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Nitrate-reducing Fe(II)-oxidizing microorganisms in the sediments of an extreme acid rock drainage affected river (Rio Tinto, Spain)

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Rio Tinto (Huelva, Spain) is an acid rock drainage-affected fluvial estuarine system transporting high loads of iron and heavy metals (HMs) to the Atlantic Ocean. Despite these extreme conditions, it has been shown that Fe(II)-oxidizing microorganisms are able to thrive in the water column [1] and in the river sediments [2]. However, it is not known if the Fe(II)-oxidizing microorganisms present in the anoxic sediments are able to use nitrate as electron acceptor and perform Fe(II) oxidation under extreme pH and salinity fluctuations. Therefore, the aim of this project is 1) to obtain mineralogical data and identify hydrogeochemical parameters of the sediments collected from the headwaters and upper estuary of the river to assess the availability of electron donors and acceptors required for nitrate-reducing Fe(II)-oxidizing (NRFeOx) microorganisms, 2) to integrate these geochemical results with the *in-situ* microbial diversity and relative abundance to identify which microniches are inhabited by NRFeOx microorganisms, 3) to quantify rates of nitrate reduction coupled to Fe(II) oxidation in microcosm experiments with freshly sampled sediment mimicking different *in-situ* conditions, and 4) to establish enrichment cultures to isolate and study the genomic potential of strains responsible for NRFeOx via metagenomic analysis. The outcome of this work will give new insights into the metabolic pathways and key genes involved in NRFeOx processes carried out by novel bacteria adapted to acidic and/or fluctuating geochemical conditions. Our work will furthermore reveal how the NRFeOx activity can influence heavy metal immobilization within the contaminated sediment by co-precipitation with biogenically produced Fe(III) (oxyhydr)oxides.

[1] Abramov et al. (2020) *Science of the Total Environment* 718, 137294.

[2] Abramov et al. (2021) *Applied and Environmental Microbiology*, AEM0229021