INVESTIGATIONS ON THE IMPACT OF NANOPARTICLES IN ENVIRONMENTAL SUSTAINABILITY AND ECOTOXICITY

INVESTIGATION DES NANOMATERIAUXS SUR LA SUSTAINABLE AMBIENTAL ET ECOTOXICOLOGIE

INDAGINI SULL'IMPATTO DELLE NANOPARTICELLE SULLA SOSTENIBILITA' AMBIENTALE ED ECOTOSSICOLOGIA

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Abstract

A special greenhouse was constructed to verify the impact of nanoparticles dispersed in air and in the soil on plant and small animal models. A 40x4m^2 greenhouse was divided in two specular parts in order to have a polluted area (B) and the reference one (A). Two different systems to spray nanoparticles (NPs) were set up: the first consists in a combustion of wood or coke perfused with an alcoholic solution containing Copper and Cobalt NPs and following emission of the micro and nanosized by-products in the greenhouse. The second system is a suitable sprayer of NPs starting from a water solution of engineered NPs of Cobalt, Nickel, Silver, Titania, Cerine. Plants (tomato, rice, tillandsia and moss) and insects (*Ceratitis capitata*) were exposed to NPs according to specific protocols, as well as aquatic marine animal models (Earth worms (*Lumbricus rubellus),* Sea urchins (*Paracentrotus lividus),* Brine shrimps (*Artemia salina),* Zebrafish (*Danio rerio*), Barnacles (*Balanus amphitrite*). The results indicate that the NPs produce some effects in photosinthesis in the plant and biological damages at the developmental stage in the sea urchins.

Keywords: *nanoparticles; nanotoxiticy; nanoecotoxicity*

Résumé

Un effet de serre a été construite afin de vérifier l'impact des nanoparticules sur les plantes et de petits modèles animaux. Appareil (40x4m^2) a été divisée en deux parties spéculaires (A,B) afin d'avoir une zone polluée et la référence. Deux systèmes différents de pulvérisation nanoparticules ont été mis en place: le premier consiste en une combustion du bois ou de coke intégré dans une solution alcoolique de Cuivre et Cobalt nanoparticules avec la suite de l'émission de la micro et nanométriques sous-produits dans la serre. Le deuxième système est un pulvérisateur approprié de nanoparticules à partir de solution d'eau des NPs

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d'ingénierie de Cobalt, Nickel, Argent, Titania, Cérine. Plantes (tomate, riz, *tillandsia* et de moss) et les insectes (*Ceratitis capitata*) ont été exposés aux NPs selon des protocoles spécifiques, ainsi que des modèles animaux aquatiques marins: vers de terre (*Lumbricus rubellus*), oursin (*Paracentrotus lividus)*, artemia (*Artemia salina)*, le poisson zèbre (*Danio rerio)*, barnacle (*Balanus amphitrite*). Les résultats indiquent que les infirmières praticiennes produire des effets dans Photosinthesis et dans la prochaine génération.
Mots clés: *nanoparticules ; nanotoxicité ; nanoecotoxicité*

Riassunto

E' stata costruita una serra climatizzata per verificare l'impatto delle nanoparticelle su modelli di piante e animali di piccole dimensioni. L'apparato della serra di 40x4m^3 è stato suddiviso in due parti speculari (A,B) in modo da avere un'area in cui spraiare nanoparticelle ed una di riferimento, pulita. Sono stati installati due diversi sistemi di diffusione di nanoparticelle: il primo consiste nella combustione di pellet di legno o carbone i quali sono irrorati di soluzioni alcooliche contenenti nanoparticelle di Rame o di Cobalto. I fumi prodotti dalla combustione vengono poi veicolati nella serra B. Il secondo sistema è uno spraiatore dotato di un ugello che spruzza nanoparticelle di circa 200nm partendo da soluzioni acquose di nanoparticelle ingegnerizzate di Cobalto, Nichel, Argento, Titanio, Cerio. Piante di pomodoro, riso, muschio e tillandsia, ed insetti (*Ceratitis capitata*) vengono esposti alle NPs per spraiatura o irrorazione secondo protocolli specifici; così come modelli di animali acquatici marini: vermi Terra (*Lumbricus rubellus*) di riccio di mare (*Paracentrotus lividus*), crostacei (*Artemia salina*), Zebrafish (*Danio rerio*), balanidi (*Anfitrite Balanus*). I risultati indicano che le NPs sono in grado di produrre alcuni effetti sul processo fotosintetico e danni allo stato di sviluppo embrionale nei ricci di mare.
Parole chiave: *nanoparticelle; nanotossicologia; nanoecotossicologia*

