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 μg 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.

Keywords: Nanoparticles; Basil (*Ocimum basilicum* L.); Pollution; Soil; Inductively coupled plasma-optical emission spectrometry

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 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.

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

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).

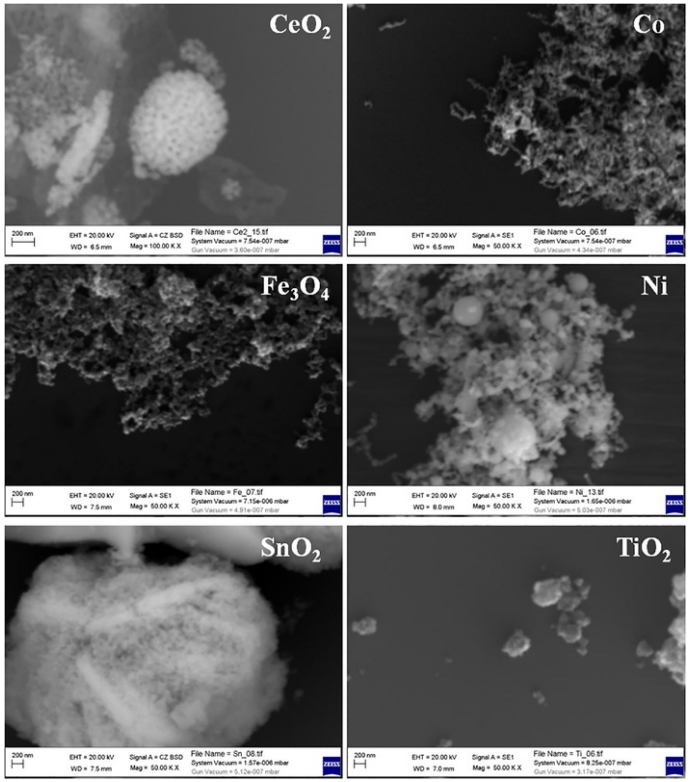

Fig. 1 Examples of pristine NPs observed by high-resolution transmission electron microscopy (HRTEM).

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 diethylene-triamine-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.

Water extraction was carried out by shaking the soil-water suspension, with a ratio of 1:10 w/v, for 16 h (Blaser et al., 2000). Both soil suspensions were centrifuged for 15 min at 1200*g*; then the supernatant were filtered through 0.45 µm filter HTTP (Millipore, USA). The concentration of elements in the soil extracts was determined by ICP-OES.

Finally, the partition coefficient (Kp) was calculated according to the following equation:

$$Kp = [metal]_{soil\ fine\ earth}/[metal]_{water\ extract}$$

where Kp is the solid/water partition coefficient ($L\,kg^{-1}$); $[metal]_{soil\ fine\ earth}$ is the total metal concentration in soil determined in AR ($mg\,kg^{-1}$) and $[metal]_{water\ extract}$ is the free ion concentration extracted in water ($mg\ L^{-1}$) at equilibrium conditions (after 16 h) (Blaser et al., 2000). The data are expressed as log Kp (Vittori Antisari et al., 2013).

2.4 Plant sampling and vegetal tissues analysis

At the end of the experiment, 28 days after the first NP treatment, each plant was harvested, separated into aerial part (stems and leaves) and root, washed with deionised water and then prepared for the following analysis: fresh and dry biomass, nutrients, total NPs component metal content, chlorophylls *a* and *b*, carotenoid and xanthophylls content and lipid peroxidation.

The root system of each plant was removed from the bulk soil and the roots were washed with distilled water and oven-dried (48 h at 60 °C) to perform dry mass.

Dry tissues of different organs of each basil plant were finely grounded and digested using a nitric acid and oxygen peroxide solution in the microwave oven according to the United States Environmental Protection Agency USEPA (2009) method, modified by Vittori Antisari et al. (2014). Approximately 0.25 g sub-sample of plant tissue was treated with 6 mL of concentrated suprapur nitric acid (Merck) plus 1.5 mL of hydrogen peroxide (Carlo Erba for electronic use). The mineralization was carried out in PTFE vessels in the microwave oven, and both the content of nutrients (Ca, Mg, K, Na, P, S) and the component metal (Ag, Co, Ce, Fe, Ni, Sn, Ti) in leaves, stems and roots, were quantified by ICP-OES. Blank and International Reference Materials (Olive leaves BCR-CRM 062) were analysed to validate the method. In addition, standard solutions (0.5 $mg\,L^{-1}$ Ag, Ce, Co, Ni, Sn) were analysed every 10 samples for quality control/quality assurance purposes.

2.5 Basil physiological parameters

Analysis of gaseous exchange and stomata conductance are early indicators of plant stress, indeed both functions change rapidly in the presence of harmful factors and they can be measured with rapid and non-destructive techniques.

The stomata conductance ($mmol\,m^{-2}\,s^{-1}$) was measured 48 h after the NP treatment at 0, 7, 14, and 21 days, on six plants per treatment with the SC-1 Leaf Porometer (Decagon Devices, Inc., USA).

