

# Development of a new plant-based biotest to assess trace element phytoavailability in contaminated soils: Selection of target-plant species for standardisation

L. Lemal<sup>1,2</sup>, M. N. Bravin<sup>2</sup>, P. Letourmy<sup>3</sup>, P. Hinsinger<sup>1</sup> and E. Doelsch<sup>2</sup>

<sup>1</sup> INRA, UMR 1222 Eco&Sols (INRA-IRD-SupAgro), Place Viala, F-34060 Montpellier, France

<sup>2</sup> CIRAD, UPR Recyclage et risque, F-34398 Montpellier, France

<sup>3</sup> CIRAD, UPR Systèmes de culture annuels, F-34398 Montpellier, France

E-mail contact: [laure.lemal@cirad.fr](mailto:laure.lemal@cirad.fr)

## 1. Introduction

While the concept of contaminant bioavailability in soil has been recently defined in the international standard ISO/DIS 17402 [1], its application at an operational level still requires the identification, the development and the standardisation of a set of tools targeted to various organisms (e.g. plants, soil micro-organisms and fauna, Human beings...). This is especially a concern for the assessment of trace element (TE) phytoavailability in contaminated soils as TE remain a major contaminant in European soils and higher plants are of primary interest for their role in human food and animal feed.

Phytoavailability of TE can be estimated with either chemical or biological methods. While chemical methods are usually the cheapest, are easy to perform and some of them are already standardised at an international level, chemical methods strictly measure TE availability in soils and thus have to be correlated with biological measurements before to be used as phytoavailability indicators. In addition, chemical methods are per se not designed to address the diversity of responses observed among different plant species or cultivars. Alternatively, a few biological methods are already standardised at an international level. However, biological methods were mainly designed to assess TE phytotoxicity while bioaccumulation remains a sound issue for a range of TE. The determination of TE accumulation in shoots is usually not sufficiently sensitive to assess TE phytoavailability compared to the amount accumulated in the whole plant (roots included). Moreover, the biological methods which are based on soil-grown plants require a tedious washing procedure to reliably measure TE accumulated in the roots. Thus, there is still a need to develop biological methods in order to properly assess TE phytoavailability, particularly in term of bioaccumulation.

Accordingly, the ongoing NormaRHIZO research project was designed to provide a strong scientific input in the development of a new plant-based biotest, the RHIZOtest, in the scope of standardisation [2]. The RHIZOtest is notably based on a complete physical separation between plant and soil compartments enabling an easy, fast and clean recovery of the roots. The present abstract introduces the first step of the project focused on the selection of the target-plant species suggested for the standardisation of the RHIZOtest.

## 2. Materials and methods

Three agricultural soils exhibiting a fairly large range of pH and a high concentration in several trace elements (TE) (Table 1) were selected for this experiment. Concentrations of TE in soils were due to anthropogenic activities for soils 1 and 3, while high concentrations in soil 2 were attributed to the natural pedogeochemical background.

	pH <sub>CaCl2</sub>	Total Cd	Total Pb	Total Zn	Origin of trace element concentration
		mg kg <sup>-1</sup>			
Soil 1	5.3	4.5	141	563	Steel industry
Soil 2	5.9	5.6	172	1408	Pedogeochemical background
Soil 3	7.1	1.7	163	432	Waste water spreading for decades

Table 1: Soil characteristics (TE, trace elements)

Ten plant species commonly cropped over the world and likely exhibiting distinct behaviours of TE uptake were tested: alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), bread wheat (*Triticum aestivum*), cabbage (*Brassica oleracea*), fescue (*Festuca arundinacea*), lettuce (*Lactuca sativa*), rape (*Brassica napus*), ryegrass (*Lolium perenne*), sorghum (*Sorghum bicolor*), tomato (*Lycopersicon esculentum*).

The RHIZOtest was deployed for each plant species and soil according to Bravin et al. [2]. Briefly, plants were first grown (“preculture period”) from seeds for two weeks in hydroponics in a cylinder closed at the bottom with 30- $\mu\text{m}$  polyamide mesh to favour the development of a planar mat of roots. Plants were then firmly pressed for 8 days on the top of a thin soil layer (“test culture period”).

