Advanced techniques to investigate the internalization mechanism of TiO_2 NPs in the roots grown in a biosolid-amended agricultural soil

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Plants play an important role in introducing the engineered nanoparticles (ENPs) into the food chain. The pathway of ENPs uptake from soil, their distribution in the edible plant parts, and their impact in the food production are important issues to be investigated. In the present study, *Pisum sativum* plants were grown at microcosm scale under medium-term TiO_2 NPs exposure, to possibly mime environmental conditions in an agricultural soil amended with biosolids from a wastewater treatment plant in Pisa, Italy. TiO_2 NPs were applied as pure rutile, pure anatase and a mixture of both crystalline phases in the biosolid amended-soil.

Micro-XRF and μ-XANES from ID21 beamline were used for Ti elemental mapping and crystalline phase identification to indicate a relative distribution/localization of TiO_2 crystalline phases within a given cross-section of roots, as well as the possible speciation and preferential crystalline phase uptake in the roots. Titanium in roots showed a main localization in the rizoderma, independently of the crystalline phase. Fewer Ti spots were found localized in the cortex or in vessel, however the roots grown in presence of a mixture of both phases showed a main presence of anatase, suggesting a preferential adsorption and translocation of this crystalline form through the roots. Our data indicated also a reduced translocation of Ti to the aerial part of the plant, confirming the chemical analysis of shoots and roots separately, which showed that Ti concentration was about 40 times lower in the upper part than in the below ground tissues.

The TiO_2 NPs were characterized on the basis of their size and shape by TEM analysis. Moreover, observations on cell ultrastructure of control and of anatase, rutile and mixture of both crystalline phases treated roots were performed. The root cells of plant grown in the presence of all NPs treatments shared the same alterations of ultrastructure: mitochondria with swollen cristae, nuclei with condensed chromatin, and part of the cytoplasm degraded, probably in consequence of an autophagic process. As detected by μ-XRF and μ-XANES, electron dense prismatic or round profiled particles of about 30-40 nm were observed mainly in form of aggregates in the intercellular spaces or crossing the wall of the cells next to rizoderma and in the cortex cells. Furthermore, the anatase treated cells were mostly damaged in respect to control and rutile treated roots, and more frequently internalized NPs were observed in these samples.