The leaf gas exchange (H_2O and CO_2 gas) was measured on attached leaf samples with an infrared portable CIRAS-2 (PP-System®, Hitchin, UK). This instrument consists of an infrared differential analyser (IRGA) connected to an automatic assimilation chamber (Parkinson's Automatic Universal Leaf Cuvette, PAR 1000 $mmol\,m^{-2}\,s^{-1}$, 26 °C, CO_2 13.63 $mmol\,L^{-1}$ and 300 $cm^3\,min^{-1}$ flow rate) and equipped with 18 mm diameter, 2.5-cm^2 area cuvette inserts. Leaf transpiration rate (E, $mmol\,m^{-2}\,s^{-1}$), stomatal conductance (Gs, $mmol\,m^{-2}\,s^{-1}$) and net photosynthesis (Pn, $\mu mol\,m^{-2}\,s^{-1}$) were measured 48 h after the NP treatment at day 14 and 21, on six plants per treatment.

In addition, at the end of the experiment leaf pigments and lipid peroxidation content were determined to evaluate possible NP impact on crop development and physiology.

Plant pigments were extracted from freeze-dried tissues according to Strickland and Parsons (1972). Briefly, 0.1 g of leaf samples, from each treated plant, were milled in a mortar and 10 mg of magnesium carbonate ($MgCO_3$) were added to neutralize solute acidity and to prevent the chlorophyll conversion in phaeophytin. Finally, 10 mL of acetone was added to the milled material and then incubated for 12 h in complete darkness. Subsequently, the samples were centrifuged at 10 °C for 10 min at 11,180*g*. Pigment content was then evaluated by measuring absorbance at the wavelengths maxima (470, 645 and 662 nm) for the solvent used in the extraction (pure acetone) (Moran, 1982). An aliquot of the supernatant was collected (1cm cuvette) to perform the spectrophotometer analysis (DU 530, Beckman Coulter Inc., USA). The supernatant solutions were diluted by adding acetone, as necessary, to obtain a

spectrophotometer reading in the range of 0.2 ~~to~~ -0.8 absorbance units at wavelengths of 645 nm and 662 nm.

The concentration of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and the sum of leaf carotenoids and xanthophylls (c + x) were calculated using the following equations of Lichtenthaler and Buschmann (2001):

- Chl *a* (µg/mL) = [11,24*(abs 662) − 2,04*(abs 645)]*dilution factor;
- Chl *b* (µg/mL) = [20,13*(abs 645) − 4,19*(abs 662)]*dilution factor;
- c + x (µg/mL) = [(1000*(abs 470) − 1,90*(abs 662) − 63,14*(abs 645)/214]*dilution factor.

Lipid peroxidation was measured as the amount of TBARS determined by the thiobarbituric acid (TBA) reaction as described by Hernandez and Almansa (2002). Fresh leaves (0.2 g) were homogenized in 1 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000*g* for 10 min. To 0.5 mL of the aliquot of the supernatant, 1.5 mL of 20% TCA containing 0.5% (w/v) TBA was added. The mixture was heated at 90 °C for 20 min and then quickly cooled on ice. The contents were centrifuged at 10,000*g* for 5 min and the absorbance was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using a TBA acid calibration curve.

2.6 Bio-concentration factor (BCF) and translocation factor (Tf)

According to Zayed et al. (1998), the metal bioconcentration factor (BCF) of root system and aerial part (stem + leaves) was calculated as follows:

BCF = (metal concentration in the harvested plant material mg kg^{-1}/metal concentration in the soil mg kg^{-1})

The translocation factor (Tf) was calculated to synthesize the capability of the species to translocate nutrients and contaminants from roots to shoots (Zacchini et al., 2009), according to the following equations:

Tf = (metal concentration in the aerial parts mg kg^{-1}/metal concentration in the roots mg kg^{-1})*100.

2.7 Environmental Scanning Electron Microscopy (ESEM) analysis

Detection of NPs inside the tissues of tomato organs were identified by FEG-ESEM (Field Emission Gun – Environmental Scanning Electron Microscope) investigations and EDS (Energy Dispersive X-ray Spectroscopy) analyses to classify univocally the engineered NPs and differentiate them from the natural particles. The electron microscope analyses were performed in Secondary Electron and Back Scattered Electron Diffraction mode, in order to obtain information on the morphology of the samples and on chemical nature of the NPs.

2.8 Data analysis

The results are presented as means (±standard deviation); significant differences were determined by one-way ANOVA and Tukey's HSD test using the R package (version 2.14.0) (The R Foundation for Statistical Computing, Vienna, Austria, 2011).

3 Results

3.1 Soil chemical characterization and accessibility of NPs component metal

Soil pH_{H2O} values (7.5 ± 0.1) was not statistically affected by NPs contamination during the experimentation, while differences were noted among the pH_{CaCl2} values, which decreased from 7.0 to 6.5 in Ag-NP treatment. The total CEC of soils of all NPs treatments decreased with respect to the control ($p < 0.05$) as follows CTR > Co > Fe_3O_4 ≥ Ag = TiO_2 ≥ CeO_2 ≥ SnO_2 ≥ Ni and the NPs addition affected both Ca and Mg exchangeable bases (data not show).

The total concentration of NPs component metal in treated soil after dissolution in *aqua regia* is shown in Table 2. The component metal concentration in the soil significantly increased over the control, except for soil polluted with Fe_3O_4 and TiO_2 NPs.

