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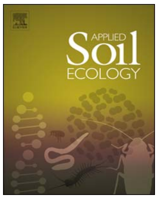

Engineered nanoparticles effects in soil-plant system: Basil (*Ocimum basilicum* L.) study case

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ABSTRACT

The aim of this study was to examine the effects of selected metal (Ag, Co, Ni) and metal oxide (CeO_2, Fe_3O_4, SnO_2, TiO_2) engineered nanoparticles on basil (*Ocimum basilicum* L.). Seedlings, grown in soil mixture (20% sandy soil, 80% peat), were exposed to nanoparticles once per week, for 4 weeks with solutions at 100 $\mu g\,mL^{-1}$ of nanoparticle component metal, to simulate a chronic exposure to NPs supplied with irrigation. At the end of the experiment (4 weeks), (i) morphological and physiological parameters of basil (e.g. dry weight, gaseous exchange), (ii) nanoparticle component metal taken up by the basil plant (namely, Ag, Ce, Co, Fe, Ni, Sn and Ti) and (iii) the content of nutrients (Ca, Mg, K, Na, P and S) in different basil organs were evaluated. The results indicated that the nanoparticle component metal was mainly accumulated in the basil roots. However, despite the capability of plant to exclude potentially toxic elements, Ag, CeO_2, Co and Ni NPs component metal translocate from the root to the shoot reaching the leaves, the edible part of the plant. Notably, also in the relative short exposure there was an accumulation of Ca in roots, suggesting that the modification of metabolic pathway in plants could be aimed at counteracting the membrane damage generated directly or indirectly by nanoparticles.

1. Introduction

Engineered nanomaterials (ENMs) have at least one dimension between 1 and 100 nm (Ball, 2002; Roco, 2003), giving them high physicochemical reactivity compared to bulk material. ENMs characterized by all three dimensions in the nanoscale (< 100 nm) are defined as nanoparticles (NPs), they can be made from a great variety of substances (organic and inorganic), their behaviours depends on chemical composition and on the size and/or shape of particles (Brunner et al., 2006). Three different type of NPs can be observed as a function of their origin: natural, incidental and engineered. Natural NPs result by natural processes (e.g. volcanic dust, soil mineral colloids, soil humic substances), while incidental NPs are involuntarily produced during anthropic activity, such as coal combustion, welding fumes, diesel exhaust particulate, etc. Nowadays, engineered NPs have attracted the most attention due to their increased use in many industrial sectors for a wide range of application, e.g. pharmaceutics, cosmetics, transportation, energy and agriculture, including consumer products (Nowack and Bucheli, 2007).

Silver (Ag-NP), iron (Fe_2O_3, Fe_3O_4, zero-valent NPs), titanium dioxide (TiO_2-NP) and cerium dioxide (CeO_2-NP) are among the most used nanomaterials in the manufacturing industry (OECD, 2010), however other metal and metallic oxides NPs are produced and can enter into the market, such as Co, Ni, SnO_2 (Fernandez-Garcia et al., 2011; Magaye and Zhao, 2012). In a future perspective, these products and/or their residues will end up in incinerator, landfill and sewage sludge, increasing the probability of the interaction with the environment. Probability models (Gottschalk et al., 2009) suggest that NPs can reach soil through the irrigation of sewage water or sewage sludge, but also with the application of fertilizers and plant protection products (Batley et al., 2013), biosolids (Benn and Westerhoff, 2008), or the flooding of floodplains (Lecoanet et al., 2004). Plants are in close contact with soil, water and atmospheric environmental compartments, which can convey ENMs (Miralles et al., 2012). For this reason, plants have been used as bioindicators to evaluate the bioavailability and mobility of pollutants in soil (Andén et al., 2004).

