ESRF	**Experiment title:** Influence of compost soil fertilization on the uptake and translocation of TiO_2 nanoparticles in sorghum and pea plants	**Experiment number**: EV140
Beamline: ID 21	**Date of experiment**: from: April 15, 2015 to: April 18, 2015	**Date of report**: 18/07/2015
Shifts: 9	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

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Report:

Introduction

The increasing production and use of TiO_2 nanoparticles (NPs) in consumer products inevitably lead to accumulation in water and soil. Soil is susceptible to the accumulation directly from fertilizers and plant protection products or indirectly from the use of wastewater sewage sludge, as well as due to nanomaterial products becoming waste. Moreover, NPs uptake by agronomic cultures and their translocation to edible parts represent a direct way to transfer them into the food chain. Soil organic matter present or added to the soil to improve fertility could be an important factor influencing NPs mobility and transference to the food chain. Apparently there is no consensus on the response of plants to TiO_2 NPs exposure, then the need to assess their impact in real soil matrixes also under common organic matter amendment (such as compost, a material rich in organic matter from vegetal origin). The aim of this study was to evaluate the uptake of TiO_2 NPs in roots, its translocation to the aerial part and the possible speciation inside the tissues of *Pisum sativum* and *Sorghum vulgare* plants growing in an agricultural soil amended or not with compost. For this purpose, we used micro X-Ray Fluorescence to investigate the distribution/localization of NPs in the different plant tissues and micro X-Ray Absorption Near Edge Structure to investigate the possible speciation and the identification of crystalline phases inside the plant tissues.

Material and Methods

The agricultural soil was sampled andin the laboratory it was treated with compost and TiO_2 NPs before sowing (35d and 24h, respectively) resulting in five different soil matrixes: *1*. control; *2*. control + compost treatment; *3*. TiO_2 NPs (100% anatase) treatment; *4*. compost + TiO_2 NPs (100% anatase) treatment; *5*. compost + TiO_2 NPs mix (anatase and rutile mixture, 1:1 ratio) treatment. NPs treatment in soil (400 mg kg^{-1}) was performed mixing mechanically the water suspensions of TiO_2 NPs (100% anatase or TiO_2 mix) with soil. Pea and sorghum plants were grown in soil matrixes for 30d. Roots and leaves of both plants were collected, washed and immediately cryo-embedded in OCT resin and shipped to ESRF-ID21 for further sectioning and freeze-drying.

Since only 9 shifts were allocated for the experiment EV-140 as a preliminary test, we decided, after discussion with the beamline scientist, to use the beam time allocated to perform firstly the analysis on roots of pea plants grew in the 5 different soil matrixes and only if beam time was still available some roots of sorghum would be analysed.

At the ID21 beamline, thin-section of <u>roots</u> (25 m) were prepared using the cryomicrotome and some were also freeze-dried. Elemental maps (-XRF) under cryogenic conditions (-160°C) and at room temperature (freeze-dried samples) for Ti and other elements (P, Ca, K, S, Cl) were recorded at various step-sizes (10, 2, 1 m^2) and dwell-times (200, 100, 60 or 50 ms) with an incident energy of 5.1 keV. Ti K-edge -XANES spectra (4.95 – 5.10 keV) were recorded in fluorescence yield detection mode on regions of interest of the maps (spots) for Ti speciation and crystalline phase identification.

Results

The cutting of samples at the cryomicrotome from ID21 permitted an *in situ* selection of the best cut sample for mapping and spectra analysis at low temperatures.

The -XANES spectra of reference materials (powder TiO_2 NPs 100% anatase, powder TiO_2 NPs 100% rutile, TiO_2 NPs 100% anatase suspended in solution of humic acid or in soil water extract) permitted to verify that soluble organic matter covering NPs did not alter its crystalline phase.

Pea roots from control soils (1: no NPs, no compost added and 2: no NPs, compost added) showed -XRF maps with few Ti spots (Fig.1: A1, B1 and A2, B2, respectively) only at the root epidermis. XANES spectra evidenced the anatase crystalline phase on a spot of the control root from soil with compost (Fig.2: spot 1), however the XANES signal of the control root from soil without compost was not exploitable. Thus, the origin of Ti in control root samples could be from soil or from compost. In soils, natural anatase is generally less abundant than rutile and in compost it could result from the different waste materials composted (household organic waste, cuttings and prunings of plants, by-products of food processing, etc). Probably -XANES on compost and soil water extracts without addition of NPs could give indications on the origin of anatase in control roots. Anyway, XANES analysis of control roots showed that also natural TiO_2 could be found into the root epidermis.

