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# 1 Uptake, translocation, size characterization and localization of 2 cerium oxide nanoparticles in radish (*Raphanus sativus* L.)

3

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11

## 12 **Abstract**

13 Due to their unique physical and chemical properties, the production and use of cerium oxide  
14 nanoparticles (CeO<sub>2</sub> NPs) in different areas, especially in automotive industry, is rapidly increasing,  
15 causing their presence in the environment. Released CeO<sub>2</sub> NPs can undergo different transformations  
16 and interact with the soil and hence with plants, providing a potential pathway for human exposure  
17 and leading to serious concerns about their impact on the ecosystem and human organism. This study  
18 investigates the uptake, bioaccumulation, possible translocation and localization of CeO<sub>2</sub> NPs in a  
19 model plant (*Raphanus sativus* L.), whose edible part is in direct contact with the soil where  
20 contamination is more likely to happen. The stability of CeO<sub>2</sub> NPs in plant growth medium as well as  
21 after applying a standard enzymatic digestion procedure was tested by single particle ICP-MS (SP-  
22 ICP-MS) showing that CeO<sub>2</sub> NPs can remain intact after enzymatic digestion; however, an  
23 agglomeration process was observed in the growth medium already after one day of cultivation. An  
24 enzymatic digestion method was next used in order to extract intact nanoparticles from the tissues of  
25 plants cultivated from the stage of seeds, followed by size characterization by SP-ICP-MS. The results

26 obtained by SP-ICP-MS showed a narrower size distribution in the case of roots suggesting  
27 preferential uptake of smaller nanoparticles which led to the conclusion that plants do not take up the  
28 CeO<sub>2</sub> NPs agglomerates present in the medium. However, nanoparticles at higher diameters were  
29 observed after analysis of leaves plus stems. Additionally, a small degree of dissolution was observed  
30 in the case of roots. Finally, after CeO<sub>2</sub> NPs treatment of adult plants, the spatial distribution of intact  
31 CeO<sub>2</sub> NPs in the radish roots was studied by laser ablation ICP-MS (LA-ICP-MS) and the ability of  
32 NPs to enter and be accumulated in root tissues was confirmed.

33

34 **Keywords:** Single particle ICP-MS, edible plants, engineered nanoparticles, laser ablation ICP-MS,  
35 ionization efficiency

36

## 37 **1. Introduction**

38 In recent years there has been a rapid increase in the development, production and application  
39 of engineered nanoparticles (ENPs), which are currently playing a growing role in a wide range of  
40 industrial and commercial applications (Stark et al., 2015; Vance et al., 2015). Due to their unique  
41 properties and novel features, the global production of nanomaterials is significantly increasing,  
42 leading to the release of ENPs into the environment, where they can interact with higher plants.

43 Among the different ENPs, cerium oxide nanoparticles (CeO<sub>2</sub> NPs) are one of the most  
44 important and promising due to their capability for undergoing a redox-cycle between the two natural  
45 oxidation states (Ce<sup>3+</sup> and Ce<sup>4+</sup>) (Cassée et al., 2011), with an estimated global production of up to 10  
46 000 tons per year (Keller et al., 2013; Piccinno et al., 2012). Cerium is the most abundant rare earth  
47 element in the earth's crust and belongs to the group of elements referred to as technology-critical  
48 elements that were considered just as laboratory curiosities, being now key components for the  
49 development of new technologies (Wojcieszek et al., 2018). CeO<sub>2</sub> NPs have special electrical, optical  
50 and thermal properties which make them widely used as polishing agents, UV-blockers, glass  
51 additives, in agricultural products and especially in automotive industry (Stanek et al., 2008; The  
52 Project on Emerging Nanotechnologies, 2019; Yang et al., 2017). Moreover, the high reactive oxygen