Table 2 Concentrations of NPs component metal Ag, Ce, Co, Fe, Ni, Sn and Ti found in basil soil. After different extraction: DTPA, H_2O and *aqua regia* (AR) at the end of the experiment. The logarithm values of partition coefficients (Kp) are reported. One-way ANOVA and Tukey's test ($p < 0.05$) were used to determine statistical significance of the differences between treatment and the control means. $P < 0.01$ **, $P < 0.05$ *; ns is not significant. DL is detection limit; the values of DL in DTPA for Ag and Sn were 0.006. 0.02 mg kg^{-1}, respectively. DL in H_2O for Ce and Sn were 0.03. 0.004 µg L^{-1}, respectively.

	DTPA		H_2O		AR		log (Kp)	
	mg kg^{-1}	SD	µg L^{-1}	SD	mg kg^{-1}	SD	L kg^{-1}	SD
Control	DL		11.8	*0.6*	0.1	*0.0*	0.9	*0.0*
Ag-NPs	0.1	*0.0*	263.0	*13.2*	61.9	*0.2*	2.4	*0.1*
ANOVA			*		**		**	
Control	0.4	*0.0*	DL		30.9	*3.0*	2.9	*0.1*
CeO_2-NPs	0.5	*0.0*	669.3	*33.5*	124.7	*0.8*	2.3	*0.1*
ANOVA	ns				**		*	
Control	0.2	*0.0*	25.3	*1.3*	7.8	*0.2*	2.5	*0.1*
Co	10.7	*0.5*	306.7	*15.3*	56.8	*1.1*	2.3	*0.1*
ANOVA	*		**		**		*	
Control	19.2	*1.0*	3868	*193.4*	12,743	*724*	3.5	*0.2*
Fe_3O_4-NPs	20.4	*1.0*	2867	*143.4*	12,576	*373*	3.6	*0.2*
ANOVA	ns		*		ns		ns	
Control	0.5	*0.0*	23.4	*1.2*	34.7	*0.2*	3.2	*0.2*
Ni-NPs	1.8	*0.1*	77.3	*3.9*	106.8	*0.6*	3.1	*0.2*
ANOVA	*		*		**		ns	
Control	DL		DL		1.1	*0.2*	2.4	*0.1*
SnO_2-NPs	DL		97.9	*4.9*	3.9	*1.5*	1.6	*0.1*
ANOVA					*		*	
Control	51.4	*2.6*	252.2	*12.6*	854.5	*95*	3.5	*0.2*
TiO_2-NPs	57.3	*2.9*	666.3	*33.3*	795.7	*4.4*	3.1	*0.2*
ANOVA	ns		*		ns		ns	

The accessibility of NPs component metal in soil was determined using solution with different ionic strength and it decreased as follows: AR > DTPA > H_2O for Co, Fe_3O_4, Ni and TiO_2 NPs (Table 2). The recovery of metals in DTPA and H_2O is comparable for Ag and CeO_2 NPs. SnO_2 NPs are only detectable in water and AR and the latter is lower than theoretical one (80 mg kg^{-1}), probably due to incomplete mineralization of SnO_2 NPs in the acid mix used for the soil analysis or re-precipitation (Ellison et al., 1998).

The log partition coefficient (log Kp) was used to evaluate the affinity of NPs component metal between solid and aqueous phase at the equilibrium. Fe_3O_4-, Ni- and TiO_2-NPs showed high values of log partition coefficient Kp (>2.8) while SnO_2-NPs treatment showed a partition coefficient log Kp equal to 1.6.

3.2 Plant growth parameters

The data obtained from the morphological parameters of basil plants are summarised in Table 3. The plants exposed to NPs did not show significant differences in vegetative growth based on the leaf counts (data not shown). However, basil exposed to Ag NPs exhibited a significant reduction of root and leaf dry matter, 30.4% and 14% respectively, as compared to the control. Fe_3O_4, Ni and TiO_2 NPs determined higher root dry matter than the control; while no significant difference was found in the stem dry weight.

Table 3 Effect of NPs treatments on dry matter of roots, stems and leaves of *O. basilicum* plants grown in pots. Means followed by a different letter are significantly different at $P < 0.05$ according to the One-way ANOVA and Tukey's test.

Treatment	Dry matter (g dw per pot)								
	Root	*SD*		Stem	*SD*		Leaves	*SD*	
Control	0.46	*0.03*	ab	0.31	*0.04*	a	1.00	*0.08*	a
Ag-NPs	0.32	*0.03*	b	0.30	*0.03*	a	0.86	*0.07*	b
CeO_2-NPs	0.45	*0.01*	ab	0.33	*0.02*	a	1.01	*0.05*	ab
Co-NPs	0.46	*0.03*	ab	0.33	*0.02*	a	1.03	*0.04*	ab
Fe_3O_4-NPs	0.47	*0.03*	a	0.33	*0.01*	a	1.02	*0.04*	ab
Ni-NPs	0.47	*0.02*	a	0.33	*0.02*	a	1.03	*0.02*	ab
SnO_2-NPs	0.45	*0.04*	ab	0.34	*0.04*	a	1.12	*0.06*	a
TiO_2-NPs	0.52	*0.02*	a	0.38	*0.02*	a	1.12	*0.43*	a

3.3 NPs metal content in basil tissues, bioconcentration and translocation factor

The NPs component metal determined in above- and below-ground organs of basil is shown in Table 4. A greater amount of NPs component metal is accumulated in basil roots, except for Fe_3O_4 and SnO_2 NPs treatment, which showed no significant differences, compared to the control. This behaviour was also confirmed by ESEM–EDS images (Fig. 2) where cluster of NPs was found on the basil roots.

