2.2 = TiO_2 nanoparticles and sludge from wastewater treatment plants: a new concern for crops?

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Contaminants of emerging concern are increasing in all the ecosystems, due to the unintentional or intentional release into the environment of new molecules/compounds or to a new employment and disposal of complex and potentially polluted matrices. In this respect, the reuse of sludge from wastewater treatment plants in farming soils is recognized as a cost-effective practice to dispose of a byproduct, that, after specific treatments and maturation, can be provided as a fertilizer, rich in organic matter and nutrients (1). On the other hand, due to the uncertainty of its contents not thoroughly tested for safety, sludge can result a possible sink of unknown priority pollutants as well as of not commonly monitored chemicals, such as nanoparticles (NPs) (2). The latter emerging contaminants are becoming a worldwide problem: nanotechnologies are being gradually more employed in all sectors of technology and innovation, but their effects on living organisms are not yet fully clear and unambiguously interpretable (3). Besides, NPs behavior is poorly estimated in the different environmental matrices, especially in agricultural soils. In such complex matrices, the bioavailability of the different nanomaterials frequently is not predictable, for the tendency of NPs to aggregate, to adsorb/precipitate on solid phase, as well as to be coated by organic molecules (4). In addition, the overall picture of their possible interactions with crop plants and with food chains is not at all clear (5).

In the present work we aimed to investigate the effects of TiO_2 NPs in a sludge amended agricultural soil to evaluate the growth and development of the crop *Pisum sativum* L. at microcosm scale under long term exposure, possibly miming an environmental occurrence. TiO_2 was spiked in two different concentrations in the form of bulk material or of NPs and by using the two main crystal forms (anatase and rutile), applied singularly or in a mixture of the two. Electron microscopy imaging studies allowed us to visualize internalized NPs in the different sub-cellular compartments of the root tissues and their effects on cell ultrastructure. Synchrotron studies demonstrated that both titanium crystal forms, especially anatase, were taken up and moved to the vascular system. Studies dealing with the possible induction of oxidative stress were performed by *in situ* histochemical techniques and by biochemical approach.

The potential fertilized value of the sludge-amended agricultural soil seemed to have no beneficial effects on the plant, but rather to induce disturbance at different levels for the selected crop. The addition of NPs in the sludge-amended soil marked oxidative damages in *P. sativum*. This result was more pronounced at the lowest NPs concentration and this fact could be probably related to the low tendency of the diluted NPs suspension to form homo- and heteroaggregates in a complex matrix, with a consequent greater bioavailability. In our experimental system, the most adverse effects on plants were mainly recorded following exposure with anatase crystal form, alone or mixed with rutile as well as in the presence of the corresponding bulk material.

These findings rise some reflections on fertilization practices of agricultural soils by treated sludge and on the exploitation of nanomaterials, which safety must be carefully evaluated, in order to establish precise regulation over their use, confinement and environmental disposal.

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