53 species (ROS) scavenging properties gives CeO<sub>2</sub> NPs the potential to be used as an antioxidant and  
54 radioprotective agent (Li et al., 2015). For instance, CeO<sub>2</sub> NPs are widely used in the production of  
55 catalysts or as a diesel fuel additive to increase fuel combustion efficiency, decrease diesel soot  
56 emissions and to reduce NO<sub>x</sub> emissions (Cassee et al., 2011; Johnson and Park, 2012). Therefore,  
57 their release into the ecosystem is unavoidable for example through deposition along roadways that  
58 leads to their translocation to the soil and thus to plants, which has raised serious concerns about their  
59 fate and impact in the ecosystem (Colvin, 2003; Gardea-torresdey et al., 2014; Johnson and Park,  
60 2012). CeO<sub>2</sub> NPs can interact with the soil and hence with plants, which in turn increase the risk of  
61 their bioaccumulation in the animal and human food chain (Colvin, 2003; Deng et al., 2014). As a  
62 results, and due to the importance of the physicochemical states of Ce to its food safety implications, a  
63 more extensive studies on the interaction of CeO<sub>2</sub> NPs and higher plants is essential for a fully  
64 understanding of their environmental impact (W. Zhang et al., 2017a).

65 Both positive and negative effects of CeO<sub>2</sub> NPs on plants have been demonstrated, depending  
66 on the plant species, size and concentration of NPs, exposure time and plant growth conditions (Ma et  
67 al., 2010; Rossi et al., 2016; Wang et al., 2012; Zhao et al., 2014). Species-dependent responses of  
68 plants for CeO<sub>2</sub> NPs treatment have been also observed by other researchers (Lopez-Moreno et al.,  
69 2010b). Additionally, it was already presented that toxicity of CeO<sub>2</sub> NPs towards plants is  
70 concentration dependent (Lopez-Moreno et al., 2010a). On the other hand, CeO<sub>2</sub> NPs were shown to  
71 enhance plant growth under certain exposure conditions (Ma et al., 2016; Wang et al., 2012). CeO<sub>2</sub>  
72 NPs have generally been considered as insoluble under environmental conditions (Walser et al., 2012)  
73 (Yokel et al., 2009). However, several studies suggest that after CeO<sub>2</sub> NPs treatment, cerium in plants  
74 could exist in the form of Ce salts or dissolved Ce; reducing agents present in plant growth media  
75 could reduce the surface Ce<sup>4+</sup> of CeO<sub>2</sub> NPs into Ce<sup>3+</sup> (Dan et al., 2016; Schwabe et al., 2014; Zhang et  
76 al., 2012; W. Zhang et al., 2017). Additionally, solubility of CeO<sub>2</sub> NPs is also pH dependent  
77 (Hernandez-viezcas et al., 2013). Dissolved form of CeO<sub>2</sub> NPs was found for example in cucumber or  
78 soybean plants (Hernandez-Viezcas et al., 2016; Zhang et al., 2012). In contrast, other studies showed  
79 that CeO<sub>2</sub> NPs did not undergo any transformation in cucumber, alfalfa, tomato and corn seedling  
80 (Lopez-Moreno et al., 2010b). Most of the studies performed showed that CeO<sub>2</sub> NPs can be taken up

81 by plants, but the majority of NPs appeared to remain in root tissues, raising concerns on their  
82 accumulation by root vegetables. However, even though the edible tissues of below-ground  
83 vegetables have direct contact with NPs contaminated soils, only little attention has been paid to this  
84 group of plants (Zhang et al., 2015).

85 In this context, radish (*Raphanus sativus* L.), a popular vegetable with high global  
86 consumption, was chosen to the study since its edible part is in contact with the soil where  
87 contamination, through atmospheric dust deposition, is more likely to happen. Taking into account  
88 different, often contradictory, results presented in previous works, the main purpose of this study was  
89 to investigate the uptake, translocation, characterization and spatial localization of a commercial  
90 suspension of CeO<sub>2</sub> NPs in a model edible plant throughout the whole process by using different mass  
91 spectrometry based techniques: the stability of the CeO<sub>2</sub> NP in the hydroponic solution was assessed  
92 by single particle ICP-MS (SP-ICP-MS); the bioaccumulation of cerium in plants tissues and the  
93 translocation factor from roots to leaves was determined by ICP-MS; the presence of CeO<sub>2</sub> NPs in  
94 radish tissues was investigated by SP-ICP-MS and their size distributions obtained; finally, the spatial  
95 distribution of cerium in roots was studied by laser ablation ICP-MS (LA-ICP-MS).