Introduction

Nanotechnological products could potentially offer great benefits to consumer through the development of products containing NPs that have novel, incredible properties that the same material, but in bulk shape do not present. The use and following exposure of nano-sized materials with a size similar that of the vital components of the human and animal life (proteins, enzymes, DNA) could also present new risks which have not been properly evacuate. From this consideration the worldwide concern of scientists about potential risks of NPs on human and environmental health (Donaldson et al., 2004; Meyer & Kuusi, 2004; RS-RAE, 2004; Ostiguy et la., 2006; Wiesner & Bottero, 2007). The broad spectrum of nanoparticles (NPs) and nanomaterials used everyday in agricultural, electronic, cosmetic and medical products, makes it urgent to assess their possible toxic impact on living organisms and in particular on human health. The NPs have a tiny dimension able to interact directly with bacteria, proteins, viruses and the DNA. There is another concern bound to their impact on the environment at the end of

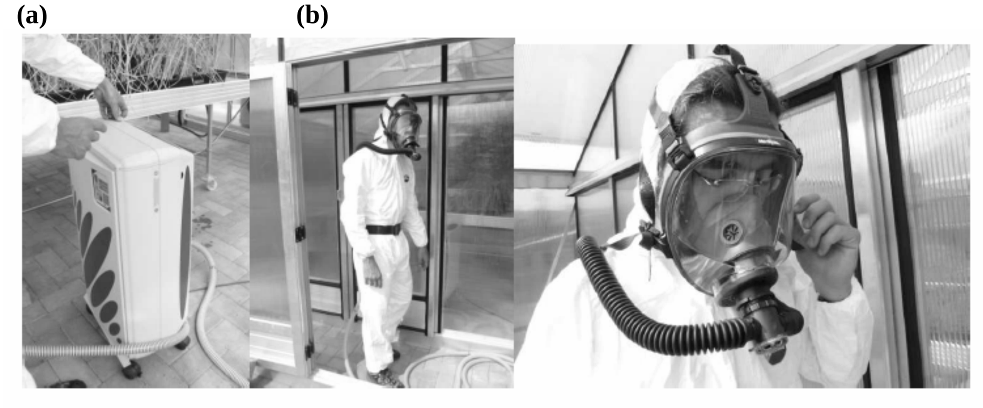
their life cycle that needs a LCA (Life Cycle Assessment). Currently nanoproducts, at the end of their use, are incinerated, so the possibility of their free and uncontrolled dispersion into the environment is real.
The article presents a facility that was constructed to verify the impact of NPs on plant models within the INESE project (investigations on the Impact of Nanoparticles in Environmental Sustainability and Ecotoxicity**)** financed by the Italian Institute of Technology.

Materials and Methods

The greenhouse. To perform toxicological investigations of NPs on vegetables and living organisms, in Modena a greenhouse (10x4x m^2) was built and provided with an automatic irrigation, air conditioning and shadowing systems (Fig.1). Into the greenhouse two cubicles were set up dividing the space to be polluted with fumigations (B) from a clean "control" area (A). The plant models are grown in both the cubicles, in order to be able to compare eventual growth or behavioral differences and to evaluate the acute effects of such NPs on leaf photosynthesis and flower/fruit development. The cubicle contaminated with NPs was equipped with two certified NPs filtering devices (so-called "absolute" filters) in order not to disperse the NPs sprayed in the environment and with no connection with the external part. In absence of data about possible risks due to an exposure to NPs, the operators who worked there protected themselves with a mask connected through a tube to an external air generator (Dragaer, Germany) and an electro filter worked at the end of every test in order to abate the nanopollution (Fig.1).

Figure 1 - *Special care was taken to protect the operators who worked in part B of the greenhouse: (a) electrofilter; (b) facial mask with external air supplier*

(a) **(b)**



The pellet stove combustor. A pellet stove was installed and connected to the greenhouse (Fig.2b). Through the combustion of wood pellet or coke added with selected metal-core NPS, 'fumigations' have been performed and were mainly aimed at characterizing the ecotoxicity of an NP-laden ambient-air upon living

