MICROSCOPIC INVESTIGATIONS CONCERNING *IN SITU* COPPER OXALATE FORMATION

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Introduction

White and brown rot basidiomycetes are important organisms for biodegradation of wood. A main distinguishing feature of brown rot fungi is their ability to produce organic acids like oxalic acid. The reaction between copper and oxalic acid can result in a detoxification of copper containing wood preservatives due to the formation of insoluble copper oxalate. The aim of investigation is to determine *in situ* Cu oxalate formation in order to localise the exact place of precipitation by means of scanning electron microscopy (SEM) analysis.

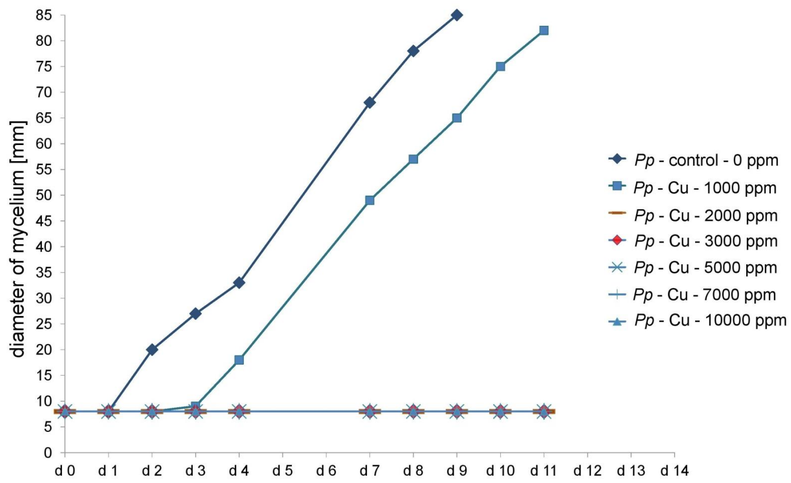

Figure 1: Growth dynamics of *Poria placenta* (*Pp*) treated with different concentrations of copper sulphate ($CuSO_4$ * 5 H_2O) on malt extract agar

Measurements

The growth dynamics provide a minimal inhibitory concentration (MIC) of 1000 - 2000 ppm copper after 7 days of growth (Figure 1).
The weight loss of samples treated with copper sulphate in comparison to untreated controls is reduced. As expected samples with a lower copper content show a higher weight loss. Figure 2 illustrates that an increasing sample volume results in a lower weight loss due to a better volume-surface-ratio.

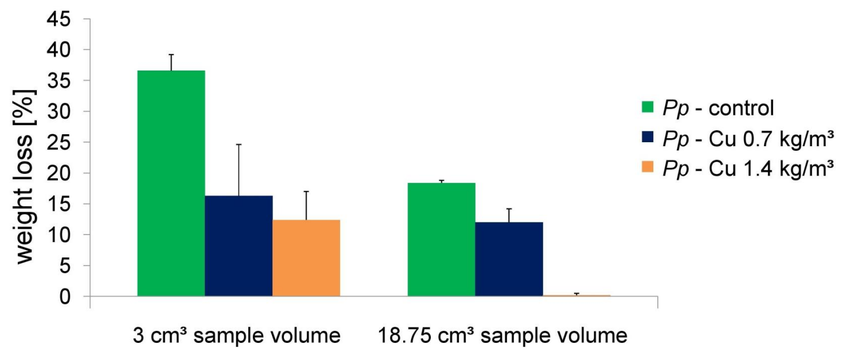

Figure 2: Weight loss after six weeks from different volume samples of *Pinus sylvestris* L. treated with two different concentrations of copper sulphate ($CuSO_4$ * 5 H_2O) [1]
Pp - *Poria placenta*; n = 4