96

## 97 **2. Material and methods**

### 98 *2.1. Samples and reagents*

99 Seeds of radish (*Raphanus sativus* L.) were purchased from Vilmorin Garden (Komorniki,  
100 Poland). Analytical or biological reagent grade chemicals and LC-MS grade solvents were purchased  
101 from Sigma-Aldrich (Saint Quentin Fallavier, France) unless stated otherwise. Ultra-pure water  
102 (18 MΩ cm) obtained with a MiliQ system (Millipore, Guyancourt, France) was used  
103 throughout. Hydrogen peroxide from Fisher Scientific (Hampton, NH) and nitric acid (INSTRA-  
104 Analysed) from Baker (Deventer, Netherlands) were used for samples digestion. A standard ionic  
105 solution of 1000 mg L<sup>-1</sup> cerium was purchased from Plasma CAL standards (Teddington, UK).  
106 Macerozyme R-10 enzyme (pectinase from *Rhizopus* sp., Sigma Aldrich) was used to digest plant

107 tissues for CeO<sub>2</sub> NPs extraction. Macerozyme R-10 is a multi-component enzyme mixture containing  
108 cellulase (0.1 unit per mg), hemicellulase (0.25 unit per mg) and pectinase (0.5 unit per mg).

109 Aqueous suspension of CeO<sub>2</sub> NPs (40 wt %) with a nominal size of 30-50 nm was purchased  
110 from US Research Nanomaterials, Inc. (Houston, TX). A diluted suspension of CeO<sub>2</sub> NPs was  
111 prepared daily in ultrapure water by accurately weighing aliquots of the stock suspension after one  
112 minute sonication. After dilution and before each analysis, the suspensions were sonicated for  
113 approximately 1 min.

## 114 2.2. Instrumentation

115 Homogenization of the plant tissues was performed by a Vibracell 75115 ultrasonic probe  
116 (Bioblock Scientific, Illkirch, France) offering a nominal power of 500 W. Incubation of samples was  
117 performed in a Grant OLS-200 water bath (Keison Products, Essex, UK). A Branson B2510 ultrasonic  
118 bath (Branson, Danbury, CT) was used for sonication of nanoparticles suspensions and enzymatic  
119 extracts before SP-ICP-MS analysis. A Digi-Prep system from SCP Science (Quebec, Canada) was  
120 used for acid digestion of plant tissues and extracts for the total cerium determination. A centrifuge  
121 5415R (Eppendorf, Hamburg, Germany) was used for the extraction procedure. pH was adjusted by  
122 using a FiveEasy pH meter from Mettler Toledo (Columbus, OH).

123 The determination of the total concentration of cerium in the plant samples was carried out  
124 using an Agilent 7500ce ICP-MS (Agilent, Tokyo, Japan) instrument fitted with Ni cones and a 2.5  
125 mm i.d. injector torch. The position of torch and nebulizer gas flow was adjusted each day of work.  
126 The working conditions of ICP MS were optimized daily using a 1 µg L<sup>-1</sup> solution of <sup>7</sup>Li<sup>+</sup>, <sup>89</sup>Y<sup>+</sup> and  
127 <sup>205</sup>Tl<sup>+</sup> in 2% (v/v) HNO<sub>3</sub>.

## 128 2.3. Single particle ICP-MS method

129 An Agilent 7900 ICP MS equipped with Single Nanoparticle Application Module was used  
130 for the characterization of CeO<sub>2</sub> NPs. The default instrumental and data acquisition parameters are  
131 listed in Table 1. During analysis <sup>140</sup>Ce with a natural abundance of about 88.5 % was monitored.  
132 <sup>140</sup>Ce is interference-free in ICP-MS analysis which makes this isotope the best choice for analysis of  
133 Ce based nanoparticles. SP-ICP-MS analyses were performed in TRA mode using a dwell time of 100

134  $\mu\text{s}$ , with a total time of analysis of 60 s. The dwell time is a critical parameter when working in single  
 135 particle mode and the use of microsecond dwell time can improve particle sizing accuracy and  
 136 counting (Abad-Álvarez et al., 2016). Gold nanoparticle standard reference material with a nominal  
 137 diameter of 56 nm (RM 8013) was obtained from NIST (Gaithersburg, USA) and was used for  
 138 determination of transport efficiency, which was calculated by the particle frequency method  
 139 described by Pace et al. (2011). The sample flow rate was calculated daily by measuring the mass of  
 140 water taken up by the peristaltic pump for 2 min (this operation was repeated 3 times). After each  
 141 sample analysis, the software automatically processed the raw data and generated the particle size,  
 142 particle concentration, size distribution and information about concentration of dissolved metal. The  
 143 size distributions were prepared in Origin 8.5 software (Northampton, MA) and adjusted to lognormal  
 144 distributions in order to obtain the median diameter.

