Esperimental Reports
EUROPEAN SYNCHROTRON RADIATION FACILITY
ESRF User Office
Experimental Method
Proposal Summary (should state the aims and scientific basis of the proposal) :
CuO NPs (nanoparticles) are used in agriculture as pesticides. In Zucchini plants they slightly reduce the biomass and the chlorophyll, but less than the corresponding salt CuSO4. However, the cellular mechanisms laying behind these phenomena are still unknown. Little is known about the fate of the pristine
CuO NP structure inside the plant and, consequently, the local structural environment around Cu atoms has not yet been elucidated. Throufg molecular analysis we can exploit this information, for example, to appreciate if the NPs induce genotoxic effects by binding into the reproductive parts of the plants (anthers). We propose to use EXAFS technique to clarify these two essential points for a safe application of CuO NPs in the environment.
Scientific background :
Copper is an essential nutrient for plants, but at high concentrations, it becomes highly phytotoxic. Cu contamination of soils is widespread as a result of mining, smelting, dispersal of sewage sludge, and other industrial activities (1). Plants that grow on copper contaminated soils can develop mechanisms to reduce copper uptake, or to tolerate and even detoxify the metal, but the observed mechanisms vary depending onthe organ studied and the administered concentration (2). By utilizing CuO NPs in agriculture as pesticides it is possible to reduce the amount of Cu wasted during application (3). Most studies on the effects of nanoparticles in agriculture have focused on the physiological aspects. Therefore, so far, we have a poor understanding of the cellular mechanisms of the CuO NP even though it is well known that copper is highly accumulated in the tissues of exposed plants and that Cu can lower the mitotic index, inhibit cell division, induce changes in the oxidant state by over-production of H2O2 and modulation of other elements such as Fe, Al, Mg and Zn (4). Copper can act through changes in H2O2-dependent peroxidases and other enzymes activity, such as PAL (phenylalanine ammonia lyase, that acts for the construction of lignin from phenolics lignols), leading to cell wall stiffening due to the formation of cross-links between lignin molecules, moreover, when exacerbated by increased NADH oxidase activity, Cu decreases cell wall extensibility leading to cell death (5). However, we still don’t know if CuO NPs have the same effect as Cu, if they can reach unchanged the cell walls of plant organs exerting damages to the cell growth or causing genotoxic damages to the reproductive organs by binding to critical molecules for the cell metabolism. Zucchini plant (Cucurbita pepo L.) is a quite diffuse edible vegetable and well known for its positive performance in studies on plant-nanoparticles interaction (6). Male reproductive parts, anthers, are easy to reach and analyse. So far we have treated zucchini plants with CuSO4 salts, CuO bulk material, and CuO NPs at concentrations corresponding to 100 mg/kg of Cu, for 45-60 days according to the level of physiological toxicity observed with the different treatments. Utilizing ICP-MS we found Cu in all plant parts, in concentrations of 65 mg/Kg in the roots, 60 mg/Kg in the leaves and shoots, 30mg/Kg in the flower (work in progress). However, we were not able to identify the cell structure where Cu localizes for each type of treatment. Experimental technique(s), required set-up(s), measurement strategy, sample details (quantity...etc) : Biological samples: Cucurbita pepo (Zucchini plants), Costata romanesco, leaves, roots, and anthers will be analysed separately, as dried fine powder in the form of a 1g tablets. Plant treatments: 100 mg/kg of Cu, for 45-60 days in the form of CuO NPs (bought from US Nanomaterials research) or 100 mg/kg CuSO4, the Cu concentrations found with these treatments are of about 65mg/kg in roots, 60mg/kg in leaves, 30 mg/kg in anthers.We expect a concentration of two order of magnitude from fresh to dry weight after drying. Model compounds: Cu inorganic salts: CuO, Cu2O, CuS, Cu2S, CuSO4, CuNO3, CuCO3, CuCl, CuCl2. CuO NPs (40nm with 80% Cu, morphology: nearly spherical, z-potential = -24).
Experimental plan:

Dried samples will pressed in 13 mm pellets and successively sealed in kapton tape. XAS measurements will be carried out at Cu K edge (8978.9 eV) in fluorescence mode for the biological samples and in

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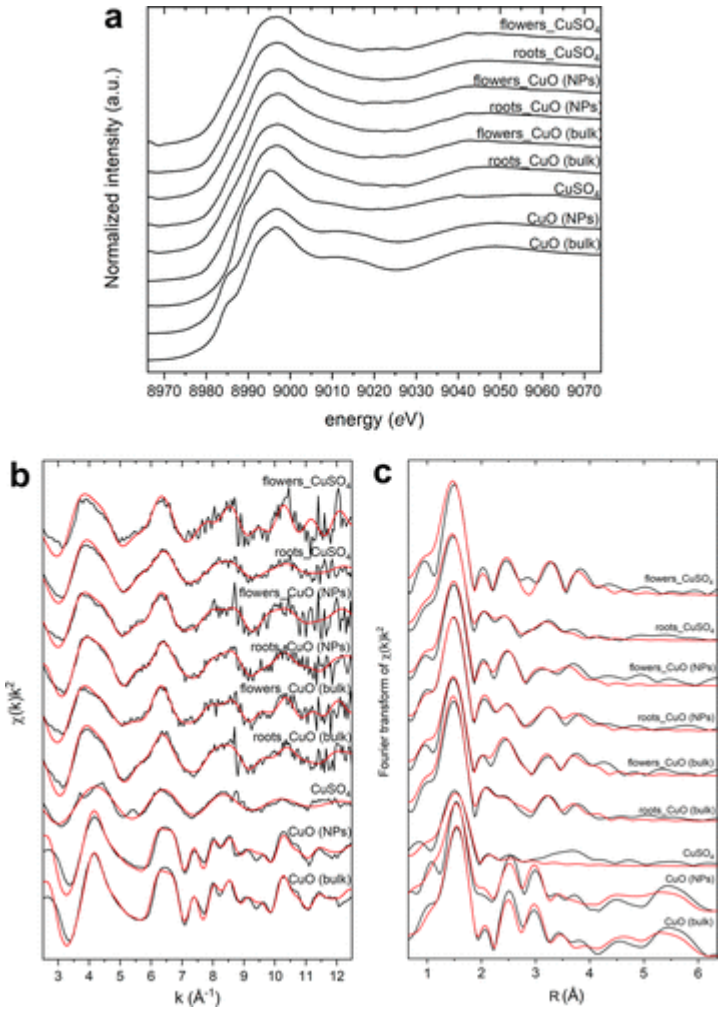
transmission mode for the Cu model compounds. A number of scans comprised between 6 and 12, depending on the concentration of Cu in the samples, will be averaged for each plant samples measured in fluorescence mode, while two scans per sample will be performed for model compounds in transmission mode. The maximum attainable k value will be limited to k=12 Å-1 due to the presence of Zn. Suitable filters will be used to damp the emission of lower Z elements (namely Mg and Fe). Measurements will be carried out at 80 K using a cold finger cryostat in order to minimize the possibility of beam damage effects. None of the samples poses any particular risk to human health; samples will be kept in sealed containers and brought back to the user's own laboratory.

Results We obtained the results below



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