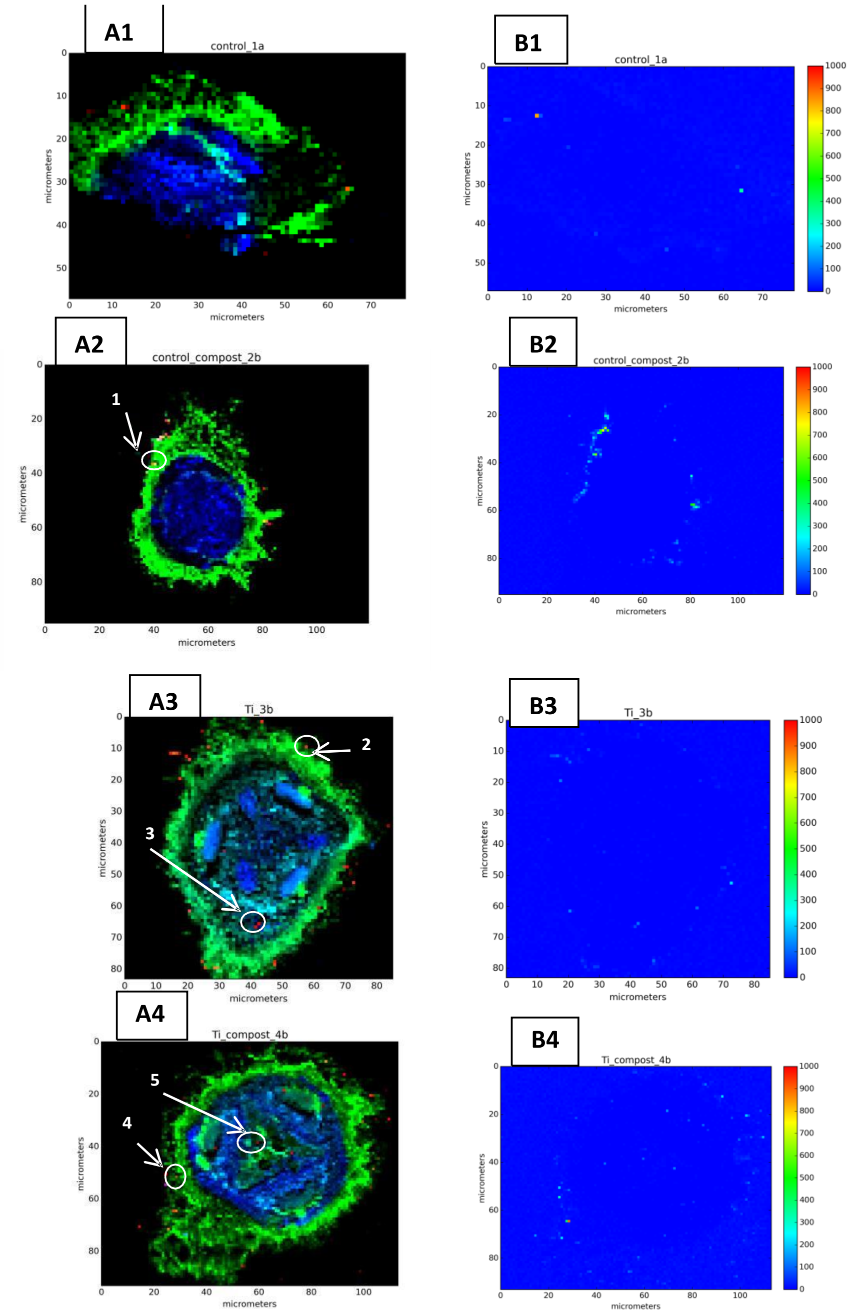
Pea roots grew in both soils (without or with compost) spiked with NPs showed -XRF maps (Fig1: A3-A5, B3-B5) with Ti spots mainly localized into the root epidermis, however some spots were also found more internalized inside the cortex, independently of the presence of compost in soil. The -XANES analysis on roots from soil treated with 100% anatase showed a main contribution of anatase phase (Fig.2). The spots 4, 3 and 5 (Fig.2) evidenced anatase phase inside the epidermis, in the cortex and in the vascular system, respectively. XANES spectra in the spots of pea roots from soil treated with a mix of anatase and rutile NPs (1:1 ratio) showed both phases predominantly localized into the root epidermis, even though some spots were observed inside the cortex (Fig.1: A5, B5). Micro-XANES in this samples revealed more anatase spots (Fig.2: spots 6, 7) than rutile ones (Fig.2: spot 8). Admitting that some translocation to stems or leaves could occur in pea plants a possible preferential translocation of rutile phase to upper parts (as observed in other plant species) was not investigated.

Conclusion

Generally the analysis of Ti elemental maps in cross sections of pea roots made it possible to see that Ti was mainly internalized in the root epidermis, however some NPs could be evidenced in the root cortex and in the vascular system of roots from soiltreated with NPs. Elemental maps showed that the presence of compost in soil does not particularly influenced the presence of NPs inside roots. Although no analysis in upper parts of plants were performed due to the limited beamtime, data gives an indication that the translocation to the aerial part is very low or even does not occur (very few spots were found in the vascular system).

