PREFACE

This book presents current research in the study of the ecology, economic uses and environmental impacts of algae. Topics include ultraviolet irradiation to control algal proliferation in the environment; alga Trachydiscus minutus as a new source of poly-unsaturated fatty acids; systematics and taxonomic keys for the marine green algal family monostromataceae; the ecophysiology of soil algae; and an evaluation of the total phenolic content and antioxidant activities of crude extracts from red alga, Corallina elongata.

Chapter 1 - In the conservation field of buildings, historical monuments and subterranean environments, very few studies highlight the importance of finding alternative methods to chemical products in protecting against algal biofilm proliferation. Indeed, biofouling by microorganisms is a well-known biodeterioration phenomenon. Algae are considered to be significant contributors to the biodeterioration of stone, and that's why they are the subject of special interest in this chapter. Conservators of monuments or art works used many chemical applications to prevent or to control the algal proliferation on stone. But, due to the changes in European standards on their use, the chemical products must be replaced by environmentally friendly processes. Ultraviolet irradiation appeared to be a suitable process to control algal proliferation in these environments.

Chapter 1 Firstly describes problematic algal biofilms (proliferation and biodeterioration) on different substrata and also the effects of applied chemical treatments. Secondly, UV light and its effects on living organisms is defined and characterized. Finally, the experimental design and the obtained results on algae harvested in caves at the cellular and molecular scales (pigment contents, photosynthetic activity, DNA damages ...) and at the biofilm scale (proliferation measurements) is discussed. This study shows that UV treatment can be used as a conservation tool to eradicate algal biofilms, which proliferate in buildings and monuments.

Chapter 2 - A new bacterial isolate *Azospirillum brasilense* NH strain isolated from salt-affected soil in the north of Algeria was applied to inoculate durum wheat (*Triticum durum*, var. waha). This strain showed already an important positive impact on durum wheat (stem height and spike length) in absence of salinity. The strain shows also a slight effect under saline conditions. Moreover, the combination of this stain with the marine alga *Ulva lactuca* illustrated the best results in absence or presence of high saltiness (160 and 200 mM NaCl). Proline and total sugar production (stress parameters) under saline conditions is inhibited by using *A. brasilense* NH, but the both components are strongly inhibited when *U. lactuca* was added. Also, rates of germination, stem height per week, height/width of the largest leaf, roots and shoot dry weight, optical density (OD) of chlorophyll a and b and total rate in chloro-

phyll, stem and roots dry weight, 1000-seed weight, total spike length, spike dry weight, seed number per spike and total weight of seeds per spike, were positively affected. It was shown that this new strain restores entirely the vegetable growth at 160 and 200 mM NaCl. This study was carried out using a reference strain *A. brasilense* Sp7 in order to compare between the tow strains. It was shown that the reference strain Sp7 is totally inhibited at 200 mM NaCl.

Thus *A. brasilense* NH restored the vegetable growth of durum wheat and reduced the proline and total sugars under saline conditions in pot and field experiments. The results obtained proved the best effect of this new strain NH on durum wheat growth and inhibition of the osmotic stress. In additional to the restoration of durum wheat growth under saline conditions by *A. brasilense NH-U. lauca*, the algal extracts remains the good stimulator of plant growth in absence of stress.

The endophytic colonization of this strain was studied in presence or absence of salinity, in axenic conditions. Wheat roots were harvested and fixed with paraformaldehyde (4%) and hybridized using the FISH technique. Cofocal laser scanning microscopy (CSLM) was performed and the in situ localization of bacterial cells were obtained in the endophytic wheat roots. The best colonization was obtained at high salinity (200 mM NaCl). This result can justify the ability of this new strain to restore wheat growth at 200 mM NaCl. Thus, the results of the work displays that the combination of *A. brasilense* NH – *U. lactuca* extracts could be a good solution to solve the problem of salinity which is the serious threat hindering a wheat culture in arid and saline soils.

Chapter 3 - This chapter aims at testing the feasibility of developing an innovative micro-algae base technology that can concurrently produce biodiesel and protect the environment. The use of fossil fuel for transportation systems is costing the world billions of dollars each year and contributes to air pollution and the global warming. Microalgae have been recognized as one of the most promising source of biofuel. However, there is no local testing or commercial production of microalgal oil in many countries so far.