Table 4 Comparison between the concentration of NPs elements of stem, leaves and root of basil affected by NPs treatments and the control. Bioaccumulation factor (BCF = [leaf] + [stem] or [roots]/[soil]). Translocation factor (TF = [leaf] + [stem]/[root]*100).

		Ag	*SD*	Ce	*SD*	Co	*SD*	Fe	*SD*	Ni	*SD*	Sn	*SD*	Ti	*SD*
		mg kg^{-1}													
Leaves	Treatment	1.4	*0.2*	1.7	*0.6*	3.3	*0.3*	131.7	*3.7*	3.8	*0.9*	0.3	*0.0*	2.87	*0.2*
	Control	0.2	*0.0*	DL		0.3	*0.0*	135.3	*4.5*	2.0	*0.2*	0.3	*0.0*	2.74	*0.0*
ANOVA		***				***		ns		***		ns		ns	
Stem	Treatment	2.1	*0.4*	DL		2.8	*0.2*	39.04	*0.6*	0.6	*0.2*	0.5	*0.0*	1.08	*0.0*
	Control	0.2	*0.0*	DL		0.3	*0.0*	45.27	*5.2*	0.4	*0.1*	0.5	*0.1*	1.33	*0.1*

ANOVA		***				***		ns		ns		ns		ns	
Root	Treatment	5.8	*0.3*	50.8	*1.2*	71.4	*8.8*	196.8	*18*	27.3	*1.2*	0.2	*0.1*	8.17	*0.2*
	Control	0.1	*0.0*	DL		1.2	*0.1*	128	*11*	1.9	*0.2*	0.1	*0.0*	3.74	*0.4*
ANOVA		***				***		ns		***		ns		***	
BCF		Ag	*SD*	Ce	*SD*	Co	*SD*	Fe	*SD*	Ni	*SD*	Sn	*SD*	Ti	*SD*
Aerial	Treatment	0.06	*0.0*	0.02	*0.0*	0.11	*0.0*	0.01	*0.0*	0.03	*0.0*	0.21	*0.0*	0.005	*0.0*
	Control	3.93	*0.5*	0.02	*0.0*	0.08	*0.0*	0.01	*0.0*	0.07	*0.0*	0.71	*0.1*	0.005	*0.0*
ANOVA		***		ns		*		ns		.		***		ns	
Root	Treatment	0.09	*0.0*	0.41	*0.0*	1.26	*0.3*	0.02	*0.0*	0.26	*0.0*	0.04	*0.1*	0.01	*0.0*
	Control	1.30	*0.0*	0.01	*0.0*	0.16	*0.0*	0.01	*0.0*	0.05	*0.0*	0.13	*0.1*	0.004	*0.0*
ANOVA		**		***		***		*		***		ns		***	
		Ag	*SD*	Ce	*SD*	Co	*SD*	Fe	*SD*	Ni	*SD*	Sn	*SD*	Ti	*SD*
		%													
Tf	Treatment	60.7	*2.2*	5.0	*2.3*	9.2	*1.6*	85.8	*7.6*	11.7	*3.8*	1635	*729*	47.0	*4.3*
	Control	304	*24*	221	*11*	53.7	*4.8*	141	*13*	127	*10*	947	*533*	111	*14*
ANOVA		***		***		***		*		***		ns		*	

DL was the instrumental detection limit for Ce 0.2 mg kg^{-1}.

One-way ANOVA and Tukey's test ($p < 0.05$) were used to determine statistical significance of the differences between treatment and the control means. $P < 0.001$ ***, $P < 0.01$ **, $P < 0.05$ *, $P < 0.1$., ns is not significant.

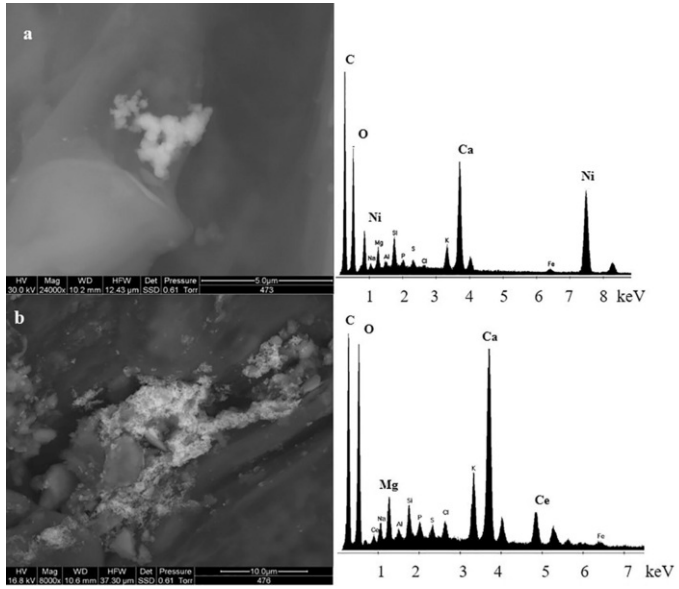

Fig. 2 ESEM-DSX images obtained by studying the basil roots. a) Basil root treated with Ni NPS, b) basil root treated with CeO_2 NPs and.