145

146 **Table 1**

147 Default instrumental and data acquisition parameters for SP-ICP-MS.

Instrumental parameters	
RF Power	1550 W
Argon gas flow rate	
Plasma	15 L min <sup>-1</sup>
Auxiliary	0.9 L min <sup>-1</sup>
Nebulizer	1.10 L min <sup>-1</sup>
Sample uptake rate	0.35 mL min <sup>-1</sup>
Data acquisition parameters	
Dwell time	100 $\mu\text{s}$
Readings per replicate	600000
Total acquisition time	60 s
Analyte	Ce
Mass (amu)	140
Density	7.13 g cm <sup>-3</sup>
Mass fraction	0.8139

148

149

150 *2.4. Laser ablation ICP-MS method*

151 A NewWave UP-213 laser ablation (LA) system (NewWave Research, Fremont, USA) was  
152 coupled to an Agilent model 7700x (Agilent, Tokyo, Japan) using a 60 cm Tygon tube (5.0 mm i.d.).  
153 The laser ablation system was operated in a focused spot mode at the repetition rate of 20 Hz with a  
154 spot size of 250  $\mu\text{m}$  and a scan speed of 100  $\mu\text{m s}^{-1}$ . The ablated matter was transported into the ICP  
155 with He gas (800 mL  $\text{min}^{-1}$ ) and mixed in a T-connector with carrier gas of ICP MS delivered at 1.1 L  
156  $\text{min}^{-1}$  aerosol (a Micromist nebulizer and a double pass Scott spray chamber were removed from the  
157 configuration). No collision/reaction gas was used during analyses. A 1.5 mm i.d. injector torch and  
158 Pt cones were used. Pulse energy was of 30% and fluence was 1.45  $\text{J cm}^{-2}$ . The ICP-MS instrument  
159 was optimized in wet plasma condition before coupling with laser in order to obtain the maximum Ce  
160 signal-to-noise ratio.

## 161 2.5. Procedures

### 162 2.5.1. Plant cultivation

163 Portions of radish seeds (ca. 1 g) were germinated in distilled water in darkness for 3 days and  
164 then the seedlings were transferred to 350 mL opaque containers with a Knop nutrient solution (pH  
165 6.8) and placed in a growth chamber. After 4 days, the  $\text{CeO}_2$  NPs suspension was added to the  
166 medium at a cerium concentration of 5  $\text{mg L}^{-1}$ . As a control variant, plants were left in the nutrient  
167 solution without the addition of cerium. Cultivation was carried out for the next 7 days in a growth  
168 chamber. The plants were grown at with a temperature varying from 21-25  $^{\circ}\text{C}$ , light intensity  
169 200 microeinsteins  $\text{m}^{-2} \text{s}^{-1}$ , 12 h photoperiod and 50-60% relative humidity. All the time the solutions  
170 in the containers were aerated. Each variant of the cultivation was performed in three replicates. After  
171 cultivation, the plants were harvested and the roots were gently rinsed with deionized water. The  
172 plants were divided into roots and above ground organs (leaves plus stems) and lyophilized. Dried  
173 plant material was ground in a mortar before further analysis.

174 For LA-ICP-MS analysis, radish plants were initially cultivated in typical garden soil in the  
175 greenhouse for 4 weeks to obtain well-formed tubers. Next, adult plants of radish, with the taproot  
176 diameter of ca. 1.5 cm were conditioned in deionized water for 24 hours and then transferred to  
177 opaque containers with suspensions of  $\text{CeO}_2$  NPs (50 mL) at concentrations of 5  $\text{mg L}^{-1}$  or 50  $\text{mg L}^{-1}$

178 for 24 and 48 h. After finishing the cultivation, taproots were gently rinsed with deionized water and  
179 prepared for further analysis. Each variant of the cultivation was performed in three replicates.