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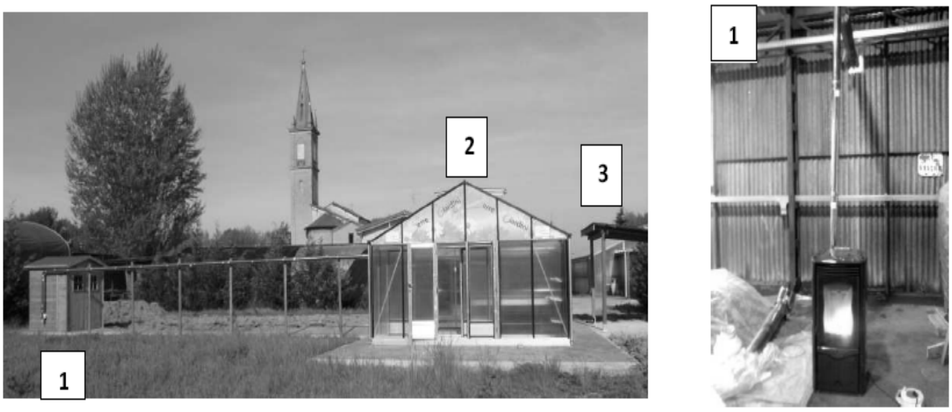
species. The stoves manufactured by Thermorossi SpA (Vicenza) correspond, ahead of the necessary technical adaptations, to the model named "Ecotherm 3001" (12 kW thermal power). The adaptation of the stoves and their technological conversion to the specific duty of the INESE project was made at the manufacturer's shop according to UNIGE-DIMSET (Dipartimento di Macchine, Sistemi Energetici e Trasporti) design specifications.

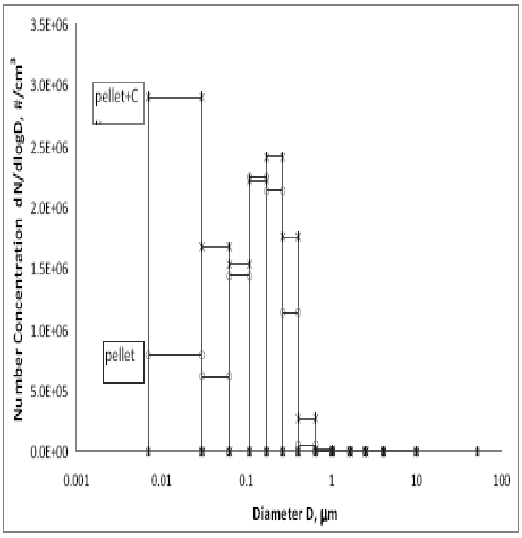
Figure 2 – *The greenhouse (2) with the generator of nanoparticles (1) and the automatic irrigation system (3).*



The same department has also carefully selected and followed the supply of two certified nano-particles filtering devices (so-called "absolute" filters), specifically conceived for guaranteeing the "absolutely safe" containment of nano-particles presence inside the rigorously confined room of the greenhouse dedicated to NP fumigation. By this means, any dangerous outside escape of nano-particles will be safely and strictly prevented. The combustor activity is documented by the NP measurements performed inside the experimental greenhouse after fumigation and the incineration of selected nanoproducts has been carried out, by conducting several incineration campaigns of wood pellets, used as fuel for the pilot stoves. In order to pursue the "evaluation of incineration residues" a proper strategy, and related provisions, had to be conceived and realized. In presence of NPs, the issue is not just that of 'filtering and separating' them (in order to monitor and analyse their concentration, typology, diameters, composition, etc.) but also that of taking care of the related health hazards for humans, animals and the environment surrounding the NP production processes. The campaigns of measures of exposure were performed by ELPI Dekati (Fig.3b) and Aerasense systems (Philips) used to evaluate the NPs concentrations and NPs size. This consists of an Electrostatic Low Pressure Instrument (ELPI, Dekati) suitable to measure the number concentration of the in-stream particles in the size range between 7 mm and 10 mm (Fig.3).

Figure 3 – *Number concentration distribution of particles measured in the greenhouse containing nanosizedby-products from a stove fuelled with pure pellet and pellet coated with Cu nanoparticles (a) and NPs measurement system (ELPI) (b)*

(a)



(b)

The nanoparticles tested. The following NPs were selected for the ecotoxicity tests:

1- Copper and Cobalt dispersed in alcohol for the combustion tests (Nanoamor, USA; size 50-120nm)

2- dry NPs of Titanium dioxide (10-30nm), Cerium oxide (size 15-30nm), Cobalt (size 28nm), Nickel (size 20nm) dispersed in distilled water (Nanoamor, USA; 5mg/l dispersion in water, sonicated)

3- Silver (colloidal dispersion in water Polytech, Germany; size:1-10nm, conc=1000ppm, pH 9).

The aerosol generator. Since the dispersion of NPs by combustion involved also the emission of gases and vapours, it was set up another systems of dispersion of engineered NPs in the cubicle B of the greenhouse.

