

Introduction

- The high porosity and large surface area of biochar make it suitable for adsorption of Heavy metals (HMs). This condition reduces the bioavailability of HMs to soil microorganisms thus potentially enhancing the remediation of contaminated soil.
- To assess the biochar performance as a soil amendment we develop bacteria based biosensors to measure metal bioavailability and toxicity at a cellular level.
- Development of Foster Resonance Energy Transfer (FRET) sensor inside the soil bacteria is a novel approach for measuring the metal bioavailability in soil. This can be used as a complementary tool to chemical analysis to monitor the level of metal bioavailable concentration during soil remediation.

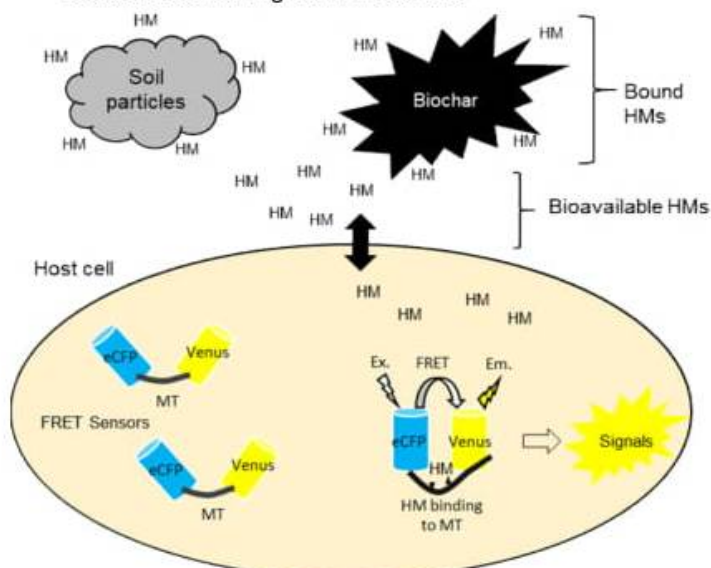


Fig 1. Overview of heavy metals interaction with soil, biochar, and bacteria biosensor.

Objectives

- To develop FRET biosensors that exploit the metal binding protein (Metallothionein/MT) for Cd, Zn, and Pb
- Application of biosensors to monitor the changes of HMs bioavailability at biochar-amended contaminated soil.

Methods

Determination of biosensor properties

Lab Test
Construction of FRET sensor inside the soil bacteria (host cells)

Assessment of Biochar Performance
(Integrate the biosensor analysis with soil microbial and plant growth)

Field Test
Biosensor application in biochar-amended contaminated soil

Current Results

Plasmid construct

Metallothionein (MT) gene was inserted between genes encoding eCFP and Venus fluorescent proteins

Protein expression

Initial expression inside *E.coli* for in vivo and in vitro characterisation of FRET protein sensors

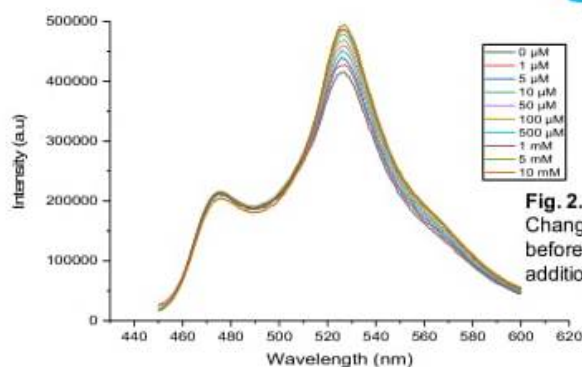
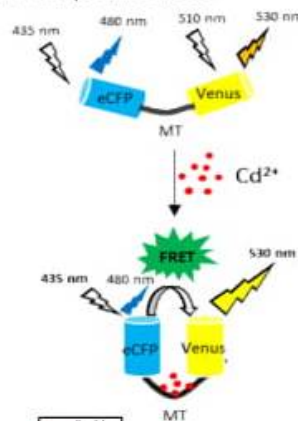
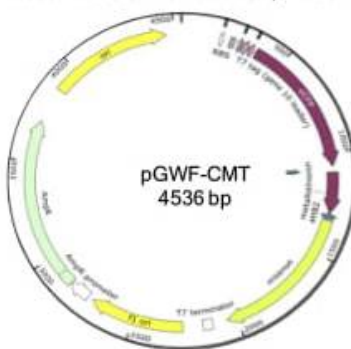


Fig 2. Changes of emission signal before and after Cadmium addition (λ_{ex} : 435 nm)

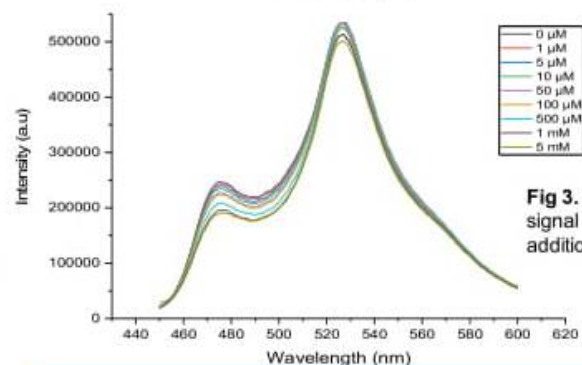


Fig 3. Changes of emission signal before and after Zinc addition (λ_{ex} : 435 nm)

Conclusion and Future Works

- FRET sensors have been successfully constructed and characterised.
- In vitro characterisation showed some responses to Cd and Zn. The emission signals increase in correspond to the metal concentrations range (min: 1μM, max: 5mM). This allow for a rapid quantification of intracellular metal concentrations.

Future works:

- FRET sensor characterization in vivo (expression inside soil bacteria e.g *Pseudomonas putida*)
- Application in contaminated soil remediation to monitor the changes of bioavailable heavy metals

References

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- Hartley, I. L., Temple, G. F., and Brasch, M. A. (2000). DNA Cloning Using in-vitro Site Specific Recombination. *Genome Research*, 10; 1789-1795
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