Micro-XANES evidenced the presence of TiO_2 crystalline phases also in the control roots grew in compost treated soil, which could be a source of NPs. Roots from soil treated with a mix of NPs rutile and anatase phases (1:1 ratio) showed a predominance of anatase to the rutile phase. It was not possible to perform any analysis on the upper parts of plants, thus a potential translocation of rutile (admitting some) as observed in other plant species was not evaluated.

This results will permit to finalize a paper regarding the impact of TiO_2 NPs in the soil–plant system particularly when soil is amended with the mature compost used in this experiment. To go deeper in our observations we intend to resubmit a proposal to the ID21 beamline.We would evaluate the effect of sewage sludge, from a wastewater treatment plant, on the uptake and translocation of NPs in crop species. Increasing amount of sewage sludge enforces the need to recycle it as a fertilizer in agricultural soils, however it is often rich in NPs.



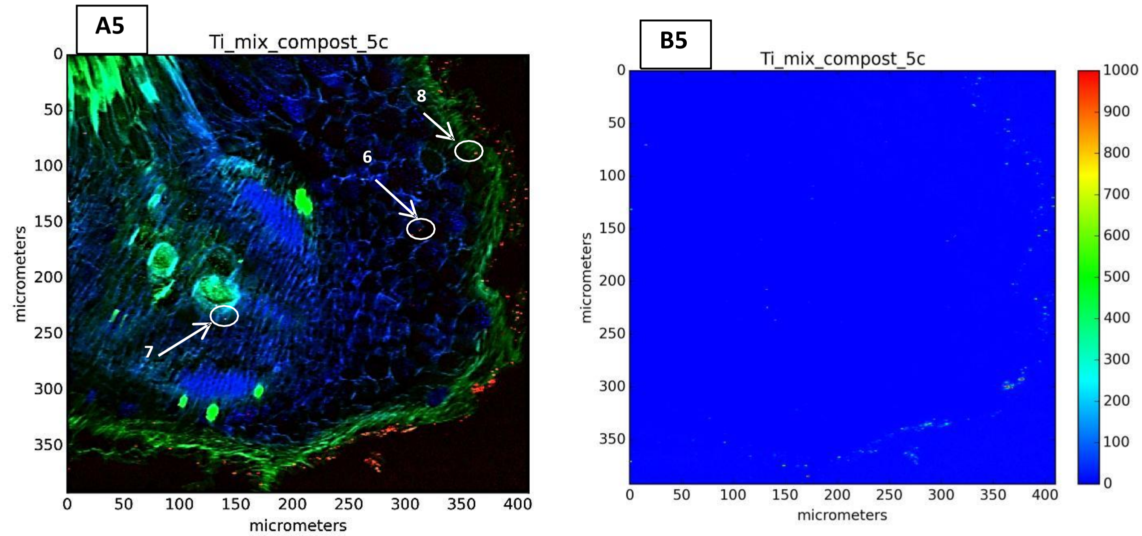


Fig 1: Micro-XRF tricolor maps (A) and Ti-K temperature maps (B) of cross sections of pea roots from the five different soil matrixes: 1-control soil;2-controlsoil +compost; 3-NPs spiked soil (100% anatase); 4-NPsspiked soil (100% anatase) +compost;
5-NPs spiked soil (anatase:rutile- 1:1ratio)+compost.

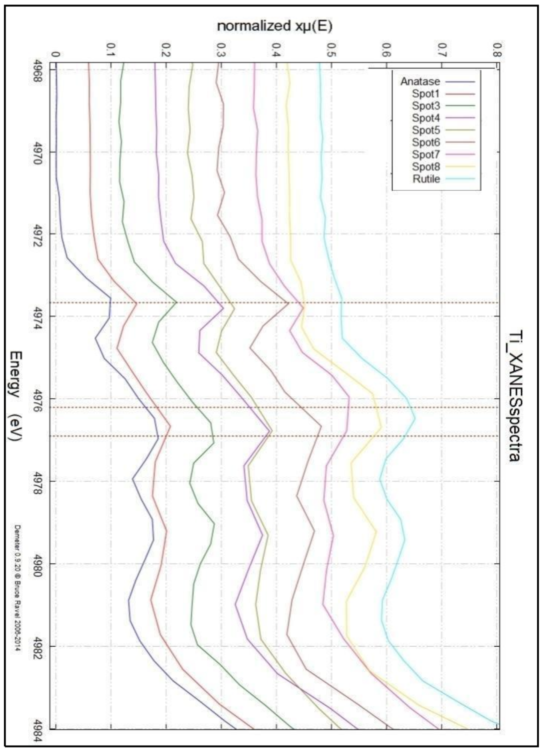


Fig 2: Micro-XANES spectra on spots of interest (1-8) from Fig 1A, zoomed in the pre-peaks.