PhD ACTIVITY REPORT – THIRD YEAR

DEPT. OF CHEMISTRY, LIFE SCIENCES AND ENVIRONMENTAL SUSTAINABILITY

XXXII PhD COURSE IN BIOTECHNOLOGIES AND LIFE SCIENCES

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Title: "*Reliable Molecular Markers to Identify Genotoxic Effects of ENMs in Plants*"

Risk assessment of potential deleterious effects of engineered nanomaterials (ENMs) on human health and environment requires implementation of reliable and versatile tests.

Genotoxicity deriving from acute and chronic exposure is one of the main issues related to ENMs. In the first and second year of my PhD, analyses on CdS QDs were conducted using the model plant *A. thaliana*, accession Landsberg erecta (Ler-0).

In particular, Random Amplified polymorphic DNA (34 primers, to investigate potential genotoxic effects on genomic DNA with the use of End Point PCR) and Real Time Quantitative PCR (6 Cp and 5 Mt gene primers, to investigate genotoxic effects on chloroplast and mitochondrion) analyses were performed on plants exposed to a range of CdS QDs concentrations (0, 40, 80, 150, 250 mg/L), and compared with effects of $CdSO_4$ (50, 100 µM) after 0, 10 and 20 days of exposure.

While RAPDs analysis permit to highlights that CdS QDs are not responsible of visible genotoxic effects on genomic DNA, RT-qPCR permitted to measure how genes change their relative quantities (RQ) in the different test conditions.

Genes *YCF1* and *PSAC* (both Cp genes) decrease significantly in RQ in plants exposed to higher concentration of CdS QDs, while Cp genes *PSBA* and *PSBD* increase significantly in RQ at higher time of exposure to CdS QDs. Mt genes *COB* and *COX* were find to be statistically more abundant in plants stressed with higher $CdSO_4$ concentrations.

In the third year of my PhD the same set of RAPDs, Cp and Mt genes were studied on plants of *A. thaliana* stressed with new types of ENMs and their relative salts: CeO_2 NPs and $CeCl_3$; Fe_2O_3 NPs, Fe_3O_4 NPs and $FeCl_3$; ZnS QDs and $ZnSO_4$. Tested concentration of each ENMs and salts was determined after repeated toxicity test to identify minimum inhibition concentration (MIC). ½ MICs

was used to stress plants for 20 d of exposure. For all ENMs ½ MIC corresponds to 500 mg/L, for $CeCl_3$ and $ZnSO_4$ salts ½ MIC = 175 mg/L, and for $FeCl_3$ ½ MIC = 75 mg/L.

All genotoxic analyses were accompanied with physiological analyses to determine chlorophyll *a*, *b* and carotenoids concentration, respiration activity and lipid peroxidation. Even in this cases RAPDs analyses permit to highlight how ENMs exposition don't cause visible genotoxic effects on genomic DNAs. Contrarily, RT-qPCR permits to highlight how CeO_2 ENMs don't influence in significative way the RQ of both Cp and Mt genes (although a general increase in RQ was observed). In the case of plants exposed to Fe based ENMs and Fe salt, all Cp and Mt genes appears statistically more abundant although differences in RQs were observed between ENMs; these results is explicable considering differences in Fe ion charges released by the ENMs. Finally, ZnO_2 QDs seem to cause a general significant increase in RQ for both Cp and Mt genes, and results similar for what observed in plant exposed to Zn salt.

Principal Component Analyses (PCA) were performed to highlights possible trends between physiological parameters and genes RQs. PCA performed between data from photosynthetic pigment concentrations and Cp genes RQs permit to see how an increase in pigments concentrations was related with an increase in RQs. This result, in conformity with literature, is explicable with the fact that ENMs can damage chlorophylls and carotenoids forcing plants to increase the number of chloroplast, and consequently the number of Cp genomes with respectively genes. PCA performed between respiration rates and Mt genes RQs highlights how an increase of respiration activity is related to an increase in RQs; similarly, at the previous situation, ENMs can damage mitochondria functionality forcing plants to increase the number of mitochondria and their relative genomes. Finally, PCA performed between lipid peroxidation data and Mt RQs highlights how an increase of oxidative stress was associated with a decrease in Mt genes RQs; according to the literature, ENMs toxicity seem to be related principally with ROS production that can damage lipids, proteins and genetic materials.

In all PCAs it's clearly possible to see how more unstable ENMs (ZnO_2 QDs, Fe_2O_3 NPs and Fe_3O_4 NPs) cluster together and with their relative salts, indicating how their behavior it's similar one each other and the release of respective metal ions it's similar to salts. Contrarily, CeO_2 NP cluster principally with control, indicating its relative stability and limited toxicity.