Ag, Co and Ni component metal concentrations in the basil stem and leaves were significantly higher than the control.

According to bioconcentration factor (BCF), the most accumulated metals in roots were Co, Ce and Ni (1.26, 0.41, 0.2, respectively) followed by Ag, Sn (0.09 and 0.04) and finally by Fe and Ti (0.02 and 0.01). However, this behaviour is not reflected on the aerial part where the BCF can be ranked as follow: Sn > Co > Ag > Ni > Ce > Fe > Ti. For the Fe and Ti, it is probably due to the high soil background that determine a lower BCF in both organs, root and aerial part.

The comparison of aerial and root BCF for Ce Co, and Ni showed that these elements were difficultly accumulated in the above ground tissues 5.1, 8.8 and 11.4% respectively. These data were in agreement with the Tf (Table 4). Only Ag could be concentrated and translocated (Tf 60.7%) in a good extent in the basil plant.

3.4 Nutrient content in basil tissues

The average concentration of nutrients (Ca, Mg, K, Na, S and P) in basil organs grown in soil contaminated with NPs is reported in Table 5.

Table 5 Comparison between the concentration of macro elements of stem, leaves and root in basil plants. Means followed by a different letter are significantly different at $P < 0.05$ according to the One-way ANOVA and Tukey's test.

		Ca	*SD*		K	*SD*		Mg	*SD*		Na	*SD*		P	*SD*		S	*SD*	
		g kg^{-1}																	
Leaves	*Control*	18.2	*0.6*	ab	2.7	*0.4*	a	4.7	*0.2*	a	0.5	*0.1*	ab	3.1	*0.1*	a	1.9	*0.1*	b
	Ag-NPs	15.7	*0.6*	b	2.2	*0.5*	a	4.9	*0.3*	a	0.6	*0.1*	ab	3.0	*0.2*	a	2.7	*0.1*	a
	CeO_2-NPs	18.5	*0.4*	a	1.9	*0.2*	a	4.5	*0.4*	a	0.8	*0.1*	a	3.0	*0.3*	a	1.8	*0.1*	b
	Co-NPs	17.4	*0.7*	ab	2.7	*0.4*	a	4.3	*0.2*	a	0.5	*0.1*	ab	3.1	*0.2*	a	1.8	*0.1*	b
	Fe_3O_4-NPs	18.7	*0.3*	a	2.3	*0.3*	a	4.8	*0.2*	a	0.5	*0.1*	ab	3.2	*0.2*	a	1.9	*0.1*	b

	Ni-NPs	18.6	*0.4*	a	2.9	*0.3*	a	4.8	*0.1*	a	0.4	*0.1*	b	3.1	*0.2*	a	1.9	*0.1*	b
	SnO_2-NPs	17.9	*0.7*	ab	2.6	*0.3*	a	4.4	*0.1*	a	0.5	*0.1*	ab	3.3	*0.1*	a	1.8	*0.1*	b
	TiO_2-NPs	18.4	*0.7*	ab	3.4	*0.3*	a	4.7	*0.3*	a	0.5	*0.1*	b	3.1	*0.2*	a	1.8	*0.1*	b
ANOVA				*			ns			ns			*			ns			***
Stem	*Control*	10.0	*0.5*	a	7.6	*0.3*	a	2.1	*0.1*	a	1.4	*0.5*	a	1.2	*0.1*	ab	0.9	*0.1*	a
	Ag-NPs	6.5	*0.4*	a	6.7	*0.2*	a	1.9	*0.2*	a	3.4	*0.8*	a	1.2	*0.2*	ab	1.1	*0.3*	a
	CeO_2-NPs	9.2	*0.2*	a	7.3	*1.2*	a	2.1	*0.2*	a	2.3	*0.7*	a	1.2	*0.1*	ab	1.0	*0.1*	a
	Co-NPs	8.1	*1.2*	a	6.9	*0.5*	a	1.8	*0.1*	a	1.8	*0.6*	a	1.1	*0.1*	ab	0.8	*0.1*	a
	Fe_3O_4-NPs	8.7	*0.6*	a	6.3	*0.6*	a	2.1	*0.1*	a	1.5	*0.1*	a	1.1	*0.1*	ab	0.8	*0.1*	a
	Ni-NPs	8.1	*0.6*	a	7.7	*0.2*	a	2.2	*0.1*	a	1.6	*0.1*	a	1.1	*0.1*	ab	0.8	*0.1*	a
	SnO_2-NPs	10.2	*1.8*	a	8.2	*1.0*	a	2.2	*0.2*	a	1.9	*0.5*	a	1.6	*0.2*	a	1.4	*0.2*	a
	TiO_2-NPs	8.9	*1.0*	a	7.1	*0.9*	a	1.9	*0.1*	a	1.3	*0.4*	a	1.0	*0.1*	b	0.7	*0.1*	a
ANOVA				ns			ns			ns			ns			.			ns
Root	*Control*	6.9	*0.4*	c	4.8	*1.1*	a	6.9	*0.2*	a	7.8	*0.4*	a	1.5	*0.0*	b	5.6	*0.2*	a
	Ag-NPs	9.2	*0.5*	ab	6.6	*0.2*	a	4.3	*0.6*	b	7.1	*0.6*	a	1.5	*0.1*	b	3.2	*0.2*	b
	CeO_2-NPs	9.1	*0.4*	ab	6.4	*0.9*	a	6.9	*0.6*	a	8.1	*0.7*	a	1.5	*0.2*	ab	5.5	*0.4*	a
	Co-NPs	8.5	*0.3*	bc	4.0	*1.0*	a	8.2	*0.2*	a	6.9	*0.8*	a	1.9	*0.0*	ab	6.6	*0.4*	a
	Fe_3O_4-NPs	9.6	*0.3*	ab	6.8	*0.8*	a	8.4	*0.5*	a	9.6	*0.3*	a	1.8	*0.0*	ab	6.7	*0.5*	a
	Ni-NPs	9.1	*0.3*	ab	5.9	*0.6*	a	7.7	*0.3*	a	8.2	*0.6*	a	1.7	*0.1*	ab	6.1	*0.3*	a
	SnO_2-NPs	8.6	*0.4*	bc	4.5	*0.5*	a	7.0	*0.5*	a	7.0	*0.6*	a	1.7	*0.1*	ab	5.4	*0.6*	a
	TiO_2-NPs	11.0	*0.4*	a	6.3	*0.9*	a	8.3	*0.3*	a	7.9	*0.9*	a	2.0	*0.0*	a	6.0	*0.4*	a
ANOVA				***			ns			***			ns			**			***