In this work, it is hypothesized that an economically viable microalgae-based biodiesel production system could be established if innovative photobioreactors were used along with suitable microalgal species rich in oil content. It is proposed to combine the biodiesel production with wastewater treatment and CO_2 reduction using microalgae. Such development will commence commercial feasibility projects. This technology is particularly useful in countries, where huge amounts of CO_2 are released to environment and huge demand on useful applications of wastewater.

In all countries, billions of dollars are spent each year for energy production. Many of the power plants consume diesel in the process of generating electricity and desalination of water. Most public transportation system uses diesel engines. It is well established that carbon dioxide emission from various transportation systems accounts for one third of the total carbon dioxide released into the atmosphere, leading to global warming. The toxic exhausted gas from vehicles also causes hazardous effects on human health.

Biodiesel, derived from vegetable oils or animal fats rather than petroleum, is an alternative fuel for diesel engines. It is renewable, non-toxic, and biodegradable. It can be used in existing diesel engines without modification, and can be blended in at any ratio with petroleum diesel. Currently, the major feedstock for biodiesel production is soybean oil. However, low oil production rates (e.g. 1 ~ 3 barrels of oil per acre of land per year) coupled with high production costs (e.g. feedstock accounts for 70-80% of biodiesel production costs)

have limited the expansion of soybean-based biodiesel to meet the growing demand by the society. Therefore, it is important to develop more economically viable sources of feedstock for biodiesel production. Microalgae are known to exhibit 10- to 20-fold higher growth rates than agricultural crop plants, and certain microalgal species can accumulate large amounts of lipids or oil (30-60% of dry weight).

In this work is proposed in order to develop a complete prototyped model for the production and testing of biodiesel. The model will consist of cultivation reactor to grow suitable microalgae species, photobioreactors with wastewater and or CO_2 as feed to the algae, oil extraction from algae, chemical reactor to convert the extracted and finally test the produced biodiesel in a conventional diesel engine.

Chapter 4 - The alga *Trachydiscus minutus* (Bourr.)Ettl, strain Lukavský et Přibyl 2005/1, Eustigmatophyceae, is described here as a new source of polyunsaturated fatty acids (PUFAs), especially of eicosapentaenoic acid (EPA). These fatty acids (FA) are valuable food additives for man, animals and in aquaculture.

T. minutus was first described as *Pseudostaurastrum minutum* by Bourrelly (1951), from a small fishpond in France, in 1951. It is now found in various water localities, e.g. ponds, lakes and streams, but usually as a rare species. Its principal mode of reproduction is by autosporogenesis, and in prolonged darkness, zoospores are produced. Only chlorophyll_*a* was evaluated; other pigments are violaxanthin, vaucheriaxanthin ester, and ß–carotene.

In laboratory cultures, biomass can reach 16 $g.l^{-1}$ dry weight (DW), consisting of 26-31% total lipids (TL) and EPA comprises up to 42% of TL. The TL content can be increased to 39% of DW by nutrient starvation (N,P,S deficiencies) resulting in an EPA content of up to 35.8% DW, and a productivity of 88 mg $l^{-1}.d^{-1}$. Targeted stereoisomers of triacyglycerols (TAGs) can also be obtained. Oil drops are prominent and an iodine value of about 216 was determined by Raman microspectroscopy, even in individual cells; this has high potential value for the food industry. *T. minutus* also contains 26% of saturated myristic fatty acid, and about 48% protein suitable for food and fuel. The optimal growth temperature was about 27 °C, but it survived below 15 and at 35 °C. Extreme temperatures resulted in increased TL content, up to 35% DW, and decreased carbohydrate (13.6% DW) and protein (30-38% DW). High temperature elevated the activity of superoxide dismutase, catalase and proteases, but glutathione reductase, and peroxidase activity decreased. Under 15 °C, all enzymes except glutathione reductase were inhibited. In the laboratory, optimal growth occurred under continuous light of 2x132 μmol photons.$m^{-2}.s^{-1}$, whereas the maximum light levels evaluated in the greenhouse were up to 640 μmol photons.$m^{-2}.s^{-1}$.