### 180 2.5.2. *Determination of total cerium content*

181 Samples of leaves and roots (0.025 g) were digested by adding 2.5 mL HNO<sub>3</sub> (c) in a DigiPrep  
182 tube and the following temperature program: 30 min of heating up to 65°C and then keeping  
183 temperature at 65°C for 4 h. Afterwards, 1 mL of H<sub>2</sub>O<sub>2</sub> (c) was added to the samples and the digestion  
184 was continued for the next 4 h using the same program. After digestion, the mixtures were cooling  
185 down and diluted with ultrapure water to a final volume of 25 mL. Further dilutions were prepared  
186 with 2% HNO<sub>3</sub> (v/v), directly before ICP-MS analysis. Two isotopes of cerium (<sup>140</sup>Ce, <sup>142</sup>Ce) were  
187 monitored during analysis. The analytical blanks were analyzed in parallel. Quantification was  
188 performed by external calibration; a 5 points calibration curve was prepared for cerium concentration  
189 in the investigated range from 0.0 to 5.0 ng mL<sup>-1</sup>. The content of cerium in each sample was  
190 calculated as the mean of results obtained for the monitored isotopes, from three replicates of each  
191 sample. In order to verify the accuracy of the method, a recovery study was performed. A known  
192 amount of CeO<sub>2</sub> NPs suspension (at the same concentration as used for plant cultivation) was spiked  
193 into the controls of roots and leaves plus stems and the acid digestion method described above was  
194 applied.

### 195 2.5.3. *Enzymatic digestion method*

196 After cultivation performed on radish seeds, plant tissues were digested enzymatically as  
197 reported in previous works (Jiménez-Lamana et al., 2016; Kińska et al., 2018). Briefly, grounded  
198 samples of leaves and roots (0.025 g) were homogenized with 8 mL of 2 mM citrate buffer (pH 4.5;  
199 adjusted with citric acid) by using an ultrasonic probe. After the end of homogenization, 2 mL of  
200 Macerozyme R-10 solution (0.01 g of enzyme powder for roots and 0.05 g of enzyme powder for  
201 leaves plus stems, dissolved in 2 mL of ultrapure water) was added to samples and they were next  
202 shaken at 37 °C for 24 hours in a water bath with continuous shaking. After digestion, the samples  
203 were settle down for approximately 15 min and the obtained suspensions were filtered with a 0.45 µm  
204 syringe filter (Sigma Aldrich). The filtered samples were next analyzed by SP-ICP-MS.

205 *2.5.4. Study of the spatial distribution of CeO<sub>2</sub> NPs in radish roots by LA-ICP-MS*

206 After the end of cultivation performed on adult plants, radish roots were washed with  
207 ultrapure water in order to remove nanoparticles adsorbed on the surface. Afterwards, fresh roots  
208 samples were cut into 150 µm thick slices using the Leica VT1000 S vibrating blade microtome  
209 (Leica Biosystems, Wetzlar, Germany). The radish slices were directly mounted onto glass slides  
210 coated with double-sided tape to enhance adhesion and kept in a fridge before further treatment. Four  
211 repetitions of each root sample were prepared. The prepared slices were next analyzed by means of  
212 LA-ICP-MS. The tissue section was systematically scanned (line by line) by a focused laser beam.

213

214 **3. Results and discussion**

215 *3.1. Cultivation of radish in the presence of nanoparticles and determination of total content of*  
216 *cerium*

217 During cultivation, radish plants appeared tolerant to the applied concentration of CeO<sub>2</sub> NPs  
218 since no visible phytotoxic effects were observed. Biomass production and tissues hydration did not  
219 differ substantially between the control and the CeO<sub>2</sub> NPs treated plants. Some additional studies on  
220 the content of photosynthetic pigments showed that plants were not affected by nanoparticles.

