ESRF	**Experiment title:** Distribution and Speciation of manganese in Mn-accumulator plants from New Caledonian lateritic soils	**Experiment number**: EC369
Beamline: ID21	**Date of experiment**: from: 19-02-2009/24-02-2009.	**Date of report**: 24/09/09
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REPORT

Aims of the experiment and scientific background

In New Caledonia, as a result of the long-term lateritic weathering of the ultramafic rocks Ni but also Cr, Co and Mn have been concentrated in the thick regolith and in soils. With such high metal content, New Caledonian soils are deficient in nutrients (N, P, K) and unbalanced for the Ca/Mg ratio. With all these soil particularities, 75 % of its flora is endemic [1] which make New Caledonia a hot-spot of biodiversity [2] where conservation is a priority. Even if opening new mine is restricted, one of the opportunities for the companies is to retreat mine tailing with phytoextraction technique. In the case of old mine, when the concentration of metals is far too low to continue the extraction, then mining companies are conduced to revegetate mine dump with endemic plant to restore the natural biodiversity.
Both purposes are very challenging since except for nickel little is know concerning the strategies developed by plants to growth on such high metal content soils. For example for Mn (one of the most abundant metal after Ni) even basic information are unknown (Mn concentration, localization and a fortiori speciation [3].
We have recently found that one of the major shrubs of the New Caledonian so-called 'maquis minier', Tristaniopsis guillainii, can tolerate manganese. It accumulated up to 1 500 mg Mn/kg of dry matter (DM) in its leaves, although only 600 mg Mn/kg DM are stored in its roots. Thus, in this present work, we were interested in investigating the Mn transfer and transformation from soil, root, twig and leaves.

Experiments

Field experiments were conducted in February 2007 in the Koniambo Massif, an isolated ultramafic massif of the West Coast of New Caledonia (20°59'S, 164°49'E). The study site was located on the Pandanus watershed. Tristaniopsis guillainii (TG) and T. calobuxus (TC), two major phylogenetically-close trees of the New Caledonian so-called 'maquis minier' were sampled as Mn-accumulators from the Myrtaceae family. Phyllanthus cornutus (PC) and P. serpentinus (PS), two endemic and frequent shrubs, were sampled as Mn-accumulator and Mn and Ni-hyperaccumulator respectively from the Euphorbiaceae family. The plants were chosen along an altitudinal sequence of soils, at site B (750 m) and site D (450 m), in order to test the effect of soil variability on bioaccumulated Mn speciation. On both sites, Tristaniopsis and Phyllanthus species lived together. The soils were coming from a typical lateritic alteration mantle truncated in the laterite at site B and in the transition laterite at site D.
At each site and for each species, five plants were sampled. For each plant, leaves (L), stems, and roots were collected, as well as their associated rhizosphere (RS) and bulk soils (BS). Rhizosphere soil was the soil surrounding the roots up to 5 cm of distance because of their nodular nature whereas bulk soil was at approximately 40 cm from the roots, in order not to be influenced by the sampled plant. Each plant part was rinsed with deionized water and separated in two halves. One half was oven-dried at 318 K during 48 h whereas the other half was cryogenized: it was frozen at 193 K for one week, transported in dry ice (193 K), and then stored at 248 K until analysis. A great care was taken to maintain samples cryogenized from field to experiments. This cryo-procedure was developed in order to avoid any possible dehydration of those fragile samples and to minimize sample preparation which could lead to a modification of metal speciation in planta.
The experiments were conducted on ID21 operated in fluorescence mode. The fluorescent lines of Mn, Ca, P and S were simultaneously acquired with an energy dispersive spectrometer associated to a multichannel analyzer. The

beam spot was focused down to 0.3x0.8 µm using the KB device. Micro-XRF maps were recorded on all biological samples using the cooled sample holder in order to prevent any dehydration of the samples. Mn K-edge micro-XANES measurements on plant and soil samples were performed on region of interests determined on the chemical maps.

Results

The overall session was perfectly conducted without any problem. All the allocated beamtime was fully optimized thanks to the help of the beamline scientists.

The main objectives of the proposal was to locate and determine the speciation of the Mn in various parts of accumulting plants. Previous results obtained on a lab –micro XRF tend to indicate that the Mn was homogeneous distributed in leaves of T. guilliani .

