

Nanoremediation: evaluation of effectiveness for heavy metal removal and ecotoxicological impact



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Introduction

In the last decades there has been a demand, by society, for healthy environments, and therefore free of contaminants. In particular, the soil / water system is an objective of maximum interest. Regarding this issue, new decontamination strategies such as bioremediation, phytoremediation and nanoremediation have appeared. The zero valent Fe (NZVI) nanoparticles have been used for the last decade for the remediation of polluted soil, groundwater, and wastewater, targeting a wide variety of common environmental contaminants, both organic and inorganic compounds.

Aim

We addressed the efficiency of the nanoremediation strategy in case of co-mingled HM pollution in a highly damaged soil environment. The objectives included monitoring the stability of the strategy considered in the long-term, as well as its impact on different soil organisms.



Sequencing

Bioinformatic

Analysis

Illumina MiSeq

Methods

Experimental set-up Lufa 2.2 soil was contaminated with Pb (2700 mg kg-1), Zn (4200 mg kg-1) and Cd (310 mg kg-1). nZVI suspension (Nanofer 25S) was added at a dose of 5% nZVI w/w. Samples were incubated for 120 days (21°C) and subsamples were collected from control and nZVI-treated samples at 0 (T1), 7 (T2), 15 (T3), 30 (T4) and 120 (T5) days post incubation.

Toxicity assays Microtox® Test

The Microtox[®] Basic Test was used to determine the toxicity impact

index (TII50) at each sampling time.

Metagenome library construction, sequencing and predictive metagenome Analysis

Total bacterial community DNA was extracted and sequenced using Illumina MiSeq technology and the CLC Microbial Genomics Module (QIAGEN) software. The PICRUSt tool was used to predict the metagenome functional content based on the 165 rRNA amplicon data sets

Results



Library

Construction

Quality Control Metrics

DNA/RNA

Samples

Results

- The most efficient immobilization was recorded 15 days post-treatment (T3), reflected in the decrease of HM toxicity towards *V. fischeri*
- After 120 days, nZVI showed an effective immobilization capacity for Pb (20%), partially effective for Zn removal (8%) and negligibly effective for Cd removal
- The overall abundance of the microbial community was

similar in both sets of samples, although an increase in the number of metabolically active bacteria was recorded at T3

- At T3: Firmicutes selectively displaced other bacterial phyla in parallel with a decrease in Gram-negative bacteria
- After T3: Predictive metagenomic analysis using PICRUSt showed differences among the predicted metagenomes of nZVI-treated soil and control soil



Table 1. Ratio of the relative abundance of the major predicted traits in the metagenomes of the nZVI-treated and untreated samples at T1, T3 and T5.

OUT ID	KEGG Description nZVI/contr			
		T1	Т3	T5
KO 2014	iron complex outer membrane receptor protein	-	0.12	0.22
KO 0799	glutathione S-transferase [EC:2.5.1.18]	-	0.12	0.20
KO 1768	adenylate cyclase [EC:4.6.1.1]	-	-	0.16
KO 3091	RNA polymerase sporulation-specific sigma factor	-	1.75	2.16
KO 0676	ribosomal-protein-alanine N-acetyltransferase [EC:2.3.1.128]	-	1.76	1.96
KO 6295	spore germination protein KA	-	1.77	-



KO 1449	N-acetylmuramoyl-L-alanine amidase [EC:3.5.1.28]	-	1.79	2.06	Biocynthesis of Secondary Metabolitus	13 7%		
KO 6407	stage V sporulation protein AE	-	1.81	2.30		9,6%	87,9% 4,5%	
KO 1534	Cd2+/Zn2+-exporting ATPase [EC:3.6.3.3 3.6.3.5]	-	-	1.75	65,3%	3,0% 2,5% 1,9%		
KO 3310	alanine or glycine: cation symporter, AGCS family	-	-	2.38				
Figure 4. Percentage of OTUs in the control (A) and nZVI-treated s microbiomes (B) classified at the phylum level								

Conclusions

> This study highlight the effectiveness of nanoremediation in multiple-metal contaminated soil (mainly for Pb) in the short term.

> The apparent lack of recovery of biodiversity after application of nZVI and the reversible nature of nanoremediation must be carefully considered to validate this technology when assurance of medium- to long-term immobilization of HMs is required





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