To evaluate the acute effects of NPs on leaf photosynthesis and flower/fruit development of *Microtom* tomato plants seedlings and *Tillandsia*, an aerosol system was designed to expose crop plants to airborne NPs. Aerosol generator emitted into an isolation plastic glove box (Erlab, Salisbury, UK; base area of 0.5m^2; approximate volume of 90 l an aqueous suspension of NPs at a rate of 0.3g/min and with a mean droplet diameter of 0.35µm. Aerosol generation lasted 5 min, therefore the amount of NPs applied in occasion of each treatment is in the order of 1.5mg.

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Figure 4 - *Number and mass concentration distribution measured in the greenhouse during the spray of Ag particles suspended in water.*

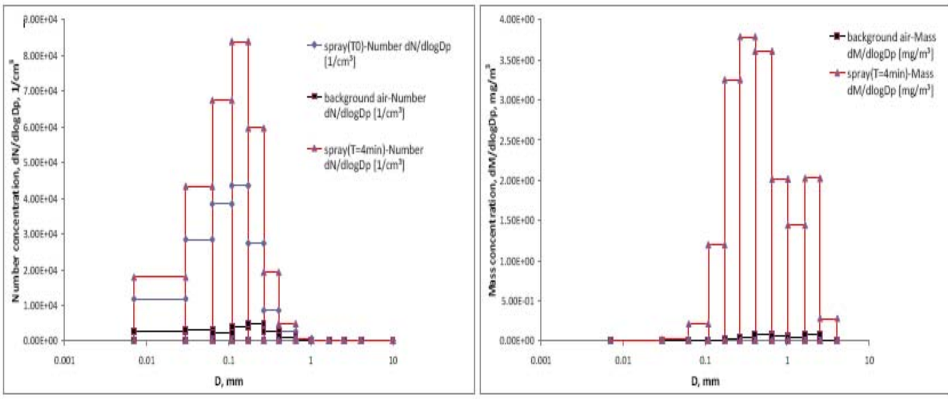

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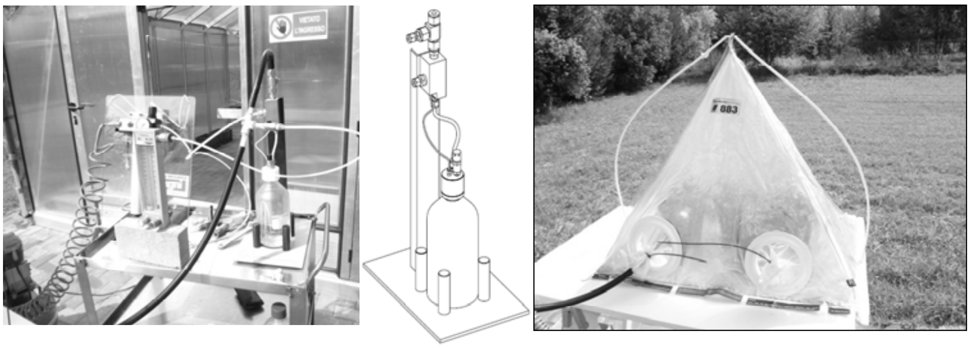
Figure 5 - *NPs Aerosolization system and its scheme and the small disposable table greenhouse where the spray of Microtom and tillandsia were performed.*



Animal models. We used soil and aquatic animal models and induced a chronic contact with NPs dispersed in soil and sea water: 1- Sea urchin *Paracentrotus lividus*, 2-Brine shrimp *Artemia salina* , 3-Zebrafish *Danio rerio*, 4-Barnacle *Balanus amphitrite.* Soil animals: 1- Worms *Lumbricus rubellus,* 2- Flyes *Ceratitis capitata.*

Both the models were exposed to different concentrations of the above quoted NPs for different times. The exposure was controlled checking the internal presence (real uptake) of NPs by means of Environmental Scanning Electron Microscopic observations and Energy Dispersive analyses of an X-ray microprobe.

Results

Preliminary data from the exposure of organisms to different NPs, in particular to Silver with which it was made the first cycle of treatment, indicating specific effects on life cycles of organisms both animal and plants: abnormalities in fertility and growth capacity, dislocation of NPs in individual cells very different from those of insertion or display. The Fig. 7 shows the presence of Silver NPs inside the tomato leaf, that probably went into through the stomata.

