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Can heavy metal pollution induce bacterial tolerance to antibiotics in soils from ancient land-mines?

Qinmei Zhong, Carla Cruz Paredes, and Johannes Rousk

Lund University, Department of Biology, Lund, Sweden (qinmei.zhong@biol.lu.se)

Soil microbial communities play vital roles in the biogeochemical processes, and they are sensitive to environmental pressure induced by environmental pollutants, including heavy metal or antibiotic contaminants. It is well known that exposure to heavy metals can increase microbial tolerance in contaminated soil. Recently it was also discovered that heavy metal exposure in agricultural soils could induce microbial tolerance to antibiotics, thus yielding human health concerns. To date, it remains unknown how wide-spread this co-tolerance is in the environment. The aim of this study was to determine the microbial tolerance under different heavy metal concentration levels, and to investigate whether increasing tolerance to metals will co-select for tolerance to antibiotic. We hypothesized that microbial tolerance to both heavy metals and antibiotics would increase with metal pollutant concentrations. The tolerance to pollutants was determined by the pollution induced community tolerance (PICT) approach and the concentration for 50% inhibition (IC_{50}) values.

To address our hypothesis, we collected soil samples from an ancient open cast land-mine in North Wales, UK, called Parys Mountain, known as the 'Copper Kingdom', where the soils cover a very wide span concentrations (c. $50 \mu\text{g} - 4000 \mu\text{g g}^{-1}$ soil) of copper (Cu), lead (Pb) and zinc (Zn) alone or in combination. The soils were very acidic with pH range from 3.49 to 4.96, and soil organic matter contents very variable, from 5 to 46 %, yielding a wide range of water holding capacities, from 0.45 to $3.47 \text{ g water g}^{-1}$ dry soil. We determined basal soil respiration, SIR-biomass, microbial growth and community composition, and bacterial tolerance to Cu, Pb, Zn, tetracycline and vancomycin.

We found that bacterial growth rates significantly decreased with increasing available Cu ($R^2 = 0.26$) and decreasing pH ($R^2 = 0.39$), but did not show any regressions against with total metal concentrations, and total microbial biomass and respiration showed similar patterns. It was possible to reliably establish accurate dose-response relationships for bacterial tolerance to metals with average R^2 values of 0.96 for Cu, 0.93 for Pb, and 0.92 for Zn with logistic curve fits. Based on these, we estimated that bacterial tolerance to heavy metals varied substantially across the sites, with average $\log(IC_{50})$ value was c. 4 log-unit Cu, 3.4 log-unit Pb, and 3.8 log-unit Zn. Metal tolerance was weakly linked to soil metal concentrations, as shown by limited linear relationship built between tolerance and soil concentrations ($R^2 = 0.25, 0.44, 0.20$ for Cu, Pb and Zn, respectively). The substantial variance in heavy metal tolerance among the sampled mining soils

provided a high power to assess if metal tolerance could induce tolerance also to antibiotics. To assess this, we established dose response relationships between bacterial growth and a common and widely used antibiotic (tetracycline) as well as an antibiotic held in reserve for human therapy (vancomycin). The toxicity estimates are still awaiting analyses, but we hypothesize a strong link between bacterial tolerance to tetracycline and that for Cu, Zn, and Pb, while a weaker or non-existent pattern is expected for vancomycin, due to its limited environmental presence.