PREFACE

This book examines microbial communities in terrestrial moss in further detail. It also discusses terrestrial mosses as trace element bioindicators; moss bioreactors; the ecological diversity and application for agricultural microbiology; small scale area investigation for heavy metals air pollution; biomonitoring of atmospheric pollution using mosses; the impact of forest decline on fine-scale vertical distribution of epiphytic bryophytes; and the use of mosses in criminal investigations.

Chapter 1 – Terrestrial moss communities are widely distributed among the Earth's major biomes, including equatorial to polar latitudes. Because of their high primary productivity, relatively dense vegetative structure, and alteration of the substratum through deposition of organic matter, moss communities often support rich and diversified microbial communities. This review encompasses soil-dwelling moss communities in low and high latitude locales, with special attention to moss-based microbial community structure (taxonomic composition and organization), function (microbial roles and interactions), and ecology (especially the dynamic interaction of microbes with mosses and their role in the carbon biogeochemical cycle). Nitrogen-fixing cyanobacteria, in addition to heterotrophic microbes, are significant members of some moss microbial communities, and consequently attention is given to the biogeochemistry of N and C.

Chapter 2 – Terrestrial mosses have been used as bioindicators since the 1960s. The first, large-scale studies which reported the concentrations of selected trace elements (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, V, Zn) were conducted in the Scandinavia in 1985 (Poikolainen et al., 2004). Since then many European scientists have employed the moss technique to solve various issues: (i) to document atmospheric chemistry, (ii) to map ecogeochemical changes in the environment, (iii) to pinpoint the major sources of atmospheric pollution, and (iv) to find contrast between background and "hot spot" element concentrations in polluted areas.

Terrestrial mosses have found practical applications in biomonitoring studies because of their morphological and physiological properties. The lack of cuticle and root system causes that their cell walls are easily penetrated by metal ions. This enables absorption of elements and particulates from rainfall, snowmelt and dry deposition. They also have a high cation exchange capacity to retain and transport many elements. Mosses are widespread, they grow in various habitats, and their annual growth increments are easily identified.

The concentrations of trace elements in moss tissues are dependent on individual moss species and their morphology, as well as bedrock lithology and many other environmental factors, i.e., topography, climate, hydrology, and edaphic (soil-related) conditions. Local topographic, climatic and edaphic variables are generally correlated with the geologic setting of study area. Different moss species show distinct variations in the concentrations of elements and some organics. This is induced primarily by diverse assimilation abilities. These facts put some constraints on the selection of a specific moss species for biomonitoring purposes. Several species (*Hylocomium splendens*, *Pleurozium schreberi*, *Scleropodium purum*, *Hypnum cupressiforme*) have successfully been used in biomonitoring programs, but all of these species must have acceptable sampling density, which is crucial to identify the species-dependent variability.

It should be stressed that the moss technique has many advantages. It is easier and cheaper than the conventional analysis of precipitation, which is based on a large number of sample collectors. This technique also allows us to monitor several sites at a low cost. Moreover, the element concentrations in moss tissues are high enough to make the analysis much simpler.

Chapter 3 – The use of moss bioreactors for the production of proteins and peptides is a recently incorporated heterologous system that was developed as an alternative for the expression of recombinant molecules that were not well obtained in mammals, insect and/or yeast systems. Moss is classified into plant kingdom, but it contains a very small genome of 511 Mbps, which facilitates its manipulation and gene transformation. Moreover, it presents less risk of contaminations with human and animal pathogens, when compared with expression systems using bacterial and mammalian cells. Nowadays, the moss species most used as a bioreactor is *Physcomitrella patens*. It was earlier used for studies on plant evolution, development and physiology. Since the last 30 years, *P. patens* started being evaluated as a new option for the production of recombinant proteins. The advantages of moss over other higher plant species is the enhanced rates of homologous recombination, giving straight-forward reverse genetics protocols by gene targeting. Furthermore, it lacks a bias for codon usage, making it less complicated to adapt foreign genes in its genome for protein expression. The cultivation of *P. patens* is also relatively simple and of low cost, when compared to higher plant species. It can grow under a wide range of pH and temperature, using light and carbon dioxide as its only energy and carbon sources. Until now, most proteins expressed in moss systems are from the human genome, with the purpose of producing biopharmaceutical molecules. Hence, it has been described the use of moss bioreactors for the production of monoclonal antibodies, vaccines-to-be molecules and other human proteins and peptides. Therefore, this report aims to describe the most recent advances on the use of moss bioreactor for the expression of proteins targeted as future biopharmaceutical molecules.

Chapter 4 – *Sphagnum* mosses serve as a unique habitat for microorganisms, which play an important role both for the host plants and the peatland ecosystems. The aim of the present study was to isolate endophytic bacteria from the tissues of *Sphagnum* mosses and to screen them for strains promising for further application in agricultural microbiology. About 50 samples of *Sphagnum fallax* (H. Klinggr.) H. Klinggr. and *Sphagnum magellanicum* Brid. were collected in the Austrian Alps, Lenindgrad Region and West Siberia in 2009–2012. Endophytic bacteria were detected inside the moss plants using fluorescent in situ hybridization (FISH) followed by confocal laser scanning microscopy (CLSM). Altogether, more than 400 isolates were obtained by cultivation on the nutrient media. Examination of the isolates for the antagonistic activity revealed that more than 50% of them could suppress the growth of phytopathogenic and toxigenic fungi. More than 30% of isolates showed some antagonistic activity against microbial phytopathogens. The isolated strains could colonize crops and promote their growth. Molecular-genetic identification coupled with physiological/ biochemical characterization showed that the dominant endophytic groups belonged to the genera *Burkholderia*, *Pseudomonas*, *Flavobacterium*, *Serratia* and *Collimonas*. The isolated endophytes were shown to be promising objects for the development of effective growth promoting and protective microbiological preparations to be used in agriculture.