To date, a wide variety of effects of NPs on plants has been observed and several endpoints have been applied: germination, seedling growth, cytotoxicity and genotoxicity (Miralles et al., 2012). In addition, ENM uptake and bioaccumulation have been investigated in crop species, such as *Triticum aestivum* (Wild and Jones, 2009), *Oryza sativa* (Lin et al., 2009), *Cucurbita pepo* and *Cucurbita maxima* (González-Melendi

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et al., 2008; Zhu et al. 2008; Corredor et al., 2009). Most of the studies assessing the phytotoxicity of ENMs in plants have been conducted with *in vitro* model (Schwabe et al., 2013; Lee et al., 2008), with the aim of understanding NPs behaviour in a standardized media. It can be misleading since the amount of nanoparticles available to soil biota and crops is affected by soil properties (Rico et al., 2011; Vittori Antisari et al., 2013). In addition, experiments carried out in aqueous suspension or Hoagland's solution usually applied high rates of NPs, ranging from 1000 to 4000 mg L^{-1} (Rico et al., 2011). Indeed, the environmental concentrations will likely range from ng L^{-1} or ng kg^{-1} for most ENMs (Mueller and Nowack, 2008). The use of higher level of NPs showed inhibition of germination and root growth of various plant species (López-Moreno et al., 2010) or caused death of almost all living cells at the root tip (Lin and Xin, 2008). Indeed, ENMs interact with plants penetrating root cells (Geisler-Lee et al. 2013), but the exact uptake mechanisms are not fully elucidated (Gardea-Torresdey et al., 2014).

Basil (*Ocimum basilicum* L.) is an aromatic herb, largely used in Mediterranean and Asian cuisine for their fresh leaves flavour or for extracting essential oil. In both cases, the edible product should not contain metal to avoid bio-magnification of trace element in food chain. This species is easy to cultivate and has a fast growing cycle; for this reason, it has been chosen as test species for the experiment.

The aim of this work was to monitor the effects of the different NPs added at chronic supplying (5 mg per pot) on the following: (i) morphological and physiological parameters (e.g. dry weight, gaseous exchange), (ii) NP component metal taken up by the basil plant (namely, Ag, Ce, Co, Fe, Ni, Sn and Ti) and (iii) the content of nutrients (Ca, Mg, K, Na, P and S) in different basil organs.

2. Material and methods

2.1. Nanoparticles characteristics

The nanoparticles examined in this study were the following: Ag, CeO_2, Co, Fe_3O_4, Ni, SnO_2, and TiO_2. Ag NPs were obtained from Polytech (Germany, type WM 1000-c), as a 1000 mg L^{-1} suspension in deionised water with polyvinylpyrrolidone (PVP) coated metallic silver (Ag); the NP size ranged between 1 and 10 nm. CeO_2, Co, Fe_3O_4, Ni, and SnO_2 powders were purchased from Nanostructured & Amorphous Materials, Inc. (Houston, USA) with at least 98% purity. TiO_2 powder was purchased from Tal Materials, INC, USA. The hydrodynamic diameter and zeta potential of nanoparticle fresh suspension was obtained with the technique of Photon Correlation Spectroscopy using a Zetasizer Nano ZS (Malvern Instruments, UK). The samples were measured 3 times, and the analysis were performed at 25 °C with an angle of 90°, the data are shown as a function of the number. Table 1 reports the NPs' characteristics.

NP suspensions were freshly prepared before material spiking as follows: NPs were weighed with an analytical scale, suspended in deionised water to bring them to the required concentrations (100 mg L^{-1}) and dispersed by ultrasonic vibration (100 W, 40 kHz; S100, Elmasonic, Germany) for one hour. The Ag solution did not need further sonication after the dilution since the PVP maintain the particles in suspension. Fig. 1 shows some examples of pristine NPs observed by high-resolution transmission electron microscopy (HRTEM) (Tecnai G2 F30 transmission electron microscope); generally, the NPs tend to form aggregates in water suspension (Klaine et al., 2008).

Table 1
NPs characteristics furnished by the supplier (shape, purity, nominal particle size, specific surface area) and measured by Zetasizer Nano ZS (Malvern Instruments, UK).

