

## MIMMICKING PYRITIC MINES CONDITIONS IN THE LABORATORY: $\text{Fe}/\text{SO}_4^{2-}$ CHRONIC EXPOSURE AND $\text{Cu}/\text{Zn}$ ACUTE TOXICITY TO FLUVIAL PERIPHYTON IN ACIDIC AND CONTROL CONDITIONS

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Several studies have highlighted the biofilm sensitivity to a large group of toxicants. Heavy metals are one of the most common inorganic pollutants in aquatic ecosystems. Most of the heavy metals in aquatic ecosystems (e.g. Cu, Mn, Fe, Zn) are essential micronutrients for algal growth. However, at high concentrations and at acidic pH these elements can be toxic to algae as well as to other aquatic organisms. In the present study, a system of indoor channels was colonized with fluvial biofilms to study the chronic effects of Fe,  $\text{SO}_4^{2-}$  and acidity and the contribution of chronic exposure to community tolerance to toxic metals by short-term Cu and Zn toxicity experiments.

Biofilms were subjected to four different treatments during 8 weeks: treatment 1: 1 mg Fe/L + 700 mg  $\text{SO}_4^{2-}$ /L at pH of 8.1-8.3; treatment 2: 0.01 mg Fe/L + 300 mg  $\text{SO}_4^{2-}$ /L at pH of 8.1-8.3; treatment 3: 0.01 mg Fe/L + 300 mg  $\text{SO}_4^{2-}$ /L at pH of 3.6-3.9; treatment 4: 1mg Fe/L + 700 mg  $\text{SO}_4^{2-}$ /L, at pH of 3.6-3.9. These treatments aimed to mimic the water conditions of the surrounding streams of Aljustrel mining area (Alentejo, Southwest of Portugal).

Short-term exposure of biofilms to Cu and Zn (24h exposure) took place after the indoor channel system experiment had finished, in vials containing the water of the respective treatment and increasing concentrations of Zn and Cu (100-8000  $\mu\text{g Zn/L}$  and 15-1200  $\mu\text{g Cu/L}$ ). Disturbances in the ecosystem would first lead to physiological and biochemical changes within the biofilm that can evolve in community changes if perturbations maintain. To evaluate these changes, the endpoints AFDW (Ash-Free-Dry-Weight), Chla (Chlorophyll a), diatom taxonomy, PhytoPAM (Pulse Amplitude Modulated) fluorometry parameters ( $F_0$ ,  $Y_{\text{max}}$ ,  $Y_{\text{eff}}$ ), enzymatic activities [catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR)] and non-enzymatic [GSH (total glutathione) and PC (total phytochelatins)] were analysed.

The main question was if pH and/or  $\text{Fe}+\text{SO}_4$  might ameliorate metal effects. Based on the results it seems that acidic pH affects CAT and GR and reduces metal toxicity in terms of low reduction in biomass ( $F_0$ ) and photosynthetic capacity ( $Y_{\text{max}}$ ) which may explain why acidic areas (as pyritic mines) with several metals in high concentrations have algal growth with typical acidic diatoms as *Pinnularia aljustrellica* found in high abundances. In addition, Fe is also affecting metal toxicity and GR. Based on  $Y_{\text{eff}}$  at the highest Cu/Zn concentration, higher inhibition is observed with Fe than without.