Plants were harvested at the end of test culture period, digested and TE concentration (arsenic, cadmium, copper, lead and zinc) were determined in plant shoots and roots. Phytoavailability of each TE was then calculated as the flux of TE to the plants during the test culture period, according to Bravin et al. [2].

### 3. Results and discussion

Excepted alfalfa, cabbage and wheat, plant species exhibited an adequate and homogeneous growth for both roots and shoots for the three soils. This means that for seven out of ten plant species the RHIZOtest enables a fair and unbiased assessment of trace element (TE) phytoavailability between soils.

As expected, plant uptake flux of TE significantly varied among the ten plant species tested. For example, Pb and Zn uptake flux varied respectively by 3- and 4-fold among the ten plant species cropped on the soil 3 (Figure 1). This confirms the need to account for a broad range of diversity in the ability of plant species to uptake TE. However, TE phytoavailability also broadly varied among TE (Figure 1) and soils.

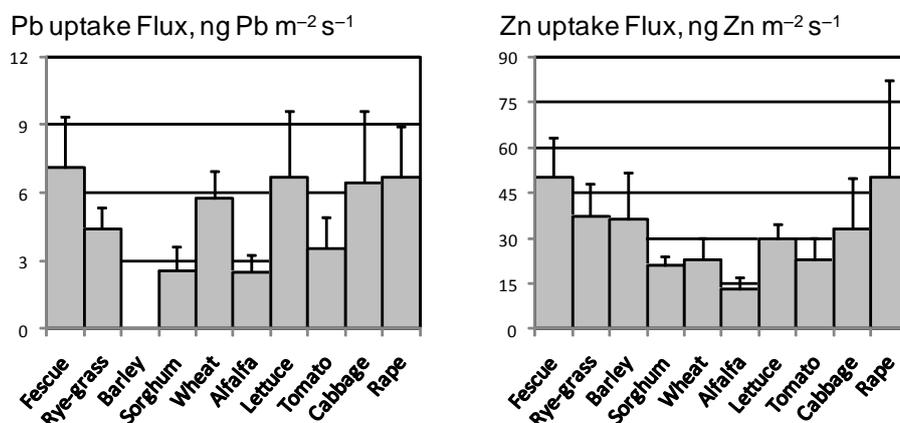


Figure 1: Lead and zinc uptake flux of in the ten plant species cropped on the soil 3 (see Table 1).

In order to classify the ability of the ten plant species to assess a rather low or high TE phytoavailability, TE uptake flux measured for each combination (plant species/TE/soil) was transformed in semi-quantitative variable by ordination and scoring. Scores were added to give a global classification of plant species. According to the precautionary-like principle, we selected the three plant species (barley, rape and fescue) from which we estimated the highest TE phytoavailability among the three soils and the five TE investigated.

### 4. Conclusions

This study supports the requirement of biological methods that enable to encompass the biological diversity in the assessment of trace element (TE) phytoavailability that chemical methods are not able to take into account. Further development of the RHIZOtest will be to validate the scope of the method in term of physical-chemical properties and level of contamination of soils for the three selected species. Such kind of validation procedure for a biotest is the unique opportunity for achieving operational methods based on a hard scientific background that could be standardised for the assessment of TE phytoavailability.

### 5. References

- [1] ISO/FDIS 17402. 2008. Soil quality – Guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soils and soil materials. Geneva, Switzerland. 35 p.
- [2] Bravin MN, Michaud AM, Larabi B, Hinsinger P. 2010. RHIZOtest: A plant-based biotest to account for rhizosphere processes when assessing copper bioavailability. *Environ Pollut* 158: 3330-3337.

*Acknowledgement* - The authors thank L. Boulonne and C. Jolivet (INRA INFOSOL) and F. van Oort (INRA PESSAC) for their help in the selection, sampling and preparation of soils. We also thank Remy Feydier for ICP-MS analysis. D. Arnal, N. Balsera, P. Cazevielle, C. Chevassus-Rosset and C. Guilleré are acknowledged for technical assistance. The French National Agency is acknowledged for funding within the frame of the programme « Contaminants Ecosystèmes Santé » (CES) 2009.