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## Heavy metal availability and their relationships with soil microbial characteristics in agricultural carbonated soils

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### ► To cite this version:

Ana de Santiago Martin, Nathalie Cheviron, Jose R. Quintana, Concepción González Huecas, Antonio López Lafuente, et al.. Heavy metal availability and their relationships with soil microbial characteristics in agricultural carbonated soils. 6th International Symposium of Interactions of Soil Minerals with Organic Components and Microorganisms (ISMOM) Soil Interfaces in a Changing World, Jun 2011, Montpellier, France. 2011. hal-02746600

**HAL Id: hal-02746600**

**<https://hal.inrae.fr/hal-02746600>**

Submitted on 15 Mar 2023

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## INTRODUCTION

Soil contamination by heavy metals (HMs) can have negative long-term toxic effects on ecosystems affecting key microbial processes and decreasing the number and activity of soil microorganisms.

Soil enzyme activities are recognized as a highly sensitive bio-indicator to HMs and the methodology for their determination is rapid, simple and inexpensive. However, natural variability within and between soils could be a decisive impediment to use simple parameters such as single enzyme activities, suggesting the use of the combination with other biochemical properties such as organic C, total N, soil microbial biomass or total DNA load.

The aim of this study was to: i) assess the impact of heavy metals on soil enzyme activities (arylsulfatase, alkaline phosphatase,  $\beta$ -galactosidase, urease and dehydrogenase) and soil DNA loads (total, bacterial and fungal DNA) and ii) evaluate the microbiological parameters measured as HMs bio-indicators and their relationships with several HM availability indexes.



## MATERIAL AND METHODS

### Soil sampling and contaminant experiment

- 10 unpolluted agricultural carbonated soils were collected down to 30 cm depth in Madrid (Spain).
- Soils were spiked with Cd, Cu, Zn and Pb at two contamination levels, corresponding to current EU legislative limits for HMs in agricultural soils - T1- and semi-maximum concentrations for sewage sludge -T2- (Table), and after soils were subjected to wetting-drying cycles for 365 contact days. [Soil samples without addition of HMs were employed as a control = T0]

mg Kg <sup>-1</sup>	Cd	Cu	Zn	Pb
T1	3	140	300	300
T2	20	875	2000	600

### Microbiological analyses

- Soil enzyme activities: dehydrogenase (DEH), arylsulfatase (ARYS),  $\beta$ -galactosidase (BGAL), urease (URE) and alkaline phosphatase (ALP) activities using standardized methods developed by the Unité PESSAC (INRA Versailles-Grignon).
- Soil DNA loads:
  - DNA extraction: by Fast DNA kit (MP Biomedical).
  - DNA quantification:
    - Total DNA load (tDNA): absorbance at 260 nm.
    - Fungal and bacterial DNA loads (fDNA and bDNA): qPCR.

### Physicochemical analyses

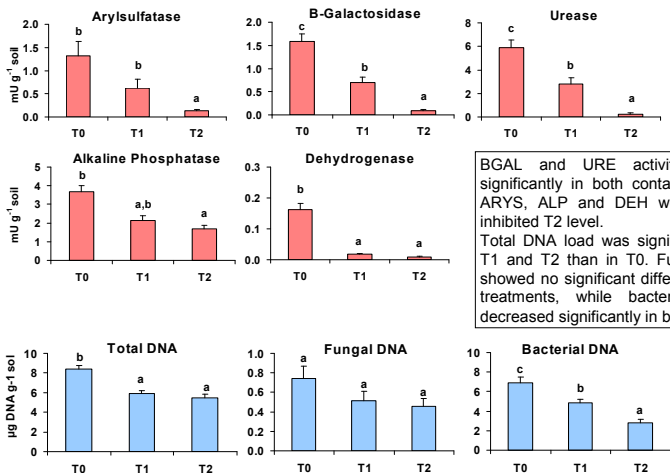
- Soil properties related with availability of heavy metals were measured such as pH, particle size, CEC, equivalent CaCO<sub>3</sub>, Fe-Mn oxides, available P, total N and several parameters related with the organic matter.

- Availability indexes: 0.1 M NaNO<sub>3</sub>, 10 mM LMWOA and 0.005 M DTPA.



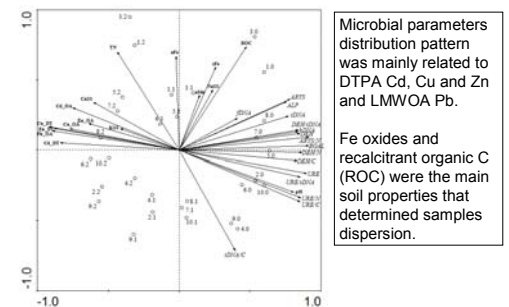
## RESULTS AND CONCLUSIONS

### Effects of HMs on microbiological parameters

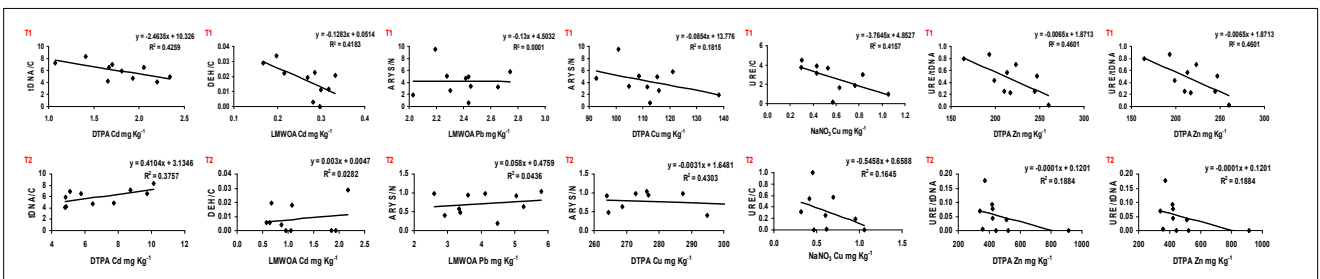


BGAL and URE activities decreased significantly in both contamination levels. ARYS, ALP and DEH were significantly inhibited T2 level. Total DNA load was significantly lower in T1 and T2 than in T0. Fungal DNA load showed no significant differences between treatments, while bacterial DNA load decreased significantly in both T1 and T2.

### Relationship between microbial parameters, availability indexes and soil properties (RDA analysis)



### Relationship between some selected microbiological ratios and availability indexes



The Pearson's correlation analysis showed no clear effects of contaminants on DNA loads and enzyme activities. In contrast, simple biological ratios were significant correlated with extractable metals.