One-way ANOVA and Tukey's test ($p < 0.05$) were used to determine statistical significance of the differences between treatment and the control means. $P < 0.001$ ***, $P < 0.01$ **, $P < 0.05$ *, $P < 0.1$ '.', ns is not significant.

The main differences were observed in the nutrient concentration of roots. Indeed, Ca was significantly higher in all treatments as compared to the control ($P < 0.001$), ranging from 21.4 to 57.5%. In addition, Ag treatments determined a decrease of Mg and S content in basil roots as compared to the control (−38.6 and −43.0%, respectively), while both control and Ag treatment showed the lowest concentration of P (1.5 g kg^{-1}, $P < 0.01$). Salt accumulation in basil roots treated with NPs was detected by ESEM-DSX (Figs. 3 and 4),

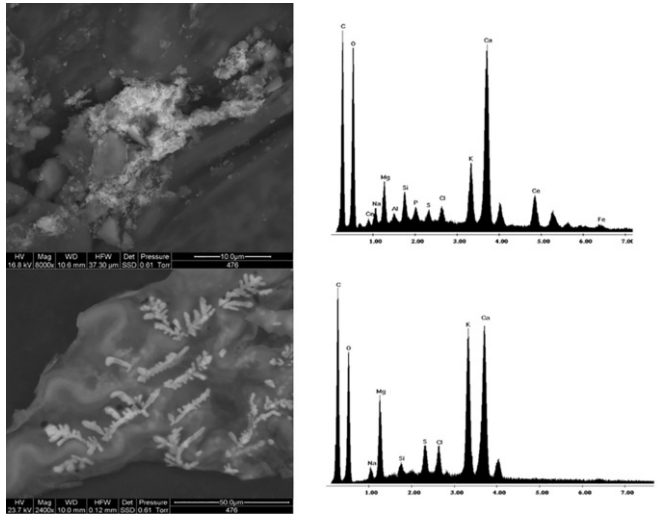

Fig. 3 ESEM-DSX images of the salt accumulation in the basil roots treated with CeO_2 NPs.

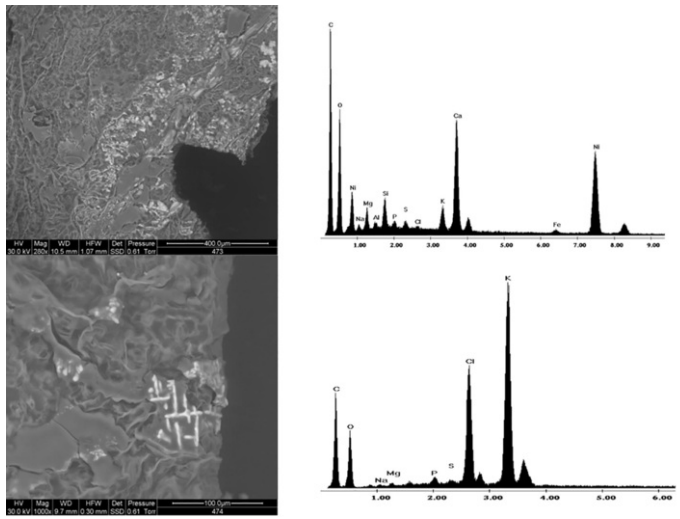

Fig. 4 ESEM-DSX images of the salt accumulation in the basil roots treated with Ni NPs.

