Peculiarities of selecting woody plants for anatomy analysis in various environments

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Abstract. Methods for studying woody plants anatomy are now very diverse. We modified the guidelines for bark studies developed for wood analysis at all stages of preparing the woody plant samples for microscope study. For the first time, we used separation to separate hard and soft fractions of the bark tissue. Correct approach to selection of plant samples and further laboratory manipulations ensures validity of the results of our study. We select the size of the sampling area depending on the type of vegetation. In every habitat, we also include transects along the gradient of height above sea level or the impact from the source of natural stress. The results of our research will help study landscape changes during exogenous geological processes and phenomena using biological indication of geosystems.

1. Introduction
Structural environmental analysis of plant wood and bark is widely used for detecting extreme natural phenomena, such as fires, volcanic eruptions, or floods. In the last few decades study of wood from trees, brush, and low shrub is used to assess the current climate changes and past climate dynamics. Environmental plant anatomy benefits from technical and theoretical advancements in optical and electronic microscopy. There are lots of methods for preparing woody plants for microscopy. Methods for studying woody plants anatomy are now very diverse [1–10]. Most of those methods were developed for studying wood. Our study of woody plants around mud and magma volcanoes have proven to be suitable for studying bark. We apply microscopy, including biochemical analysis, to a specific object of woody plants study, namely, the bark. In our method, maceration of bark tissue is based on the method by Ge Wang et al [4]. This type of maceration was developed for analysis of wood fibers of mallow hemp, tracheids of the Chinese spruce (Picea brachytyla) and sclereids of bamboo.

Bark of woody plants is a very complex object for anatomical studies. The bark includes tissues, cell groups, and individual cells, all very different in terms of their physical and chemical properties. Some are very dense and sturdy, like sclerenchyma, phellem, and rhytidome, others are soft and water-saturated, like parenchyma and conductive elements of the phloem. Significant difference in mechanical and other properties of bark cells at a distance of 100–500 microns makes it very difficult to make a microslide or take a macerated sample. It also complicates further chemical manipulations required to obtain a microslide of the required quality. Therefore, we have developed special methods allowing us to single out a specific tissue complex in the bark and obtain a panoramic slide which would include various types of tissue. For instance, we separate conductive and non-conductive phloem both for maceration.
and for preparing permanent slides in the tree bark (figure 1). Bark from brush and low shrub, as well as bark from year-old shoots of the trees, does not require such separation, because cells and cell groups with different physical and chemical properties are never found in the same area of 50–100 microns. This provides for a good monolithic slide.

We developed some methods to adapt wood maceration guidelines of Wang et al [4]. For each type of woody plant, we found the optimal time of exposure to the macerating fluid, to separate the phloem into parts. We developed the guidelines for separating dense groups of cells and conductive elements and the parenchyma (figure 2). This helps us avoid errors when analyzing anatomical features of the bark [11–18].

We practice a comprehensive approach to studying the relation between geological environment and vegetation. Proper assessment of geological environment is critical for understanding structural changes in plants. That is why geochemical, geomorphological, and general geological characteristics of the habitat of the plants we study are of utmost importance in our approach [19–21]. Study of living and fossil organisms provides a picture of the mechanisms, age, and scope of geological processes [22–25].

![Figure 1. Separate conductive and non-conductive phloem.](image1.png)

![Figure 2. Separating dense groups of cells and conductive elements and the parenchyma.](image2.png)

2. Materials and methods
Correct approach to selection of plant samples and further laboratory manipulations ensures validity of the results of our study. In the field, we take samples from three different specimens of tree, brush, and low shrub in the habitats we want to study. In extreme conditions, plants often grow in pessimum areas. In areas of volcanic activity manifested by gas and hydrothermal vents, the vegetation is sparse, therefore sampling area has to be expanded. The further from the source, the less the effect on the plants. First, we take samples from specimens closest to the source, where we can find sparse groups and synusia with areas of exposed substrate. Impact of the negative factor may vary depending on the wind direction, microrelief, and other landscape features.

We are governed by the methods and guidelines accepted in geobotanical studies when taking samples of woody plants for structural analysis [26–31]. Depending on the type of vegetation, we select the size of the sampling area, as small as 5×5 m for low shrubs and free-growing plant aggregations or as large as 20×20 m up to 100×100 m in the forest communities. Sampling areas are located inside the perimeter of gas and hydrothermal vents, thermal lakes, or...
stream banks. In every habitat we also include transects along the gradient of height above sea level or the impact from the source of natural stress. In each sampling area we select three sites where we sample woody plants, usually selected at random.

