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Modification of soil microbial activity and several hydrolases in a forest soil artificially contaminated with copper

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Soils have long been exposed to the adverse effects of human activities, which negatively affect soil biological activity. As a result of their functions and ubiquitous presence microorganisms can serve as environmental indicators of soil pollution. Some features of soil microorganisms, such as the microbial biomass size, respiration rate, and enzyme activity are often used as bioindicators of the ecotoxicity of heavy metals. Although copper is essential for microorganisms, excessive concentrations have a negative influence on processes mediated by microorganisms. In this study we measured the response of some microbial indicators to Cu pollution in a forest soil, with the aim of evaluating their potential for predicting Cu contamination.

Samples of an Ah horizon from a forest soil under oakwood vegetation (*Quercus robur* L.) were contaminated in the laboratory with copper added at different doses (0, 120, 360, 1080 and 3240 mg kg^{-1}) as $CuCl_2{\cdot}2H_2O$. The soil samples were kept for 7 days at 25 ºC and at a moisture content corresponding to the water holding capacity, and thereafter were analysed for carbon and nitrogen mineralization capacity, microbial biomass C, seed germination and root elongation tests, and for urease, phosphomonoesterase, catalase and ß-glucosidase activities. In addition, carbon mineralization kinetics were studied, by plotting the log of residual C against incubation time, and the metabolic coefficient, qCO_2, was estimated.

Both organic carbon and nitrogen mineralization were lower in polluted samples, with the greatest decrease observed in the sample contaminated with 1080 mg kg^{-1}. In all samples carbon mineralization followed first order kinetics; the C mineralization constant was lower in contaminated than in uncontaminated samples and, in general, decreased with increasing doses of copper. Moreover, it appears that copper contamination not only reduced the N mineralization capacity, but also modified the N mineralization process, since in the contaminated samples all of the inorganic nitrogen was present as ammonium, probably because of inhibition of nitrification.

There was a marked decrease in biomass-C with addition of copper, and the decrease was more acute at intermediate doses (average decrease, 73%). Despite the decreases in microbial biomass and mineralized C, the value of qCO_2 increased after the addition of copper.

Urease activity was strongly affected by the presence of copper and the decrease was proportional to the dose; the activity at the highest dose was only 96% of that in the uncontaminated sample. Phosphomonoesterase activity was also affected by addition of copper; the reduction in activity was less than for urease and the greatest reduction was observed for the dose of 1080 mg kg^{-1} of copper. Catalase activity was affected by the contamination, but no clear trend was observed in relation to the dose of copper. ß-glucosidase was scarcely modified by the contamination but an increase in activity was observed at the highest dose of copper.

Seed germination was not affected by copper contamination, since it only showed a clear decrease for the sample contaminated with the highest dose of copper, while root elongation decreased sharply with doses higher than 120 mg kg^{-1} of copper. The combined germination-elongation index followed a similar pattern to that of root elongation.

For all investigated properties showing a reduction of more than 50%, the response to copper contamination was fitted to a sigmoidal dose-response model, in order to estimate the ED_{50} values. The ED_{50} values were calculated for microbial biomass, urease, root elongation and germination-elongation index, and similar values were

obtained, ranging from 340 to 405 mg kg^{-1} Cu. The ED_{50} values may therefore provide a good estimation of soil deterioration.