3.5 Basil physiological parameters

Leaf gas exchange were measured with both Leaf Porometer and CIRAS-2, however due to the intrinsic heterogeneity of the stomata conductance (Weyers and Lawson, 1997) no significant differences were found.

Plant pigment concentrations are summarised in Table 6. No significant differences were found except for chlorophyll *b* which in plants treated with Ni and SnO_2 showed a lower concentration compared to the control.

Table 6 Comparison between the concentration of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), the sum of leaf carotenoids and xanthophylls (c + x) and relative ratio. Moreover, lipid peroxidation with TBARS in basil leaves was evaluated. Means followed by a different letter are significantly different at $P < 0.05$ according to the One-way ANOVA and Fisher's test, ns is not significant.

	Chl *a*	SD	Chl *b*	SD		x + c	SD	a/b	SD	(a + b)/(x + c)	SD	TBARS	SD

	mg g⁻¹ ww											µg g⁻¹	
Control	0.827	0.06	0.359	0.02	a	0.280	0.01	2.31	0.03	4.23	0.18	2.040	0.44
Ag-NPs	0.869	0.10	0.364	0.04	a	0.277	0.02	2.39	0.06	4.45	0.22	2.129	0.79
CeO_2-NPs	0.825	0.04	0.352	0.02	a	0.279	0.01	2.34	0.04	4.21	0.08	2.120	0.20
Co-NPs	0.843	0.05	0.373	0.02	a	0.275	0.01	2.26	0.04	4.43	0.05	1.548	0.14
Fe_3O_4-NPs	0.795	0.06	0.350	0.02	a	0.270	0.02	2.27	0.05	4.24	0.09	1.932	0.30
Ni-NPs	0.783	0.06	0.323	0.03	b	0.265	0.02	2.43	0.03	4.17	0.09	1.691	0.25
SnO_2-NPs	0.697	0.05	0.298	0.02	b	0.237	0.01	2.34	0.03	4.21	0.11	2.094	0.95
TiO_2-NPs	0.853	0.09	0.371	0.04	a	0.281	0.03	2.30	0.04	4.36	0.08	1.879	0.39
ANOVA		ns		*			ns		ns		ns		ns

Lipid peroxidation, measured with TBARS, is a classical marker of oxidative stress, but the accumulation of metal arising from the NPs in basil leaves did not show any significant difference compared to the control (Table 6).

4 Discussion

Soil pH (7.5 ± 0.1) and cation-exchange capacity, measured on the bulk were not affected by NP contamination. However, the soil CEC decreased over time, highlighting a detrimental effect of NPs to the soil cation holding sites to retain the nutrients, but also the colloidal soil capacity to protect groundwater from nanoparticles contamination (Vittori Antisari et al., 2013).

We showed via ICP-OES that a high amount of NP component metal remained in the soil underlining their low mobility, except for Fe_3O_4 and TiO_2-NPs. Indeed, NPs metal components were hardly extracted in DTPA and water and Fe_3O_4, Ni and TiO_2 NPs showed high values of log partition coefficient Kp (>2.8), suggesting that they are characterized by low geochemical mobility in water. The log Kp of Ag (2.4) is higher than that was previously observed in bare soil (Carbone et al., 2014), probably because the high content of peat had a greater capability than the natural forest soil to adsorb and stabilise the NPs. Conversely, CeO_2 and SnO_2 NPs showed a Kp lower than that observed by Vittori Antisari et al. (2013) in bare soil, possibly due to the presence of basil roots, which produce root exudates that mobilize and/or bind metals (Mench and Martin, 1991; Uren et al., 1989).

Exposure to NPs affects plant growth in different manner. In particular, AgNPs treatment decreased the grown of basil. Similar results were obtained by Qian et al. (2013) in *Arabidopsis thaliana,* where seedlings fresh weight decreased significantly in Ag NP treatments compared to the control, and by Song et al. (2013) in *Lycopersicon esculentum*, where root elongation and average biomass showed significant decrease at the lowest concentration (50 mg L^{-1}). Geisler-Lee et al. (2012) observed that AgNPs can be accumulated in the cell wall *Arabidopsis thaliana* roots determining a toxic effect to this tissue and the phytotoxic effect could be explained by both chemical (silver dissolution) and physiological interaction (disruption of cell-cell signalling). Conversely, Colman et al. (2013) who exposed five commonly occurring plant species (*Carex lurida*, *Juncus effusus*, *Lobelia cardinalis*, *Microstegium vimineum*, *and Panicum virgatum*) to AgNPs contaminated biosolid slurry. Only *Microstegium vimineum* showed sensitivity to the AgNP treatment, growing 32% less aboveground biomass in the slurry + AgNP treatment. This suggest that different species of plants have different susceptibilities to Ag induced toxicity (Yin et al., 2012).

Probably, AgNP treatment provided a slow release of Ag^+ and the content of organic matter in soil could play an important role about the time/dose of release (Colman et al., 2012).