Material	Shape	Purity (%)	Nominal particles size (nm)	Specific surface area ($m^2 g^{-1}$)	Average hydrated diameter (nm)	Z-potential (mV)
Ag	–	–	10	–	60.3	−32.5
CeO_2	Spherical	99.9	15–30	30–50	133.1	44.5
Co	Spherical	99.8	28	40–60	102	24.6
Fe_3O_4	Spherical	99.0	20–30	> 40	1407	10.6
Ni	Spherical	99+	62	6.2	682.2	27.9
SnO_2	Faceted	99.5	61	14	40.2	−47.7
TiO_2	–	–	20–160	–	999.0	−11.6

2.2. Experimental design

The experiment was carried out growing *O. basilicum* plants in the greenhouse at 25–20 °C day-night temperature, with a 14-h photoperiod. The seedlings were placed in pots of 250 cm^3 filled with the soil mixture made of 10% carbonate sand 10% silica sand and 80% neutral sphagnum peat which represents an excellent growth medium due to high moisture and nutrient-holding capacity with an uniform and slow breakdown-rate of physical structure (Ball et al., 2000).

Forty-eight pots (6 pots for control test and each NPs) were placed in a randomized block. After two weeks of adaption, the seedlings were spiked once per week with 50 mL of Ag, CeO_2, Co, Fe_3O_4, Ni, SnO_2 and TiO_2 NP solutions at 100 mg metal L^{-1} concentrations, to simulate a chronic dose of NPs supplied with irrigation. The contaminated water were added on the soil surface avoiding contact with the aerial part of the plants. For the control test only water was supplied. The treatment was repeated for 4 weeks adding 20 mg of NPs element per pot; the nominal concentration was 80 mg of NPs element per kg^{-1} of soil. Every week the plant growth was documented through leaf counting, whereas the physiological status was evaluated measuring the stomata conductance (see below) at 48 h after treatment. In addition, the photosynthetic efficiency (see below) was assessed after 48 h from the 2nd and 4th treatment.

2.3. Soil analyses

At the end of the experiment the soil was air dried, sieved (< 2 mm) and finely ground with a mixer mill (MM 200, Retsch, Germany) for chemical characterization.

Soil pH was determined potentially in a both soil/distilled water and soil/$CaCl_2$ 0.01 M salt solution (1:2.5 w/v) suspension with a glass electrode (Compact Titrator, Crison, Spain). The soil cation exchange capacity (CEC) was determined in a soil/hexamminecobalt trichloride solution 0.05 N (1:20 w/v) under 2 h of horizontal shaking (ISO, 2007). Suspension were filtered (Whatman®42) and analysed by inductively coupled plasma with optical emission spectrometry (ICP-OES, Ametek, Arcos Spectro).

The metal contents was determined according to Vittori Antisari et al. (2013). Briefly, the soil (0.25 g) was treated with *aqua regia* (2 mL HNO_3 65% plus 6 mL HCl 37%, suprapur grade Carlo Erba) in a microwave oven (Start D 1200, Milestone, USA) and the metal concentrations were determined by ICP-OES. The analysis of each sample was replicated three times and compared with analyses of the International Reference Materials (BCR 141) and laboratory internal standards (MO and ML), which was run after every 10 samples to check changes in sensitivity. Controls with only reagents were also determined.

In this study, according to Semple et al. (2004), we differentiate between bioaccessibility, assessed by chemical extraction techniques, and bioavailability, assessed by quantifying the concentration of ENMs in organisms.

Due to the soil pH (7.5) the amount of accessible metals in soil samples was determined by soil extraction using 0.005 M diethylenetriamine-penta-acetic acid (DTPA) solution with a 1:2 ratio w/v according to Lindsay and Norvell (1978). After two hours of shaking, the soil suspension was centrifuged for 15 min at 1200*g* and filtered through Whatman® 42 and the concentration of elements in soil extracts was determined by ICP-OES.