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Can Microalgae Revitalize Phytotoxic Soils?

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Abstract Microalgae represent a variable group of photoautotrophic microorganisms that include both prokaryotic cyanobacteria and eukaryotic algae. Compared to higher plants, algae have faster growth, higher photosynthetic efficiency and the ability to colonize different ecological niches. The advantage of using microalgae for bioremediation or recultivation compared to higher plants mainly lies in their simple cell structures, easier access to CO_2 and nutrients and ability to grow in extreme environments.
The goal of this study was to design and apply biotechnologies for the revitalization of phytotoxic soils based on the use of cyanobacteria and microscopic algae. Microalgal strains isolated from biocrusts from the Lítov post-mining dump (Northern Czechia), or others coming from algae collections were tested in laboratory experiments for the growth on soils. Their physiological state (photosynthetic activity, cell growth and division), and growth conditions (temperature, light intensity, pH, nutrition) were monitored and optimized. The lab-scale experiments were followed with selected strains (*Nostoc, Desmonostoc, Trichormus*) in greenhouse experiments on phytotoxic soils from the Lítov Dump by various ways of application. Soil analyses were conducted to evaluate the initial physical, chemical, and biological soil quality. Soil pH was lower than 3, microbial respiration and biomass were low, but not as low as could be expected for toxic substrates. Content of available nutrients, especially that of dissolved nitrogen and phosphorus, was very low. All strains tested in greenhouse experiments grew on the phytotoxic soil. The most successful was *Trichormus* applied with expanded clay.

Keywords: post-mining revitalization, phytotoxic soils, biocrusts, cyanobacteria, algae

1. Introduction

Microalgae, including cyanobacteria and eukaryotic algae, have shown potential in bioremediation and soil revitalization due to their rapid growth, high photosynthetic efficiency, and ability to thrive in extreme environments (Leong et al., 2021; Gonçalves, 2021).
Biological crusts (biocrusts) are soil communities formed by living organisms (Belnap et al., 2003; Weber et al., 2022). Biocrusts are often found in arid regions where they cover the surface where other vascular plants do not occur. Biocrusts can act as a natural barrier to wind and water erosion and prepare the site for colonization by other organisms (Belnap, 2006). Microalgae as an integral part of biocrusts can contribute to the initial stages of reclamation thanks to their ability to photosynthesize and rapidly colonize new substrates. They not only initiate the process of soil formation but also ensure its long-term stability. Microalgae facilitate the accumulation of organic matter, improve soil structure and create favorable conditions for subsequent colonization by bacteria, fungi and higher plants (Song et al., 2022; Ramakrishnan et al., 2023; Saini et al., 2024). The most common pioneer species of microalgae are *Microcoleus*, *Nostoc* or *Klebsormidium* (Garcia-Pichel, 2023).
Land revitalization is becoming increasingly relevant against the backdrop of rising global efforts focused on the environment, restoring biodiversity, and mitigating the consequences of land degradation (Pedrinho et al., 2024). An example of degraded land are dumps on which overlying rocks exposed by open-pit mining of brown coal are deposited. The composition of these substrates is different from common soils (Frouz et al., 2008). They are often composed of a high proportion of clays with low biological activity and extreme pH. Such areas do not overgrow by vegetation, which brings several secondary problems (Frouz et al., 2005).
This study aimed to explore the use of microalgae for the revitalization of phytotoxic soils in the Lítov Dump by evaluating their growth and impact on soil quality.

2. Methods

2.1. Laboratory tests - strain selection

Microalgal strains of genus *Nostoc*, *Desmonostoc*, *Anabaena*, *Trichormus* and *Klebsormidium*, isolated from biocrusts from the Lítov Dump, or coming from algae collections were cultivated in liquid media and tested for the growth on soils. Photosynthetic activity was determined by FluorCam as quantum yield (QY=Fv/Fm), viability by microscopic techniques, biomass growth gravimetrically as dry matter (DM) at 105 °C. Growth conditions (temperature 25-27°C, light intensity 10-25 $\mu mol/m^2/s$, pH3-5, media 1/2ZBB, BG11, BBM, 1/2SŠ) were tested and optimized.

2.2. Soil analyses

Soil samples collected from the Lítov Dump and used later for greenhouse experiments were analyzed. Organic matter (OM) content was determined based on loss on ignition at 450 °C for 5 h. Dissolved organic C (DOC), dissolved N (DN), and dissolved P (DP) were extracted in deionized water and analyzed in leachates using a TOC-LCPH/CPN analyzer (DOC and DN) and spectrophotometry (DP). pH and conductivity were assessed using a glass electrode. To determine the microbial respiration, a 10-g quantity of the fresh sample was placed in 100-mL glass vessels; the lid on each vessel was tightly closed for 15 h before a syringe was used to withdraw a 10-mL gas sample, which was stored in a 3-mL evacuated vial. The gas samples were analyzed with a gas chromatograph. Microbial biomass C and N (Cmic and Nmic) were extracted using the fumigation-extraction method and measured using a TOC analyzer.

2.3. Greenhouse growth experiments

The experiments in the greenhouse were carried out in plastic dishes filled with soil substrate (Fig. 1). Algal strains *Nostoc* 123, *Desmonostoc* 125 and *Trichormus*, selected and cultivated during laboratory experiments were applied in form of suspension, with jute fabric, and with expanded clay. The greenhouse was equipped with dataloggers for monitoring temperature, light, humidity. Growth of the biocrusts was evaluated visually. The physiological state and photosynthetic activity of the biocrusts were monitored by measurement of variable chlorophyll fluorescence using handheld fluorometers.

3. Results

Soil samples collected from the Lítov Dump were analyzed. A large variability was seen in OM, Cmic and Nmic content and content of available nutrients, mainly DOC and DN. Soil pH was low as expected (lower than 3). Microbial respiration and biomass were lower than in uncontaminated soils, but not as low as could be expected for toxic substrates. Content of available nutrients, especially that of DN and DP, was very low.

All the strains used in the greenhouse experiments *Nostoc* 123, *Desmonostoc* 125 and *Trichormus* were able to grow on the phytotoxic soil. The most successful was *Trichormus* applied with expanded clay.

Figure 1. Greenhouse growth experiments with microalgae on phytotoxic soils.

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