221 The determination of the total cerium content in radish tissues was the first step done after the  
222 plant cultivation with the CeO<sub>2</sub> NPs suspension, in order to investigate its distribution among the  
223 different tissues. Control and CeO<sub>2</sub> NPs treated samples of leaves (together with stems) and roots of  
224 radish were analyzed by standalone ICP-MS after samples mineralization. The quantification limit,  
225 calculated as 10 times the standard deviation of the blank (n = 3) divided by the sensitivity, was 0.43  
226 ng g<sup>-1</sup>. The results of cerium content were obtained from three independent experiments and are  
227 presented in Table 2.

228

229 **Table 2.**

230 Total cerium content in radish tissues from control and CeO<sub>2</sub> NPs treated plants (mean ± standard  
231 deviation).

	Control plant samples / $\mu\text{g g}^{-1}$	CeO <sub>2</sub> NPs treated samples / $\mu\text{g g}^{-1}$
Leaves + stems	< LOQ	32.9 $\pm$ 0.4
Roots	< LOQ	1948 $\pm$ 9

232

233 The obtained results showed that cerium is accumulated in analyzed radish tissues. However,  
234 the majority of cerium was found in radish roots and only minor part of cerium was transported to  
235 above ground organs, which is in good agreement with many studies as discussed below. The  
236 translocation factor (TF), defined as the ratio of cerium content in leaves with respect to roots, was  
237 0.017. It has been reported that the translocation from roots to shoots is independent of the cerium  
238 content in the roots and of the supplied form of cerium (Yang et al., 2017). Taking into account that  
239 roots as edible part of radish are in direct contact with the soil where contamination is more likely to  
240 happen, the results of the total content are especially important from the point of view of food safety.  
241 To determine the accuracy of the proposed acid digestion method, a recovery study was made.  
242 Control plant samples (leaves plus stems, and roots) were spiked with a known amount of CeO<sub>2</sub> NPs  
243 and the acid digestion method was applied. Good recovery values were obtained for both leaves plus  
244 stems (103.2%) and roots (102.7%). Regardless of the total content of cerium determined in plant  
245 tissues in previous works, its translocation from roots to the aerial part was always in very low level.  
246 For instance, Yang et al. (2017) reported that the TF of cerium from roots to above ground organs in  
247 *Arabidopsis thaliana* plant is 0.042, while Rossi et al. (2016) found a TF of around 0.002 after  
248 analysis of *Brassica napus* L. In the case of desert plant mesquite, the TF of cerium was 0.2 and 0.25  
249 for plants treated with 500 and 4000 mg L<sup>-1</sup>, respectively (Hernandez-Viezcas et al., 2016). In the case  
250 of plants grown in soils, a TF of 0.02–0.03 was found in kidney bean plants (Majumdar et al., 2016).  
251 It should be also mentioned that no translocation of cerium to above ground organs was observed in  
252 the case of tomato or wheat plants (Antisari et al., 2015; Schwabe et al., 2015). The TF is dependent  
253 also on the size of CeO<sub>2</sub> NPs. It was showed that after analysis of pumpkin leaves and roots, the TF  
254 for CeO<sub>2</sub> NPs of 9 nm was 0.0004 whereas no cerium translocation was observed in the case of NPs of  
255 64 nm (Schwabe et al., 2015).

256 3.2. Study of ionization efficiency of CeO<sub>2</sub> NPs in the plasma

257 The calculations behind SP-ICP-MS theory lie on some assumptions, being a plasma  
 258 ionization efficiency for nanoparticles comparable to the corresponding dissolved species one of them  
 259 (Pace et al., 2011). However, while this has proven to be true for silver or gold nanoparticles (Hu et  
 260 al., 2009; Laborda et al., 2011), it might not be the case for nanoparticles of a different nature, like it  
 261 has been reported for selenium nanoparticles (Jiménez-Lamana et al., 2018), and needs to be first  
 262 investigated. A ionization efficiency lower than 100% was reported for CeO<sub>2</sub> NPs with sizes from 1 to  
 263 10 nm (Sánchez-garcía et al., 2016). However, this value might be different in the case of the CeO<sub>2</sub>  
 264 NPs used in this study, since the ablation and ionization may strongly vary while increasing the size  
 265 of the nanoparticle. In order to calculate the ionization efficiency, the response of the ICP-MS towards  
 266 the nanoparticulated form of cerium was studied, by determining and comparing the total cerium  
 267 concentration of digested nanoparticles and a suspension of undigested nanoparticles. The calibration  
 268 was achieved with aqueous standards of cerium in 2% HNO<sub>3</sub> for the digested nanoparticles and in  
 269 ultrapure water and in 2% HNO<sub>3</sub> for the direct analysis of the suspension, in order to investigate the  
 270 influence of the medium on the cerium sensitivity. The concentrations determined in ultrapure water  
 271 and in 2% HNO<sub>3</sub> for the undigested CeO<sub>2</sub> NPs suspension were  $9.7 \pm 0.9\%$  and  $10.2 \pm 1.2\%$  with  
 272 regard to the concentration determined after acid digestion, respectively (Table 3).

