

## **Environmental availability of Copper in deeply sourced seeps and its influence on Copper binding protein distribution**

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Copper is incorporated as a metal cofactor in a number of copper metalloproteins involved in processes like electron transfer, substrate oxidation, monooxygenation, and denitrification. Besides its role as a metal centre in enzymes relevant for life and biogeochemical cycles, copper requires a series of processes to regulate its homeostasis, due to its toxicity at high concentrations. Copper environmental availability has changed through time, mostly influenced by the rise in oxygen after the Great Oxidation Event, which most likely influenced the biological use of this element. Today's oxic conditions are favourable for the occurrence of copper proteins, but still there are a number of physicochemical variables like pH, redox potential, and other water chemistry parameters, that can influence copper availability in the environment, and consequently affect metalloproteins distribution. At plate margins, geothermal activity associated with volcanism can result in the release of copper from rocks and minerals, as well as the generation of copper-rich fluids and melts. These fluids can migrate upward through the crust, leading to the formation of copper deposits, eventually reaching the surface through deeply-sourced seeps. The wide range of physicochemical conditions found in geothermal systems represent an ideal model to study how changes in copper bioavailability can influence copper-dependent metalloenzymes. In this study, we combine metagenomic data from more than 50 globally distributed deeply sourced seeps located in Italy, and in Central and South America, which range in temperature from 22.9 °C to 88.9 °C and in pH from 0.85 to 9.99, with geochemical and geophysical