BioRefine Project: Detection of bioavailability of Metallic Trace Elements in soils by the use of microbial biosensors

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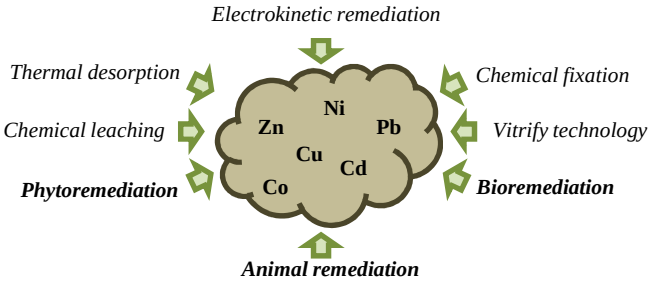
Introduction

Zinc, lead and cadmium are the main Metallic Trace Elements (MTEs) found in soils contaminated by the mining industry in Europe. MTEs are spread in the environment because of the disruption of biogeochemical cycles caused by human activities. Due to their low mobility and biodegradability, they accumulate in soils where they are strongly bound to particles. It has become necessary to understand interactions between MTEs and the environment and to implement remediation actions. This work is focused on remediation monitoring techniques by using whole cell microbial biosensors able to detect zinc, lead and cadmium. Biosensors provide a signal in response to the bio-available concentration in MTEs, which are valuable for the design of efficient techniques involving bioremediation. Whole cell biosensors used in this work are based on *Escherichia coli* strains carrying a fluorescent reporter system. The reporter element contains a promoter sensitive to MTEs and a gene coding for the Green Fluorescent Protein (GFP). MTEs activate the synthesis of GFP, which is a very stable protein, causing the accumulation of GFP inside the cells. Then, fluorescence can be measured by flow cytometry. In this study, two biosensors were investigated: *E. coli* pP_{zraP}gfp and *E. coli* pP_{zntA}gfp. The last strain provided a linear response to zinc up to 20 mg/l and a curvilinear response to cadmium up to 0.15 mg/l. No detection was highlighted regarding lead. In practical cases, soils and wastes are contaminated by several types of MTEs. Consequently, combined contaminations were also tested. This work allowed highlighting that the strain *E. coli* pP_{zntA}gfp can be used to assess the bioavailability of cadmium in soils, although the experimental procedure must be improved. This work is supported by the **BioRefine Project,** a European project in which various member states focus on recovery of inorganics from organic wastestreams. We gratefully acknowledge the **INTERREG IVB NWE** programme, which financed the **BioRefine Project** (ref. 320J-BIOREFINE).

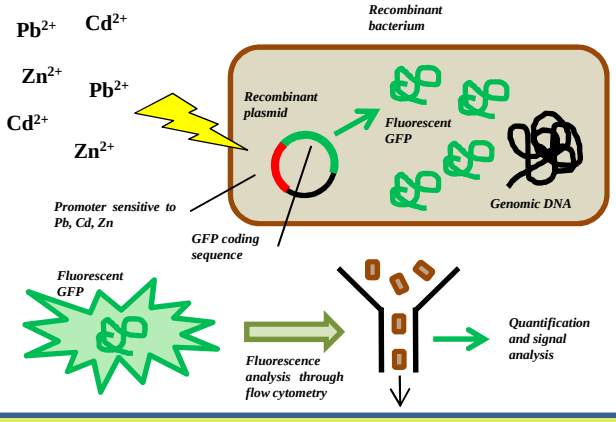
Soils contaminated with MTEs can be treated by various methods



Some of those techniques require a follow-up

⚠ **FOCUS ON BIOSENSORS** ⚠

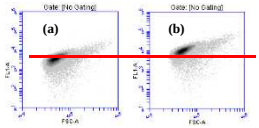
Some GFP-based biosensors can detect heavy metals quantitatively



Methodology of the work

1) Check of the biosensors *E. coli* pP_{zraP}gfp and *E. coli* pP_{zntA}gfp in LB medium (microplate assays)

→ Assay with Zn at 0,65 mg/l (a)
→ Assay with Zn at 10,65 mg/l (b)

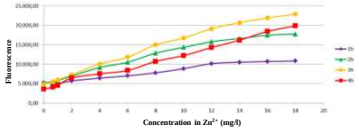


→ **Good sensitivity to Zn for *E. coli* pP_{zntA}gfp**

→ **BUT no results for *E. coli* pP_{zraP}gfp**

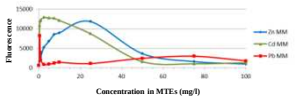
2) Check of the incubation time and linearity (*E. coli* pP_{zntA}gfp)

→ Linear signal from 0 to 20 mg/l in Zn^{2+} (LB medium)
→ Maximal fluorescence after 3h



3) Check of the effect of Pb^{2+} and Cd^{2+}

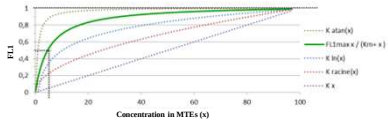
→ Linearity of the signal according to Zn^{2+}, Pb^{2+} and Cd^{2+} (a)
→ Linear signal from 0 to 2,5 mg/l for Cd^{2+}, and very low signal for Pb^{2+}



4) Linearization of Lineweaver-Burk

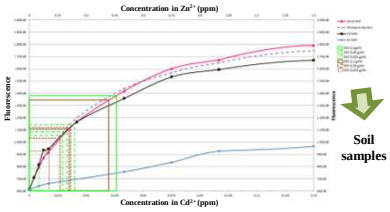
→ $FL1_{max}$ is the maximal induction
→ K_m is the affinity

$$FL1\,[MTE] = FL1_0 + (FL1_{max} - FL1_0)\,\frac{[MTE]}{K_m + [MTE]}$$



5) Assays on polluted soils

→ Soil samples must be diluted in liquid
→ The ratio Zn^{2+}/Cd^{2+} must be close to 10
→ For this ratio, only Cd^{2+} is detected
→ *E. coli* pP_{zntA}gfp can detect concentrations in Cd^{2+} close to 0,2 mg/kg
→ Bioavailability can be assessed on the basis of the equation of Michaelis-Menten



→ **Soil samples**

Acknowledgements

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