273

274 **Table 3.**

275 Determination of cerium concentration in digested and undigested suspension of CeO<sub>2</sub> NPs (mean  $\pm$   
 276 standard deviation).

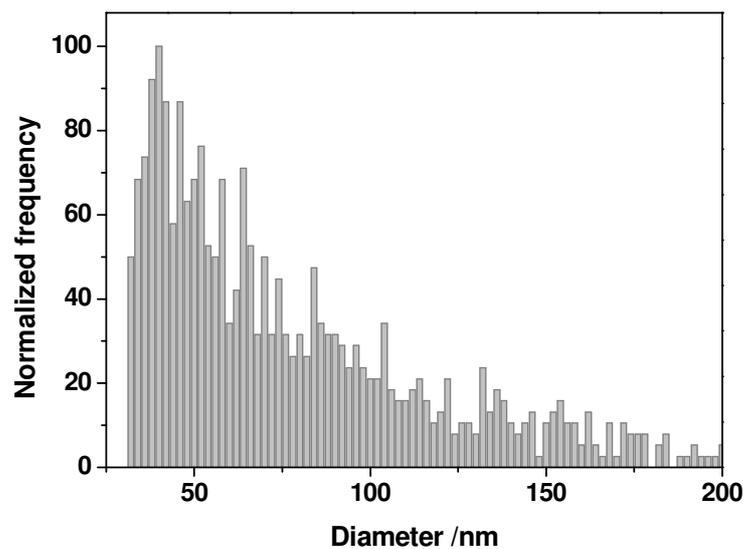
Sample	Total Cerium content / mg L <sup>-1</sup>
Acid digestion	$237.9 \pm 3.0$
Suspension in ultrapure water	$23.1 \pm 2.1$
Suspension in 2 % HNO <sub>3</sub> (v/v)	$24.4 \pm 2.9$

277

278 These results show that ICP-MS sensitivity depends on the physicochemical form of cerium  
 279 and thus a particle ionization efficiency, defined as the ratio of the ionization efficiency of the particle  
 280 to the ionization efficiency of the corresponding dissolved metal solution (Pace et al., 2011), needs to  
 281 be taken into account. According to the results obtained in this study, a particle ionization efficiency

282 of 10% was applied for sizing CeO<sub>2</sub> NPs. In addition, it was shown that the medium has no significant  
283 influence on the ionization of CeO<sub>2</sub> NPs.

284 The suspension of CeO<sub>2</sub> NPs used in this study was then analysed by means of SP-ICP-MS at  
285 a concentration of around 1x10<sup>8</sup> NP L<sup>-1</sup> taking into account the ionization efficiency calculated. The  
286 size distribution obtained (Fig. 1) showed a polydisperse distribution with its central part within the  
287 size range provided by the manufacturer: 30-50 nm.



288  
289 **Fig. 1.** Size distribution of the commercial suspension of CeO<sub>2</sub> NPs used in the cultivation studies.  
290

### 291 3.3 Investigation of CeO<sub>2</sub> NPs stability

#### 292 3.3.1 Stability in growth medium

293 Before the analysis of plant samples, the stability of the suspension of CeO<sub>2</sub> NPs in the  
294 growth medium was investigated. A suspension of CeO<sub>2</sub> NPs of 5 mg L<sup>-1</sup> was spiked into the nutrient  
295 solution and the size distribution of the nanoparticles was determined by SP-ICP-MS immediately  
296 upon preparation and after 1, 2, 4 and 7 days. Each suspension analysed was diluted accordingly in  
297 order to measure the same number of events. The median diameters obtained from the corresponding  
298 size distributions of nanoparticles at the different times analysed are shown in Table 4. From the  
299 results obtained, it can be concluded that CeO<sub>2</sub> NPs undergo agglomeration after 1 day of cultivation  
300 in growth medium, with an increase of the median diameter from 56.0 nm to 128.3 nm, whereas the

