ESRF	**Experiment title:** Relation between manganese (Mn) speciation and Mn toxicity to microorganisms and plants in soil	**Experiment number**: 26-01-877
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Report:

Introduction: scientific background and objectives

Manganese is an essential micro-nutrient for all organisms, but it can become toxic to soil organisms at high concentrations. Manganese redox states range between 0 and +7, with +2, +3 and +4 the most commonly encountered redox states in natural environments. In soil, Mn^{III} and Mn^{IV} occur as insoluble oxides. At low pH or under reduced conditions, Mn^{II} is the stable form which has much higher solubility than $Mn^{III/IV}$. The Mn redox chemistry therefore plays an important role in Mn uptake and bioavailability. Because of its complex chemistry, Mn speciation is difficult to study. Sample preparation, e.g. drying, may alter the speciation. Synchrotron techniques can be used with minimal disturbance of the sample. Therefore, X-ray absorption near edge structure (XANES) spectroscopy has been used to obtain information on Mn oxidation states in soils (Guest et al., 2002), but no attempt was yet made to relate the speciation to bioavailability.
In this experiment, the oxidation state of Mn in soils was determined with XANES in three soils with different treatments. The measurements were carried out at beamline BM26a (DUBBLE). The aim of our study is to relate the chemical speciation of Mn to its availability and toxicity. The experiment is part of a large project within the framework of the environmental risk of Mn in Europe in order to fulfill the European REACH regulations.

Materials and Methods

- ***Soils and soil treatments***. In total, there were 36 soil samples:

- 3 soils: Kasterlee ('K', sandy soil, pH 4.7, 1.7% organic C (OC), 150 mg Mn/kg); Woburn ('W', pH 6.1, 4.3% OC, 370 mg Mn/kg); Ter Munck ('T', 1.1% OC, pH 6.7, 460 mg Mn/kg)
- 2 Mn rates: without or with addition of $MnCl_2$, at a dose near the EC50 for plant growth (=concentration where plant growth is reduced with 50% compared to control). After the $MnCl_2$ addition, soils were air dried and stored for 2 months.
- 6 incubation scenarios:
    - incubated at field capacity (=not water saturated) for 7 days
    - incubated water saturated for 7 days at 20°C, without or with addition of (5%) peat or (1 g/kg) hay
    - incubated water saturated for 2 months without or with addition of (5%) peat

The effect of organic matter is assessed because organic matter plays a key role in driving redox reactions in soil.

- ***XANES measurements and data analysis***. The XANES data were collected at BM26 (DUBBLE). The BM26 beamline uses a double-crystal Si(111) monochromator to select the energy of the X-rays, and a nine-channel monolithic Ge detector to collect the fluorescence radiation. The signal was measured at the Mn K edge. The soil samples were measured in fluorescence mode. Reference samples were measured in transmission mode, and for a few samples also in fluorescence mode. Spectra were recorded from 130 eV below to 550 eV above the Mn K edge (~6550 eV), using 0.5-eV steps in the edge region. A Cr filter was used for fluorescence measurements, to reduce the scattering background. Depending on the Mn concentration of the sample, 2 to 10 replicate scans were made.
The wet soil samples were mounted between two sheets of Kapton tape, using a metal sample holder with window. The soils at field capacity were wetted to saturation immediately before the measurement, to obtain more homogeneity and conditions similar to those of the saturated soils. For one sample, the spectra were also recorded at field capacity, and it was found that wetting the sample did not cause a shift in the spectrum.
The reference samples were pressed in pellets with a thickness of 0.6 mm after dilution with BN to a concentration yielding an edge step of circa 1. Reference compounds included $MnCl_2 \cdot 4H_2O$, MnO, Mn_3O_4, Mn_2O_3, $MnPO_4$ and MnO_2, as well as natural Mn minerals: rhodonite ($MnSiO_3$), rhodocrosite ($MnCO_3$), manganite (MnOOH), psilomelane ($Ba(Mn^{2+})(Mn^{4+})_8O_{16}(OH)_4$) and pyrolusite ($MnO_2$).
The spectra were averaged and normalized. Reference compounds to carry out linear combination fitting (LCF) were selected based on principal component analysis and target transformation; only one compound was selected if spectra were similar (e.g. Mn_2O_3 and MnOOH). Following reference spectra were used for the LCF: rhodocrosite ($MnCO_3$), $MnSO_4$ (from the Lytle database), Mn_2O_3 and MnO_2.
- ***Toxicity tests and chemical tests*.** Plant assays (2 weeks growth) were carried out on the three soils amended with $MnCl_2$. The soils were at field capacity during the whole growth period or saturated for 7 days before harvest. The chemical analyses included determination of total (hot acid digestion), extractable (NH_4Ac pH 3, and $CaCl_2$ 10 mM), and soil solution Mn, and measurement of the Mn diffusion flux with DGT (Diffusive Gradient in Thin films).