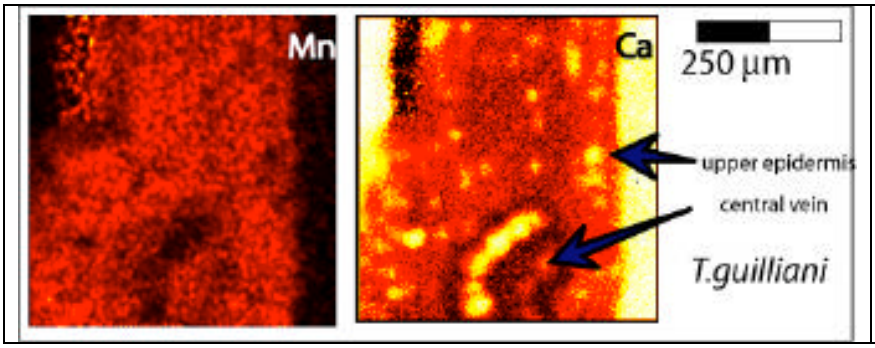

Figure 1 : Mn and Ca chemical map of a cross section of T. guilliani leaf. (10 µ X-ray spot size) using a lab. XGT 5000 (Horiba)

Micro-XRF image (normalized to the density of matter (total fluoresence)) obtained on ID21 clearly indicate that Mn is mainly located in the upper and lower epidermis with relative constant content (even lower) in other tissues (figure 2). The impressive spatial resolution of the Mn map enables to locate Mn not only at the tissue level but almost at the cellular level (in some non degraded parts). The methodology developped to maintain the samples under cryogenic condition from the sampling site to the beamline after cryo-microtome sectionning was necessary to avoid the destruction of the vacuolar part of the cells. Indeed it has been postulated many times that metals may be accumulated in the vacuolar zone. Unfortunately in our case we failed to keep this 'water reservoir' intact. THen it was only possible to detect Mn in the wall of the cells.

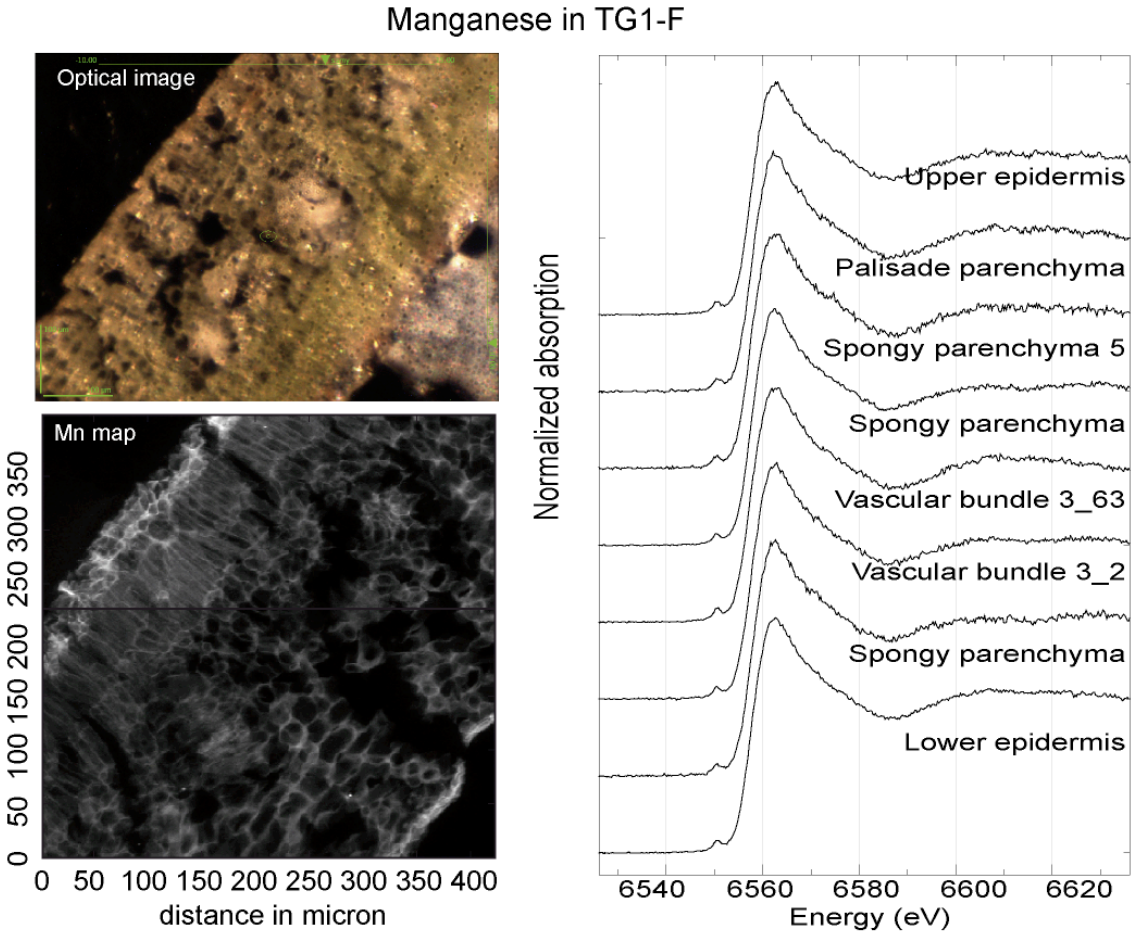

Figure 2 : optical, Mn chemical images of T.guilian leaf section (cryo-microtome). Combined to micro-XANES spectra of the various parts of the leaf.

In all parts of the leaves the Mn speciation was similar. When compared to reference compounds we were able to determine that Mn was under the 2+ form and complexed to carboxylic acids.

