The influence of technogenic pollution on the *Pinus sylvestris* pollen chemical composition

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Abstract. The elemental, fatty acid composition and content of polyphenols in the Pinus sylvestris L pollen, growing within the territory of Ulan-Ude and the Republic of Buryatia background territories, were determined. It is shown that with technogenic pollution in pollen, the content of iron, nickel, lead, cadmium and mercury increases, and the content of manganese decreases. In the fatty acid composition under stress conditions, an increase in the content of unsaturated fatty acids, and in particular, trans- and cis-forms of oleic acid, is observed. A decrease in the content of polyphenolic compounds in samples from contaminated areas was noted. Thus, the chemical composition of pine pollen can be used as an indicator of anthropogenic (technogenic) impact and early diagnosis of the stress state of the forest during forest pathological monitoring.

1 Introduction

Scotch pine, a forest-forming species of Siberia, is widely used as a bioindicator due to its high sensitivity to pollution. The generative organs of coniferous plants are most sensitive to stressful environmental conditions. The generative sphere of Scots pine, which forms the future offspring, is subject to the influence of air pollutants [1]. The formation of the microstrobils (male cones) buds begins a year prior to "pollination", after the linear growth of shoots has ended, allowing toxicants to have a prolonged effect on these organs. Air pollutants have been shown to reduce pollen quality in the common pine, as well as in other pine species [1-3]. There is a high sensitivity of conifer pollen grains in assessing the degree of impact of adverse environmental factors, as well as the possibility of bioindication of anthropogenic pollution, even in the absence of symptoms of damage to the assimilation apparatus [4]. A positive correlation has also been found between heavy metal concentrations and the number of pollen grain anomalies [5].

2 Material and methods

Plant material was collected in late May-early June 2019-2021 within the territory of Ulan-Ude city (n=7) and background areas of the Republic of Buryatia (Kabanskii and

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Pribaikal'skii districts) (n=12). The city of Ulan-Ude has been included in the Priority List of Russian cities with the highest level of atmospheric pollution for more than 10 consecutive years.

Before elemental analysis, the samples of dried plant materials were decomposed with concentrated nitric acid using microwave digestion system MARS 6. The content of elements was determined by atomic absorption spectrophotometry using Solaar M6 (Thermo Scientific, USA). The mercury content was determined by the cold steam method using a VP-100 hydride attachment to an atomic absorption spectrophotometer Solaar M6 (Thermo Scientific, USA).

Lipid fractions were isolated by a modified Bligh and Dyer method [6] followed by acid methanolysis. The isolated lipids were analyzed after preliminary derivatization. The lipid fraction was mixed with HCl solution (2 mL, 2 M) in MeOH in a screw-cap vial, saponified by heating at 90°C for 2 h, and cooled. The reaction mixture was evaporated under a stream of Ar to 0.5 mL, treated with distilled H2O (1 mL), and extracted with hexane (3 × 0.5 mL). The combined hexane fraction was analyzed by GC-MS on an Agilent 6890 gas chromatograph with a quadrupole mass spectrometer (MSD 5973N) as the detector. The percent composition of the mixture constituents was calculated from GC peak areas. Qualitative analysis was based on comparison of retention times and total mass spectra of the corresponding pure compounds using NIST14.L library data and standard mixtures Bacterial Acid Methyl Esters (CP Mix, Supelco, Bellefonte, PA, USA) and Fatty Acid Methyl Esters (Supelco 37 comp. FAME Mix 10 mg/mL in CH2Cl2).

The total phenolic content was determined quantitatively using the Folin Ciocalteu reagent, with Gallic acid as the standard. A 0.5 mL aliquot from each extract was added to 2.5 mL Folin-Ciocalteu's reagent solution (0.2 M) (Merck, USA). The solution was left to rest for 5 min before being added with 2 mL of 7.5% p/v sodium carbonate. The resulting color absorbance was measured at 760 nm in a UV-Vis spectrophotometer after15 min reaction time at 40 ºC. To obtain the phenolic compounds' concentration data, a calibration curve was constructed using different concentrations of gallic acid (10 to 70 mg·L-1).

The results are presented as arithmetic means ± standard deviations (SD). The calculation of means and SDs was carried out in Microsoft Excel. Differences were found to be significant at $p < 0.05$.