Collection of woody plants along the transect enables us to see how they adapt to various degrees of stress. Transect length and width, as well as the space between transects, are determined in situ. Samples of woody plants sprouts need to be dug out carefully, taken out, packaged, and transported. The time from the moment a plant is taken out of the substrate until the moment it is placed in the fixative solution should be as short as possible. A sample of brush or low shrub must be placed whole in a plastic bag along with the substrate remaining on its roots. Branches and fragments of larger shrub or splinters of tree trunks must be cut as long as practical (up to 20–30 centimeters), so that it is possible to cut out the middle part of a branch or trunk with live tissue before placing it in the fixative solution. This is especially important when studying bark.

It is important that the samples are taken at the end of the vegetation period. The best microslides of the highest quality come from samples taken in autumn, when the cambium becomes inactive until spring. On Sakhalin this period is usually the second half of September and all of October, in the southern Kurils it is October. Unfortunately, major comprehensive expeditions to remote areas such as the Kurils are rare in autumn. If samples are taken in summer, every stage of the process of making microslides, from identifying the plant age to placing the slide into the mounting medium, is more difficult.

It is important to choose the right stems ideal for studying their internal structure, especially young ones (1–10 years). We use classifications by I. Serebryakov [32] M. Mazurenko and A. Khokhryakov [33] to select and determine the types of shoots. For structural analysis, we use long branching shoots. Those are side shoots adding to the green matter of the assimilatory organs and seed productivity, because they contain the leaves and most of the flowers and blossom clusters. Long branching shoots develop from wintering buds. Forming shoots are not included in our samples. Such shoots are large due to wide germination of bark and wood because of intense growth, especially in their first few years. Undamaged shoots without visible stripping by insects are placed in the fixative. We take the first year-old stems when the buds open, and the last samples are taken at the end of the vegetation, after defoliation. Sampling frequency is about 7–10 days. This allows us to observe initiation, formation and development of bark tissue and elements, such as periderm, cortex and rays sclerification, initiation of the axial parenchyma layers in the conducting phloem, speed and intensity at which the conducting tissue grows and develops, the character of the phloem elements, generation of crystals. Sampling frequency depends on the species and its growth speed. The time between sample collection and fixation should not exceed 2–3 hours. After that the shoots can be stored in a fridge up to 8 hours, in a ziplocked plastic bag ensuring a moist chamber. Young shoots from the first batches of samples are covered with epidermis, so the best way to preserve those is by placing them in ethyl alcohol (50 %) right after sampling. For year-old stems and older stems covered with periderm, 70–95 % ethyl alcohol will do as well at the end of the vegetation season. Young stems covered outside with a thin layer of periderm should be fixed in 70 % alcohol. Samples covered with thick layer of periderm or crust and containing large sclerified areas in the non-conducting phloem can be placed in 95 % alcohol.

We are especially thorough and careful when taking woody plants samples in volcanic landscapes. Growth and development of plants as they adapt to adverse impact differ functionally and morphologically from plants of the same species growing in normal (background) natural conditions. In extreme conditions the plants tend to change from normal growth strategy to survival strategy. The plant body changes drastically, up to complete change of life form. For instance, impact of magmatic volcanoes or cold seashores causes the brush to take on a low, pillow-like shape closer to the substrate. Perennial branches of such plants become geophitization and burrow into the soil [17, 18, 34]. In such cases we take the whole specimen with the shoots buried into the soil and developing roots. The trees become crooked and turn into dwarves; they develop several skeletal axes and take on a bush form. The lower part of such plants is also covered under a layer of
substrate [35], so first we need to clean the plants from the topsoil or substrate. Next, we cut off several large skeletal axes with a saw and preserve them as per our standard method.

Special care and lots of labor are required when taking samples for anatomical analysis from trees. We pick trees with the top shaped evenly (in the top, middle, or lower third of the trunk). Trees must be about the same age. The age of the tree is determined using a core taken with an increment borer. For us to assess age-related parameters and condition of the plant, the samples must be as comprehensive as possible. This means choosing an age increment ensuring that structural features of the bark and wood change in a successive order from the latest year-old shoot to the base of the stem or stipitate, with account for geophytization (burrowing of stems and stipitate into the substrate). We found this was a very common occurrence in woody plants growing in volcanic landscapes. Low shrubs are sometimes found in groves or parts thereof.

An important condition for preparing samples is ensuring that containers for fixation are airtight and leak-proof. The containers must be secure enough during transportation and must remain tight for several months.

3. Conclusion
So, we modified the guidelines for bark studies developed for wood analysis at all stages of preparing the woody plant samples for microscope study. We take a comprehensive approach to studying the relation between the geologic environment and the vegetation. We developed guidelines for taking woody plant samples for certain life forms. When selecting samples, we take into account the place of the stems in the system of shoots, its spatial location, and their location in the substrate (geophytization). We have a set of measures to preserve living bark tissue to the maximum extent possible before office studies. For the first time, we used separation to separate hard and soft fractions of the bark tissue.

Living organisms are involved in shaping the Earth surface, so the results of our research will help study landscape changes during exogenous geological processes and phenomena using biological indication of geosystems.

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