

**DETERMINATION OF THE MINIMUM INHIBITORY  
CONCENTRATION OF Fe<sup>3+</sup>, Cu<sup>2+</sup> AND Zn<sup>2+</sup> IN *PSEUDOMONAS  
FLUORESCENS***

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High concentrations of metals present in the environment, due to human activities or to natural occurrence, create selective pressure for microorganisms that have developed several resistance mechanisms. Regulation of the pyoverdine synthesis in *Pseudomonas fluorescens* is a good example of a biochemical behavior change due to the alteration of an environmental parameter. Pyoverdine is the main siderophore secreted under iron-limiting conditions by fluorescent *Pseudomonads* for Fe<sup>3+</sup> uptake. This low-molecular-weight molecule also chelates other metals with lower affinity. To study the interactions between different metals and bacteria, the first step is to determine their minimum inhibitory concentration (MIC).

*P. fluorescens* DSMZ50090, grown in DSM1 broth (peptone 5.0 g/L, meat extract 3.0 g/L) at 20°C, was spread on DSM1 agar plates. Sterilized filter paper disks (diameter 2.5mm) were put on the middle of the plate and soaked with 160 µl of solution of metals (CuSO<sub>4</sub>, ZnSO<sub>4</sub>, FeSO<sub>4</sub>, FeCl<sub>3</sub> 0.25-1.5M). The plates were then incubated at 20°C for 24 hours, and the zone of clearance surrounding the disk was measured.

The area of the inhibition halo allowed us to calculate the values of MICs for CuSO<sub>4</sub>, ZnSO<sub>4</sub>, FeCl<sub>3</sub>, Fe<sub>2</sub>[SO<sub>4</sub>]<sub>3</sub>, that are 46.30, 54.40, 65.35, 74.11 mM respectively. The higher the concentration of the metals on the disk, the larger the inhibition halo. Regarding Fe<sup>3+</sup>, a counter ion effect has been observed for Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup>.