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How do microorganisms influence trace element uptake by plants? Screening in an agar model rhizosphere.

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Trace elements (TE) are essential for humans and plants, but they may be toxic if their concentration is too high. For this reason, the management of TE in soils is very important. In some cases it may be necessary to increase the uptake of nutrients or TE by plants, for example in a biofortification perspective. Conversely, in some other cases TE uptake by plants should be decreased, for instance to avoid heavy metals entering the food chain via edible crops.
Microorganisms living in the rhizosphere affect trace element (TE) uptake by plants. However, due to the complexity of this space and the variety of microorganisms that occur there, it is difficult to isolate the effect of any particular strain. To overcome this hurdle, we developed a system in which we grew plants under sterile conditions in agar and inoculated their rhizosphere with a single, well-defined microbial strain. For many years, agar has been used as a growth substrate for microorganisms and plant tissues. It is cheap, easy to use, and can be autoclaved to ensure its sterility. Because of its widespread use, an experiment conducted using this substrate can be reproduced under the same conditions in any laboratory. In contrast to soil, there is little interaction between the trace elements and the agar matrix.
There are many studies investigating the influence of microorganisms on TE uptake by plants. However, so far only a small variety of microorganisms has been tested on few plant species. Therefore, the first objective of our research was to develop a method to rapidly screen a large variety of microorganisms on various plant species. Once this goal was achieved, we sought to study the effect of single, well-defined microbial strains on TE uptake by sunflower and wheat.

The substrate for plants growth was a 10% agar solution prepared with modified Hoagland's solution and a TE solution containing 1 mg/kg Pb and molar equivalents of Cu, Ni and Zn. The agar solution was autoclaved and poured into sterile, transparent plastic boxes, whose lid was equipped with a filter allowing gas exchanges without contamination by external microorganisms. The seed surface was sterilised and the plants grew one week in agar before their rhizosphere was inoculated with LB broth containing a pure bacterial strain or agar plugs colonized by fungal hyphae. We tested 14 strains, with 5 replicates per treatment and a control where the system was inoculated with sterile LB broth. The plants grew for 2 weeks in a climate chamber and their shoots were analysed for their TEs by ICP-OES. Samples of agar and roots were collected to confirm microbial colonization of the rhizosphere, respectively sterile conditions in the control treatments.

Concerning the method development, the plants grew without visible toxicity in all the boxes, and the analysis of root and agar samples indicated that the controls were sterile and the strains inoculated were growing along the roots. More than 90% of the TE and nutrients added to the system were in the liquid fraction of the agar medium, thus available for root uptake.
The screening showed that the microorganisms in general decreased TE uptake by wheat and sunflower, although some of them had an opposite effect on the plants. However, with the same plant species, the microorganisms had a consistent effect on all TE tested, i.e. a given single strain caused the same effect (increase or decrease of TE uptake) on all TE tested. In sunflower, 3 microorganisms (Paenibacillus polymyxa, Pythium ultimum and

Rhizoctonia solani) decreased Cu and Zn uptake by 50% compared to the control treatment. These three species are common soil microorganisms. All three are known to exude auxin, a phytohormone. This hormone can modify root morphology and physiology and thus may affect TE uptake by plants. R. solani and P. ultimum are root pathogens. Their effect was opposite to what we expected. If roots are damaged, TE should have flooded into the plant and accumulate in the tissues, but this was not the case. One explanation could be the biosorption of TE by these microorganisms, reducing the uptake by plant. Conversely to sunflower, none of the microorganisms tested showed a significant effect on TE uptake by wheat.

With our research, we created an agar system allowing the screening of several microbial strains for their effect on plant TE uptake. Future work will involve screening of several other strains in a wide range of conditions in agar. A method validation with a pot experiment is also needed, as some interactions in this artificial rhizosphere may be different from those that would take place in soil.
We will also pursue the investigation of two interesting mechanisms revealed by the screening: the effect of pathogens and phytohormone-exuding microorganisms on TE uptake by plants.