Nanoparticles of Fe_3O_4, Ni and TiO_2 do not affected the aboveground growth; however, they determined an increase of the root dry matter of basil plant. Previous studies on the impact of copper on chamomile and soybean showed that the contamination enhanced the lignification of plant cells as response to the stress by the activity of peroxidase enzymes in the presence of excess H_2O_2 (Lin et al., 2005; Kováčik et al., 2010). The same behaviour has been observed in the shoot of *Thymus daenensis* exposed to Ag NPs (Bernard et al., 2015) and *Brassica juncea* L., *Arabidopsis thaliana, Vigna radiate, Glycine* max, *Cicer arietinum* treated with CuO NPs (Nair and Chung, 2015; 2014a; 2014b; 2014c; Nair et al., 2014). However, contrasting results were found in rice plants where CeO_2-NPs decreased the lignification despite the enhanced peroxidise activity and H_2O_2 content (Rico et al., 2013a). In contrast to previously published data by Wang et al. (2012) and Priester et al. (2012) regarding the impact of CeO_2 NPs treatment on tomato stem, root elongation, and in soybean dry biomass, in this experiment the basil growth was not affected by the CeO_2 NPs treatment. Cerium component metal was found in basil leaves, in agreement with previous studies where Ce was detected in the above-ground organs of corn (Zhao et al., 2012), rice (Rico et al., 2013b) and tomato (Wang et al., 2012).

Plants exposed to the NPs showed an increase of the component metal concentration in the root tissues, except for Fe and Sn-NPs, which was accompanied by a significant increase of the component metal in stem and leaves for (Ag and Co NPs) and only in leaves (Ce and Ni NPs). Roots could be the main route of the plant's exposure to NPs (Anjum et al., 2013) probably due to absorption of cluster NPs on the roots. The exudates, mucilage, mucigel and organic acids released by roots in the rizosphere could play an important role in the dissolution of NPs and increase the uptake into the plant. Ag was detected in leaves of *A. thaliana* and *Populus deltoides × nigra* (Wang et al., 2013), in tomato fruit (Vittori Antisari et al., 2014) and in lettuce leaves (Doolette et al., 2015). Wang et al. (2013) observed that the mass of silver accumulated in poplar tissues cannot be explained solely by uptake of Ag^+ released in the hydroponic solution, which suggests specific AgNP effects including possible nanoparticle uptake as suggested by other studies (Dimkpa et al., 2013; Geisler-Lee et al., 2013).

To the best of our knowledge, this is the first report about the interaction between Co- and SnO_2-NPs and *O. basilicum;* our work showed that these treatments do not have a detrimental effect on plant growth compared to the control.

Co and Ni NPs component metal were found in basil leaves as observed in stem and leaves of tomato (Vittori Antisari et al., 2014). Both are essential micronutrients, such as essential component of several enzymes and co-enzymes (López and Magnitsky, 2011), probably for this reason these elements were translocated in leaves.

Sn did not accumulate significantly in basil plant treated with SnO_2-NPs compared to the control, and Sn concentration in soil was lower than expected; probably the acid mixture used for the mineralization of soil and plants do not dissolve SnO_2-NPs completely or there was a Sn re-precipitation (Ellison et al., 1998).

Translocation to the shoot is species-specific (Wang et al., 2013), is generally limited and depends on the NPs' primary diameter (Zhang et al., 2011). The accumulation of Ti in basil roots growing in the presence of TiO_2-NP was not accompanied by Ti translocation into shoots, this is in contrast to previously published data by Servin et al. (2013) who found Ti uptake in *Cucumis sativus*. These differences may be due to plants were grown in hydroponic system and sandy loam soil, respectively; indeed, differences in species and medium might account for our differing results (Anjum et al., 2013).

The bioconcentration factor can give valuable information regarding the capability of the species to extract metal from a contaminated matrix. Our results showed that NPs are preferentially accumulated in the roots and in low extent in the aerial part, except for Ag-NPs. These data are confirmed by the translocation factor which about the 60% of Ag-NPs are translocated from the roots to the shoot; while this amount range from the 5 to the 12% for Ce, Co and Ni. The results obtained on Ag NPs translocation agreed with those reported in the literature (Dimkpa et al., 2013), suggesting two different pathway. Ag NPs could disrupt the plasma membranes and subsequently reduce the soil/plant barrier (Hall, 2002) and reach the leaves; or Ag ions can be absorbed by metabolic pathway and then can be reduced to the elemental form in plant as has been demonstrated with other plants (Haverkam and Marshall, 2009; Beattie and Haverkamp, 2011).

5 Conclusions

Our results demonstrated that, despite the capability of plant to exclude potentially toxic elements, Ag, CeO_2, Co and Ni NPs component metal could be translocated from the root to the soot reaching the leaves, the edible part of the plant, representing a way to introduce nanoparticles in the food chain.

Notably, also in the relative short exposure there was an accumulation of Ca in roots, suggesting that the metabolic alteration in plants could be aimed at counteracting the membrane damage generated directly or indirectly by NPs.

Nevertheless, further studies are required to evaluate the impact of these NPs over several generation and their fate in food chain.

Uncited references

Cornelis et al. (2011).

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Answer: ESEM-DSX images obtained by studying the basil roots. a) basil root treated with Ni NPS, b) basil root treated with CeO_2 NPs.