Results and Discussion

To assess whether no beam damage occurred, a series of five quickscans (100 s) was run on one soil sample. No shift in the spectrum was observed. However, when five consecutive normal scans (15 min) were run, a small shift in the spectrum towards lower energies was observed (Figure 1). This was also reported by Ross et al. (2001), who attributed this to reduction of the Mn oxides, with soil organic matter presumably acting as electrondonor. However, the change was minor compared to the difference between samples, and carrying out the LCF on the first, last and average of five scans indicated that this shift had only a minor effect on the estimated Mn oxidation state (at most 0.05 difference in average oxidation state). For all samples, at most (and mostly less than) five consecutive scans on the same spot were run, and the beam damage should therefore have had negligible effects on the overall results.

Figure 2 shows the normalized spectra of the reference compounds used in the LCF and of selected soil samples. Table 1 gives the LCF results for all samples. Both visual observation and the LCF results indicated the predominance of Mn^{II} in soil K (all treatments) and in all soils that were water saturated for 2 months. In the $MnCl_2$ amended soils at field capacity, Mn^{II} dominated in soil W, but $Mn^{III/IV}$ dominated in soil T, indicating that oxidation of the added Mn^{II} salt occurred in soil T (during the 2-months dry storage and 7-days incubation at FC), but not in soil W. Addition of hay promoted reduction of $Mn^{III/IV}$, but addition of peat surprisingly seemed to slow down the reduction rate, as indicated by the lower Mn^{II} fractions for the peat treatments compared to the corresponding treatment without peat.

The plant experiment showed that Mn in the plant was proportional to the Mn concentration in soil solution. A 50% yield reduction occurred at an internal shoot concentration of 4000 mg Mn/kg dry weight or at a pore water concentration of circa 480 mg/liter (all soils) or at a total Mn concentration in soil of between 500 (low pH soil) and 2000 mg Mn/kg (high pH soil). Differences in toxicity between soils and treatments could be related to differences in speciation, but were generally small, since most added $MnCl_2$ remained as Mn^{II} in the toxic range. These results are currently being written up for publication.

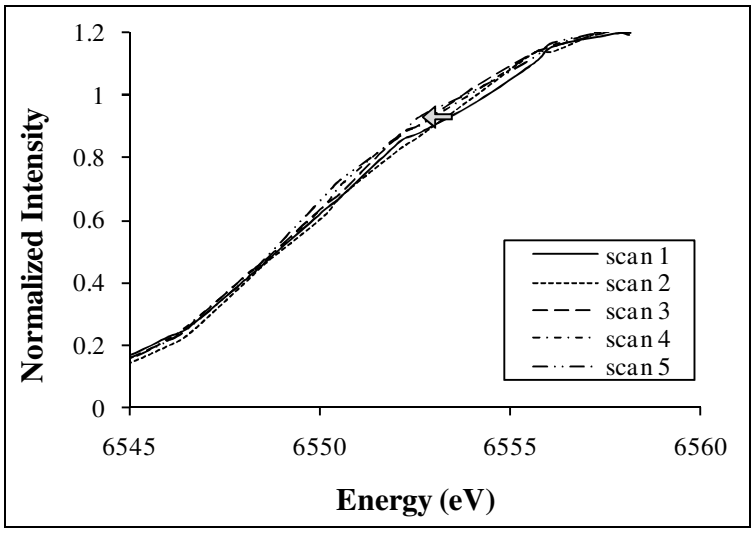

Figure 1 Repeated Mn XANES scans on soil W (7 days saturated). The grey arrow indicates the shift of the spectra over the consecutive scans.

Table 1 Results of the linear combination fitting of Mn XANES spectra, using $MnSO_4$ and $MnCO_3$ (Mn^{II}), Mn_2O_3 (Mn^{III}) and MnO_2 (Mn^{IV}) as end-members. The soil samples were incubated at field capacity (FC) or water saturated for 7 days (7d) or 2 months (2m), without or with addition of 1g/kg hay (+h) or 5% peat (+p).

