COPPER ACCUMULATION IN MICROFUNGI FROM A MINE SITE

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Heavy metal pollution exerts a selective pressure on soil communities, in which a population shift from bacteria to fungi has been observed (Gadd, 2007). In turn, metal species and concentration influence the fungal biota: for instance, in copper contaminated soils, the genus *Penicillium* seems to prevail over other genera (Gadd, 2007).

Our research was carried out in the abandoned Libiola copper and iron sulphide mine (Eastern Liguria, NW Italy) where soils are acidic and enriched of several heavy metals, namely copper, because of acid mine drainage processes (Marescotti *et al.*, 2008).

Soil samples were collected over several seasons from three sites within the study area, characterized by different vegetation types (bare ground; herbaceous and shrub cover; arboreal vegetation). Diluted suspensions of soil samples were plated according to Gams' dilution plate technique (1987) on Rose Bengal Agar and MEA. Identification of fungal colonies was performed according to classical mycology methods (observation of macro- and micromorphological characters and trophic/physiological requirements) for most species, while for some *Aspergillus* spp. identification through sequentiation of β-tubulin and calmodulin genes was necessary.

Three strains (*Trichoderma harzianum* Rifai LB8, *Aspergillus alliaceus* Thom et Church LB33, *Clonostachys rosea* (Link) Schroers, Samuels, Seifert et W. Gams LB38) were then tested for copper resistance and accumulation. 1µl of conidial suspension at $8 \cdot 10^5$ conidia/mm^3 was inoculated in triplicate in liquid MEA added with $CuSO_4$ $5H_2O$ in order to reach Cu concentrations up to 500 mg kg^{-1}. After 4-week incubation in shake flask cultures, mycelia were harvested by filtration, thouroughly washed with deionized water, dried and powedered for subsequent analysis by AAS-spectrophotometry.

36 fungal strains belonging to 25 different species were isolated in pure culture slants. Most belong to the genus *Penicillium* (*P. waksmanii* Zaleski*, P. minioluteum* Dierckx*, P. pseudostromaticum* Hodges, G.M. Warner & Rogerson etc.), in accordance with the high concentration of Cu in the soil, and subordinately to the genus *Aspergillus* (*A. alliaceus* Thom & Church*, A. carbonarius* (Bainier) Thom*, A. tubigensis* Mosseray). Other species occurring are *Rhizopus oryzae* Went & Prins. Geerl.*, Clonostachys rosea*, *Botrytis cinerea* Pers., of which the latter are well-known copper tolerant. The isolation of the uncommon teleomorph *Eurotium amstelodami* L. Mangin is of particular interest.

Both LB8 and LB38 were able to grow at at 200 mg kg^{-1} Cu, while *T. harzianum* was the only one growing at 400 mg kg^{-1} Cu. Both strains accumulated Cu at a concentrations 18000 - 24000 mg kg^{-1}, showing a remarkably high bioconcentration factor that hints at their possible use for mycoremediation purposes. These findings also suggest that tests of copper resistance and accumulation on other species found in the Libola mine could yield interesting results.

Gadd G.M., 2007. Geomycology: biogeochemical transformations of rock, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycological research 111: 3-49.

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Marescotti P, Carbone C, De Capitani L, Grieco G, Lucchetti G, Servida D., 2008. Mineralogical and geochemical characterisation of open pit tailing and waste rock dumps from the Libiola Fe-Cu sulphide mine (Eastern Liguria, Italy). Environ. Geol. 53:1613–1626.

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