Chapter 5 – Application of several moss species for monitoring of the anthropogenic impact on heavy metals air pollution in a small scale area was studied. Mosses were reviewed for their potential to reflect metal air pollution. The attention was focused on their quantification ability, underlying the metal accumulation in the moss plant tissue. Potential "hot spots" were selected in areas of lead and zinc mines (case study at Zletovo mine and Sasa mine), copper mine (Bučim mine), ferronickel smelter plant (FENI industry) and the abandoned As-Sb-Tl mine at the Allchar area, as the main metal pollution sources in the Republic of Macedonia in the period between 2010-2012. There is continuous distribution of dust from ore, flotation tailings and slag surface. This results in air-introduction and deposition of higher contents of certain metals. Several moss species (*Hypnum cupressiforme*, *Campothecium lutescens, Scleropodium purum* and *Homolothecium sericium*) were used as plant sampling media. Determination of chemical elements was conducted by using both instrumental techniques: atomic emission spectrometry with inductively coupled plasma (ICP-AES) and mass spectrometry with inductively coupled plasma (ICP-MS). Combination of multivariate techniques (PCA, FA and CA) was applied for data processing and identification of elements associated with lithogenic or anthropogenic origin. Spatial distribution maps were created for determination and localization of narrower areas with higher contents of certain anthropogenic elements. In this way, influences of selected human activities in local (small scale) air pollution cases can be determined. Summarized data reveal real quantification of elements distribution not only in order to determine the hazardous elements distribution, but also present complete characterization of elements deposition in mines/smelter plant environs.

Chapter 6 – Due to their peculiar morphological and physiological characteristics (lack of a protective cuticle and thick cell walls, numerous cell wall constituents with negatively charged groups, mineral nutrition obtained mainly from wet and dry deposition) mosses are extensively used as biomonitors of environmental contamination for a wide range of contaminants, i.e. radionuclides, heavy metals and toxic nitrogen, sulphur and organic compounds and also as reference organisms in the assessment of radiation exposure to non-human biota. Mosses usually show considerable longevity, which assists their use as long-term integrators of atmospheric deposition. The use of mosses as biomonitors of atmospheric contamination is reviewed in this chapter, with the special reference to terrestrial ecosystems in Serbia, including data on radionuclide and heavy metal content in mosses, their spatial patterns and species- and site-specific dependencies.

Chapter 7 – The vertical distribution of epiphytic bryophytes on tree trunks may serve as an indicator of diversity, and requires delineation. This study assessed changes in the vertical distribution of epiphytic bryophytes to determine how forest decline affects the diversity of these non-vascular plants.

Epiphytic bryophytes growing on the trunks of *Pinus jezoensis* var. *hondoensis* trees were examined along four 50-cm-high zones from ground level to a trunk height of 2 m in six forest patches at different states of forest decline (no decline, slight decline, severe decline, and serious decline). Overall, epiphyte species richness and trunk coverage decreased with the process of forest decline. Using two-way indicator species analysis and detrended correspondence analysis, epiphytic bryophyte flora diversity appeared to shift from higher trunk zones in healthy forests to lower trunk zones in heavily degraded forests. These changes might be explained by an increase in drought stress and light intensity caused by forest decline, which might have a greater effect on the composition of epiphytic bryophytes inhabiting the lower zones of trunks. Therefore, the epiphytic bryophytes occupying the lower trunk zones in forest patches where forest decline continues require careful monitoring and conservation. As the fine-scale distribution of epiphytic bryophytes may be easily surveyed without any special equipment or machines, these plants could be used to regularly monitor and evaluate the impact of forest decline on epiphytic bryophytes.

Chapter 8 – Forensic Botany is the application of botanic knowledge to criminal investigations. It is a relatively new science and it includes several subdisciplines, such as Palynology, Dendrochronology, Limnology, Systematic, Ecology and Molecular Biology. Plant material is everywhere and it is frequently found in association with forensic cases, especially outdoor. Nevertheless its potential applications are often unknown or underestimated by the investigators and prosecutors, so botanical traces are usually overlooked. Instead a systematic analysis of botanical evidence can provide useful information about manner and time of death, find out and dating clandestine graves, identify the primary crime scene and help discovering the offender. Botanical evidence is mainly used in forensic field to provide linkages between crime scenes, individuals (victims or suspects) and objects through morphological, anatomic and genetic study of pollens and spores, seeds, living or decayed leaves, plant fragments or algae. Mosses are particularly useful for this purpose. There are over 10.000 species of mosses worldwide; they are ubiquitous (even if they prefer humid and shadowy zones) but their growth substrates and environmental conditions are variable; so they represent good "biological indicators", more reliable than other plant organisms for geolocation. Mosses can grow directly associated to human remains or objects; moss fragments are resistant to decay and they can heavily attach to clothes or shoes, where they can be discovered and identified by genetic analysis even after a long time. Moss spores found in evidentiary materials or human remains might be used to establish in which season a crime occurred. In the last years the use of mosses for forensic purposes expanded; in recent studies the regular growth rate of some species of mosses was used in estimating the minimum post-mortem interval of skeletal remains. In this chapter the authors give an overlook on possible application of moss in Forensics.