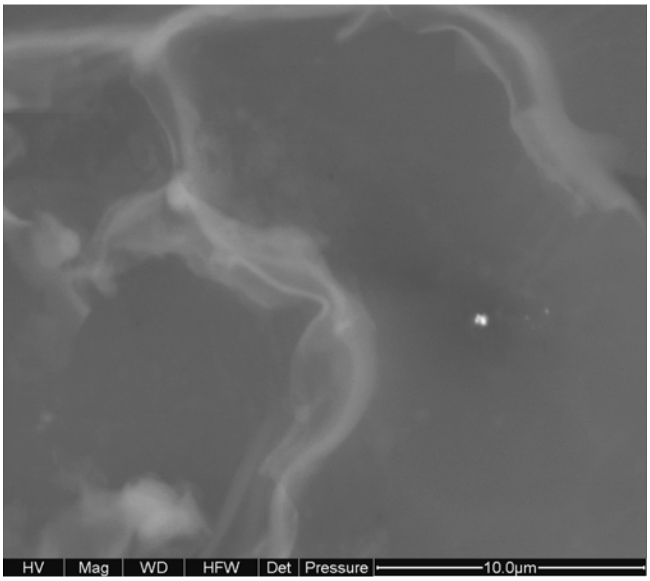
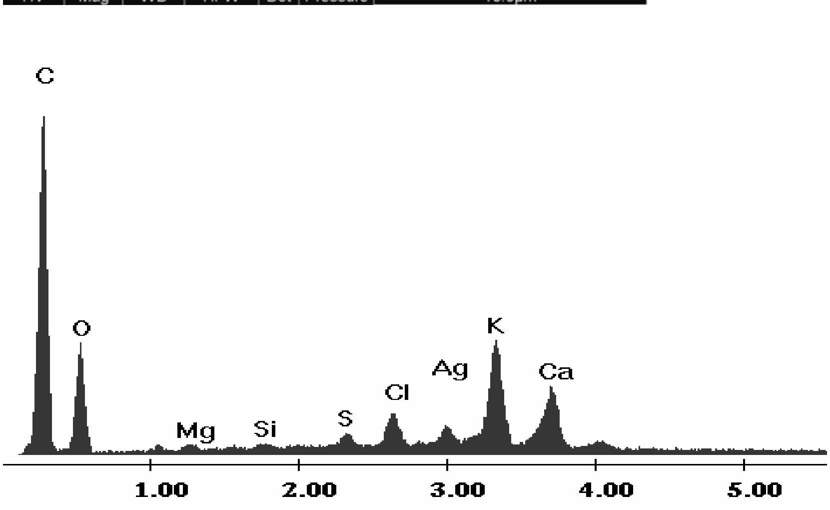


Figure 7

Silver NPs found in core of a leaaf exposed to NPs aerosolization with the EDS spectrum of the Silver nanoparticle and the biological substrate containing Carbon, Oxygen, Silver, Potassium, Silver, Calcium Chlorine, Sulphur and Magnesium



Organisms selected for in vivo testing were all proved to be responsive to NPs exposure. The experiments have proved to be even more effective in long-term: the effects of NPs are revealed very strong on successive generations to those of application. The first generation usually can adapt to their presence and sometimes, as in the case of the application of the Silver on plants, gain from its antibacterial

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action. The same bactericidal action may be due to the effects of a decrease in the presence of intestinal flora within earthworms' intestines subjected to experiments.

Discussion

The tests on plants will continue for a year more, sowing seeds on contaminated soils and trying to figure out whether it is more effective spraying specimens or their contamination by NPs irrigation. For example, in the case of tomato plants sprayed by NPs, we had problems locating the NPs in the leaves surface, while they were found inside the leaf tissue and in correspondence of stomata rhyme. We are analyzing leaf sections to ascertain the location of the substances inside the leaf and possibly of the seeds and fruits grown after and during the treatments. The decreased fertility in animal organisms, their ability to interact with the environment and the potential for seed germination of the treated plants are some of the registered outcomes, and further testing may reveal additional alterations. As for the experiments on earthworms, for example, a difference in lipids concentration is noticed (concentration saturated fat / unsaturated) in those fed with NPs contaminated soils, and from the literature we know that this is a stress index . The contamination of soils with NPs, already present in nature because of the use of pesticides containing the same, threatens biodiversity and ecological edaphon soil function: we continue to test the soils treated with NPs and the responses of microbial biomass and its functions in the soil, then test their DNA. After a only 30 days treatment with nanoparticles of silver, a change in the microbial fauna in the soil was diagnosticated, and this is very interesting considering the fact that pesticides on sale containing nanoparticles: this can produce changing in soil biodiversity, with all the consequences of the case.

Acknowledgements

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