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## ACCEPTED MANUSCRIPT

# Speciation of Nickel and Zinc, its short-term inhibitory effect on anammox, and the associated microbial community composition

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#### Highlights

- The short-term effects of Ni and Zn on anammox biomass were determined
- Several inhibition models were tested to evaluate inhibition.
- The degree of inhibition was evaluated based on bioavailable fractions.
- Candidatus Kuenenia was the dominant anammox-related organism in the samples.

#### Abstract

This study provides insight into the short-term effects of nickel and zinc on anammox. The impacts of these heavy metals are evaluated based on their potentially bioavailable fractions, including the intracellular, surface-bound, soluble, free-ion, and weak (labile) complexes of heavy metals, in the presence of certain inorganic/organic species. Results showed that the IC50 values for soluble, intracellular, cell-associated, surface-bound, and free-ion Ni concentrations are 5.99, 0.250, 0.930, 0.680, and 1.36 mg/L, respectively. The inhibitory effect of Zn is found to be lower with respect to Ni, with IC50 values of 6.76, 11.9, 15.1, and 2.71 mg/L for the soluble, intracellular, cell-associated, and free-ion Zn concentrations, respectively. This is the first detailed evaluation of anammox inhibition based on the fractionation of heavy metals. Metagenomic analysis reveals that *Candidatus Kuenenia* constitute approximately 89% of the entire Planctomycetes population, whereas *Candidatus Brocaida* are detected in relatively low fractions (3%).

Keywords: Anammox, nickel, zinc, inhibition, next generation sequencing

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