Treatment	H (pH 4.7)			W (pH 6.1)			T (pH 6.7)		
	II	III	IV	II	III	IV	II	III	IV
Unamended soils									
FC	0.53	0.37	0.10	0.10†	0.74	0.16	0.12†	0.53	0.35
7d	nd			0.12†	0.72	0.16	0.41	0.43	0.16
7d+h	0.74	0.19	0.07	0.25	0.60	0.15	0.75	0.17	0.08
7d+p	nd			0.11†	0.77	0.12	0.20†	0.50	0.29
2m	nd			0.94	0.06	0.00	0.74	0.26	0.01
2m+p	nd			0.77	0.23	0.00	0.59	0.26	0.15
Soils amended with $MnCl_2$									
FC	1.00	-	-	1.00	-	-	0.30	0.51	0.19
7d	1.00	-	-	1.00	-	-	0.69	0.17	0.14
7d+h	1.00	-	-	1.00	-	-	1.00	-	-
7d+p	nd			1.00	-	-	0.82	0.09	0.09
2m	1.00	-	-	1.00	-	-	1.00	0.00	-
2m+p	nd			1.00	-	-	0.87	0.13	-

†$MnCO_3$ identified as dominant Mn^{II} species (Other samples: $MnSO_4$ dominant Mn^{II} species)

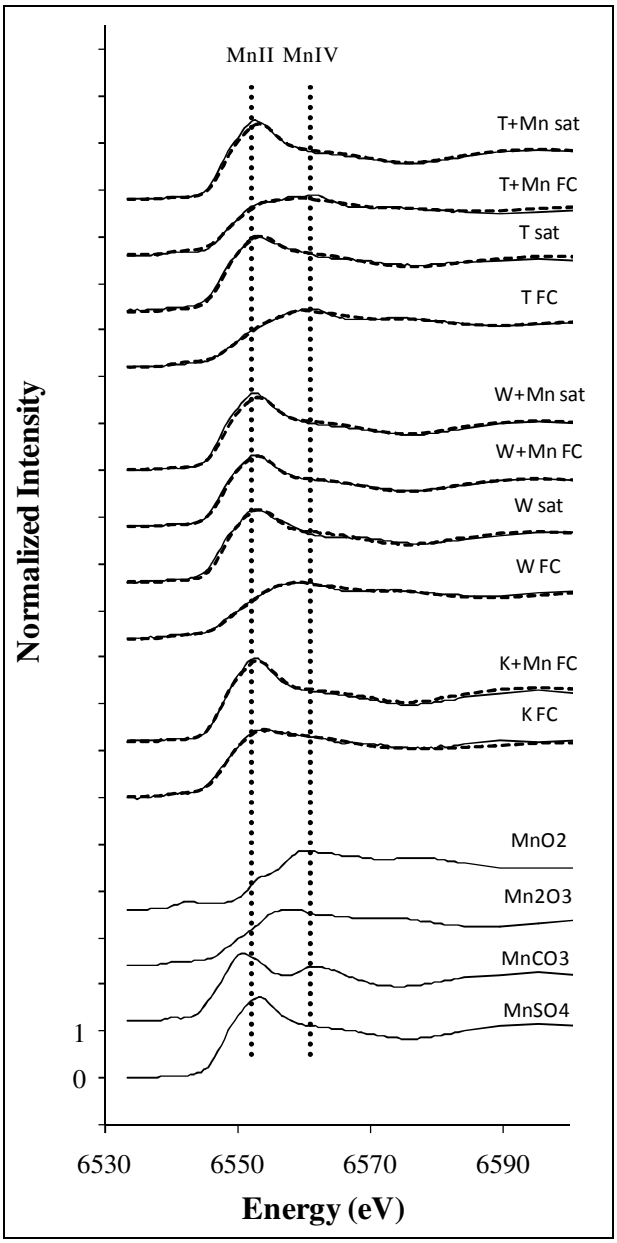

Figure 2 Bulk Mn XANES spectra of selected Mn standards and soil samples. Dashed lines give the LCF fits. The soils (K, W, T) were unamended or amended with $MnCl_2$ (between 1000 and 2000 Mn/kg), and were at field capacity (FC) or incubated water saturated for 2 months (sat). Vertical dotted lines indicate the approximate white line position of Mn^{II} and Mn^{IV}.

References

Guest GA, Schulze DG, Thompson IA & Huber DM (2002) Correlating manganese absorption near-edge structure spectra with extractable soil manganese. Soil Sci Soc Am J 66: 1172.

Ross DS, Hales CH, Shea-McCarthy GC & Lanzirotti A (2001) Sensitivity of soil manganese oxides: XANES spectroscopy may cause reduction. Soil Sci Soc Am J 65: 744-752.