of palisade parenchyma decreases and spongy increases. Under the influence of polymetallic soil contamination thickening of leaves occurs due to increase of palisade or spongy mesophyll in the leaf structure during ontogenesis. A study of the anatomical structure of the leaf of Vaccinium myrtillus in the vicinity of the waste incineration plant in Murmansk revealed a significant increase in leaf thickness due to an increase in the thickness of palisade and spongy mesophyll [3]. It is known that the reaction of various plant species to environmental pollution is ambiguous and this is due to their norm of reaction. Change in the intensity of development of photosynthetic leaf tissues of species, growing under extreme environmental pollution conditions, is connected with a disturbance of growth and development processes. On the whole, this is a demonstration of the adaptive reaction of the assimilation structural and functional complex, which ensures the growth of birch under extreme environmental conditions [12].

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