FESEM – Field Emission Scanning Electron Microscopy

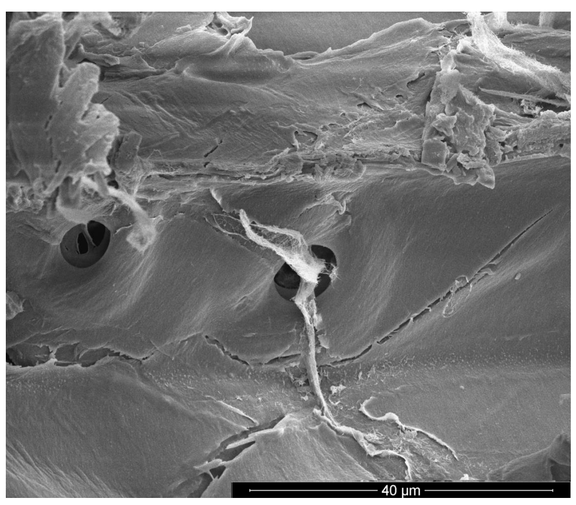

Figure 3: Untreated control - *Pinus sylvestris* L. with hyphae of *Poria placenta* growing through a pit of a tracheid.

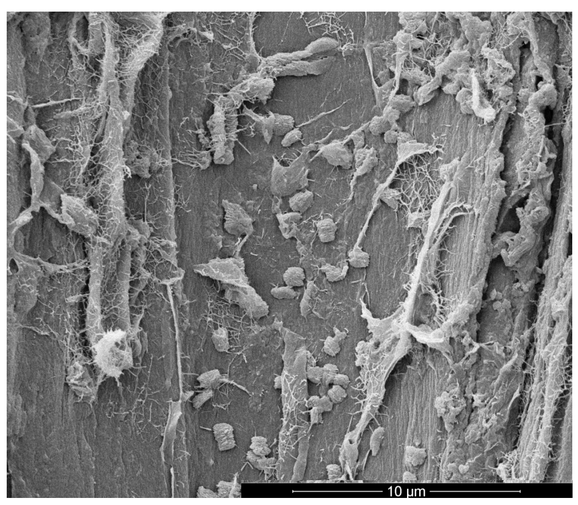

Figure 4: Surface of *Pinus sylvestris* L. Containing 0.7 kg/m³ of copper. Copper oxalate crystals of different sizes were found.

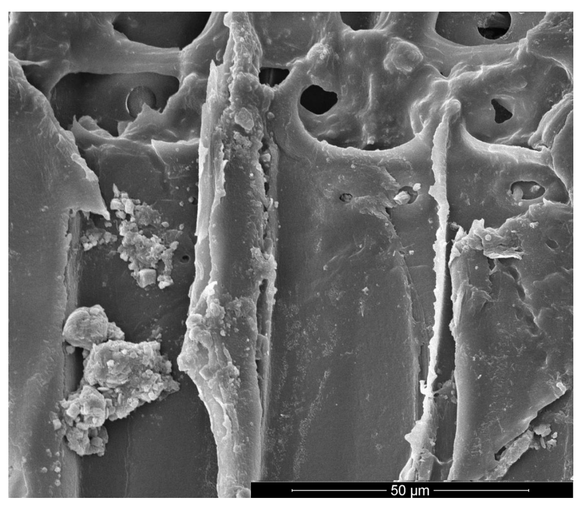

Figure 5: Surface of *Pinus sylvestris* L. containing 1.4 kg/m³ of copper. Fewer copper oxalate crystals are found.

In untreated control samples hyphae of *Poria placenta* could be observed, but no copper oxalate crystals were found (Figure 3). On the surface of samples treated with two different concentrations of copper sulphate, precipitation of copper containing crystals was observed. Copper oxalate crystals were found in wood rays as well as in tracheids (Figure 4 and 5). These statements are supported by results obtained by means of light microscopy and energy-dispersive X-ray analysis with images of element distribution (not shown here).

Summary

1.) First results of growth dynamics and weight loss confirm that *Poria placenta* is characterised by a certain copper tolerance.

2.) Imaging processes like SEM show copper oxalate formation in wood rays and tracheids in samples treated with copper sulphate.

3.) Copper oxalate formation is reduced with a higher concentration of Cu in wood preservative.

Perspectives

1.) Additional investigations concerning different types of copper containing wood preservative.

2.) A more detailed focus whether initial copper oxalate formation activities take place in the hyphae.

References:
[1] EN 113: 1996. Wood preservatives - Method of test for determining the protective effectiveness against wood destroying basidiomycetes - Determination of the toxic values