In an orbicular cultivation unit inside a greenhouse in N.Greece (vol. 200 litre, area 2 m^2) *T. minutus* produced 0.6 mg.m^{-2}/day i.e. 3.4 mg.l^{-1}.day^{-1} of EPA. In SW. Bohemia, using a thin-layer inclined photobioreactor (volume 150 litre, area 15m^2) in a greenhouse, *T. minutus* produced up to 0.25-0.45 mg.m^{-2}.day^{-1}, i.e. 20-30 mg.l^{-1}.day^{-1} of EPA. *T. minutus* proved to be tolerant of manipulation, e.g. centrifugation, oscillations of light, temperature and CO_2 concentration, contamination with another algae etc. Sedimentation followed by centrifugation and lyophylization, or drying in the sun on plastic foil was suitable for harvesting. Flocculation also has potential (Přibyl pers. com.) and no parasites have yet been observed in *T. minutus.*

T. minutus was successfully immobilized in agar or alginate. Its potential for further research and exploitation is high and comparable with, or better than other algae used for biotechnological production of PUFAs, and especially EPA.

Chapter 5 - Marine green algal family Monostromataceae consists of single cell-layered green seaweeds distributed throughout the world, yet identification of it being one of the most challenging. Monostromatic green seaweeds are all edible and are commercially cultivated in Japan for centuries. A thorough understanding of its systematics is indeed a necessity for any empirical investigation. Working dichotomous taxonomic key for this family is presented for the first time for the ready benefit of field phycologists. Recent advances in the systematics of this family with a special emphasize on the Japanese varieties are also presented.

Chapter 6 - In most biological textbooks the term "alga“ is used to designate a group of photosynthetically active organisms that are bound to aquatic habitats and lack the classical differentiation into root, stem and leaves. Only experts are aware of the fact that algae are very successful competitors in terrestrial and aerial habitats as well and that they can survive also under aphotic conditions. Accordingly, there is ample information available on taxonomy, physiology and ecology of aquatic algae and on their application in biotechnology whereas on aeroterrestrial algae comparably few data exist. This is the more astonishing since aeroterrestrial algae have to cope with much more diverse and quickly changing environmental stressors than aquatic algae do that live in a more or less constant environment. Aeroterrestrial algae are specialists for interfaces, they settle e. g. substrates exposed to the air such as soil, tree barks, rocks, fences, roofs, facades and rubber seals of car windows. There environmental conditions such as light, UV, temperature and moisture can change quickly. What is more, those algae contribute a significant part to CO_2-fixation in their ecosystems, the exact amount of which still needs thorough investigation. Thus the study of the ecophysiology of aeroterrestrial algae, of their adaptive features and their potential biotechnological applicability is one of the most promising fields in current phycological research.

In this chapter an overview will be given on what is known on the ecophysiology of soil algae, i. e. on that part of the aeroterrestrial algae that lives in soil, and on current research on their environmental impact as well as on their potential use in biotechnology.

Chapter 7 – This chapter evaluates the total phenolic contents and antioxidant activities of crude extracts from a red alga, *Corallinaelongata*. The phenolic compounds in the extracts have been identified and quantified by reverse-phase high-performance liquid chromatography. Phloroglucinol was by far the predominant polyphenol found in the extracts, followed by catechin and epicatechin. Extracting solvents such as water, methanol and water/methanol (1/1) significantly affected the total phenolic amounts determined using the Folin-Ciocalteu method. The total phenolic contents ranged from 443.0 to 487.4 mg gallic acid equivalents per 100 g of dry plant material. The antioxidant efficiency of the extracts was evaluated by measuring the ability to scavenge the stable free radical 1,1-diphenyl-2-picrylhydrazyl, finding that it ranged from 37.0 % to 66.4% (inhibition percentage). The high correlation between the yield of extraction, the total phenolic content and the radical scavenging activity was found, allowing us to conclude that phenolic constituents are mainly responsible for the observed antioxidant activity in the extracts. Because of the wide range of biological activities of phloroglucinol and the other polyphenols detected in the extracts, *Corallina elongate* appears to have many potential industrial uses.