3 Results and discussion

In pine pollen from background areas, chemical elements were arranged in the following order: K>Mg>Ca>Mn>Fe>Zn>Na>Cu>Cr>Ni>Pb>Cd>Hg. In areas with high levels of anthropogenic pollution within the city, iron and manganese are interchanged, which indicates the reaction of plants to technogenic pollution. Similar trends have been observed in the needle tissue of the common pine [7, 8]. Moreover, samples collected within the city of Ulan-Ude showed increased levels of nickel, lead, cadmium, and mercury (Fig. 1).

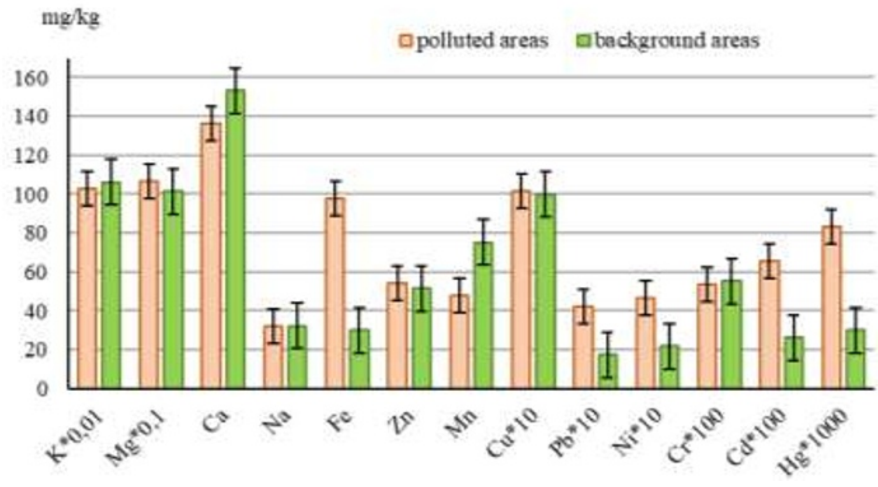

Fig 1. Elemental composition of pine pollen from urban and background territories.

The processes of pollen germination and pollen tube growth are provided by activation of many biochemical processes, including lipid metabolism, as lipids and their constituent fatty acids serve as an important energy source. The analysis showed that the lipid content in pine pollen samples from different areas varied from 1.83 to 2.26%. The total content of saturated fatty acids (SFA) ranged from 45.63 to 51.27%. Among SFAs, 16:0 (17.18-22.81%), 18:0 (3.28-6.98%), 20:0 (3.65-6.31%), and 3-OH 12:0 (9.72-10.70%) predominated in all samples. The total content of unsaturated fatty acids (UFA) was in the range of 48.73-54.37%. Dominant UFAs were 18:2n9,12 (22.18-25.27%) and cis18:1n9 (17.72-22.20%). Comparative analysis of pine pollen fatty acid composition showed an increase in the UFA content, in particular trans- and cis-forms of oleic acid - in the vicinity of Ulan-Ude, where the scotch pine experiences the greatest stress. This is consistent with literature data indicating that the percentage of unsaturated fatty acids increases in cell membranes under different types of stress, making the membrane more fluid [9]. It is also noted that an increase in UFA content in plant tissue membranes enhances plant resistance to pathogens and stress caused by high concentrations of heavy metals [10].

To counteract free radical oxidation, plants produce various antioxidant compounds (flavonoids, tannins, tocopherols, carotenoids, hydroxy- and amino acids, ascorbic acid, and other organic compounds). Phenolic compounds, or polyphenols, are some of the most common secondary metabolites in plant cells and are an important part of the plant's antioxidant system [11]. A comparative assessment of the content of polyphenolic in ethanol extracts from scotch pine pollen showed that in urban samples, the content of polyphenols compounds (mg eq. of gallic acid/g dry weight) was 1.4-1.5, which is lower than in samples collected in background areas (2.2-2.9). Researchers have previously noted that in environments with pollution, a decrease in the level of phenolic compounds may be associated with both their activity in free radical reactions and the suppression of biosynthesis [12].

4 Conclusion

Thus, the chemical composition of pine pollen may reflect the level of atmospheric pollution and be used as an indicator of anthropogenic (technogenic) impact and early diagnosis (even in the absence of symptoms of damage to the assimilation apparatus) of forest stress during forest pathology monitoring. Moreover, the study results can be useful when choosing areas for pine pollen harvesting in order to obtain biologically active additives.

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