In the case of soil we have shown using bulk XAS (on the Fame beamline) than Mn was under the 3+ and 4+ forms. We have taken great attention to analyse the Mn speciation in the root where soil residues where absent. In the next figure it can be seen that Mn is mainly located at the border of the section of the root. To try to decipher whether Mn comes from the presence of soil particles or not, the Cr map has been shown. Indeed Mn, Fe and Cr can be associated in the soil particles. In most of the Mn map, Cr is not overlapping. Then XANES have been recorded on various parts of a section of the root. As in the case of the leave, the comparison of all the spectra of Bio-Mn in the root to reference spectra indicated that Mn is mainly under the 2+ form and complexed to organic acids.

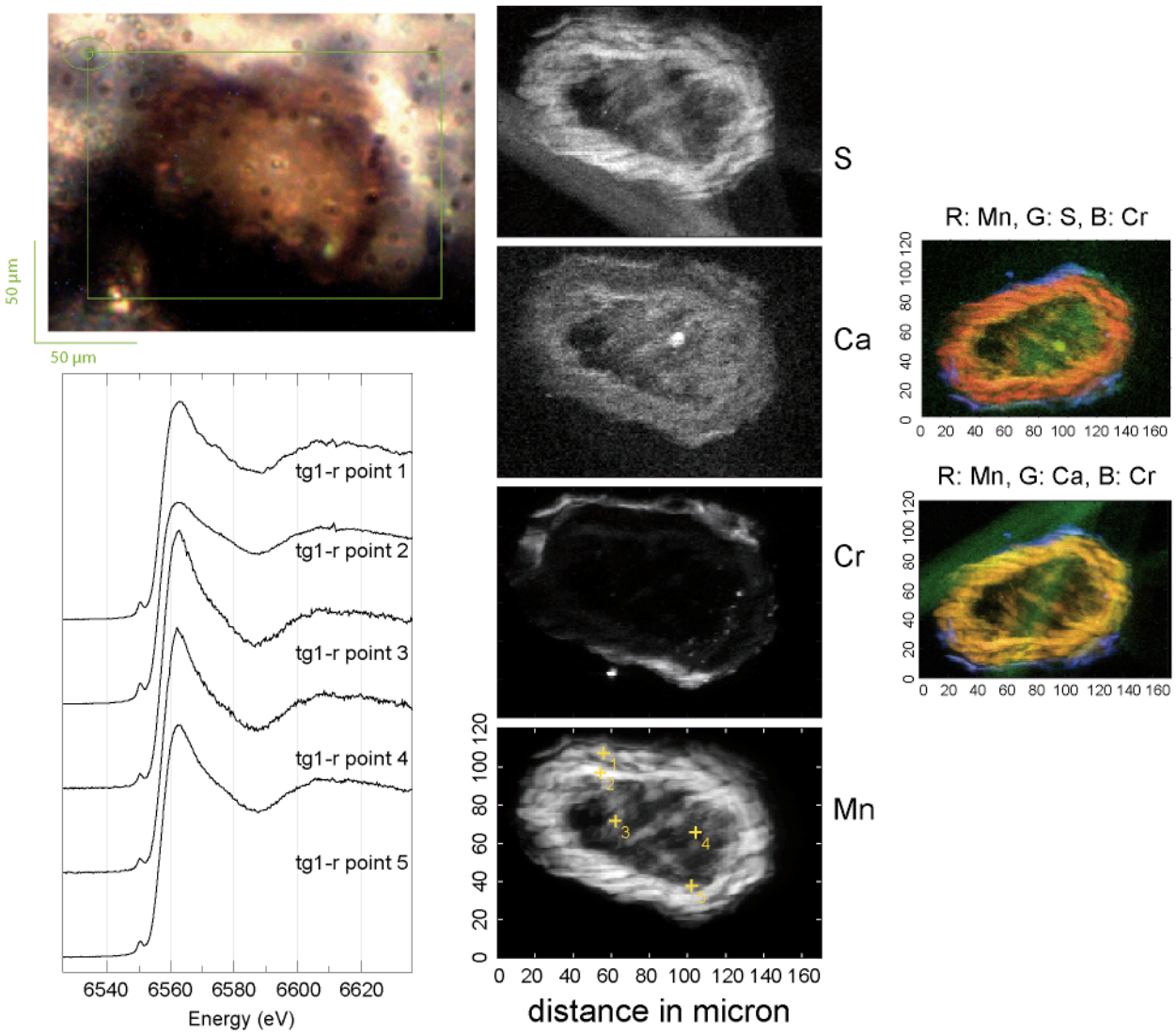

Figure 3 : optical, Mn chemical images of T.guilian root section (cryo-microtome). Combined to micro-XANES spectra of the various parts of the leaf.

From al other images of leaves, tig and roots for different plant species it came out that the biological mechanisms developped by the plant to accumulate Mn are not so different. Indeed it seems that Tristaniopsis and Phyllantus behave the same. More over we have shown that the modification of the redox state of Mn from soil to plant mainly occurs in the outer part of the root (bacteria or mycoryze).

References

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