301 size distribution does not vary significantly from 1 to 7 days of cultivation (Fig. S1). The  
302 agglomeration of CeO<sub>2</sub> NPs has already been observed in hydroponic solution, without the addition of  
303 a stabilizing agent (Schwabe et al., 2013; Schwabe et al., 2014). Remarkably, no background signal  
304 corresponding to dissolved cerium was observed on the SP-ICP-MS time scans at any of the  
305 incubation times tested in the present study. This finding can be explained by two facts: on one hand,  
306 the occurrence of CeO<sub>2</sub> NPs agglomerates prevent from cerium dissolution, since agglomeration  
307 reduces the effective specific surface area of nanoparticles, which results in less cerium dissolution;  
308 on the other hand, the release of cerium ions is size dependent: in a study performed with CeO<sub>2</sub> NPs  
309 in Hoagland medium, Schwabe et al. (2014) showed that the smallest particles (9 nm) released the  
310 highest amount of cerium, whereas the release of cerium by the largest particles (64 nm) was almost  
311 negligible.

312

313 **Table 4.**

314 Median diameters of CeO<sub>2</sub> NPs suspended in nutrient solution after different contact times.

Incubation time	Median diameter / nm
0	56.0 ± 1.9
1 day	128.3 ± 19.1
2 days	119.1 ± 4.6
4 days	112.5 ± 3.9
7 days	134.6 ± 22.7

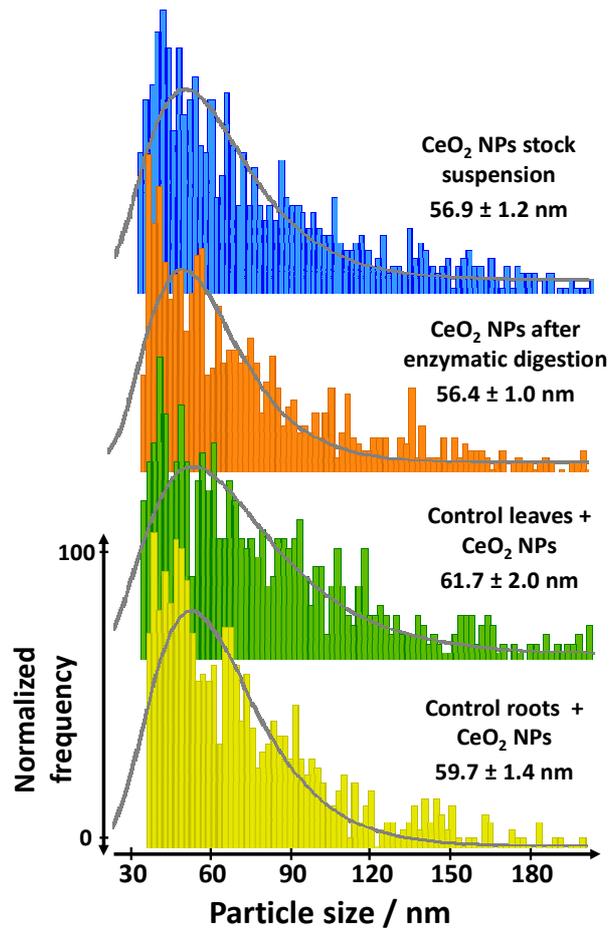
315

316 *3.3.2. Influence of enzymatic digestion and plant matrix*

317 The influence of the enzymatic procedure on the size distribution of the CeO<sub>2</sub> NPs suspension  
318 was also studied. For this purpose, a suspension of CeO<sub>2</sub> NPs (5 mg L<sup>-1</sup>) with no plant tissue was  
319 treated with the same conditions of enzymatic digestion. As it can be shown in Fig. 2, the size  
320 distribution obtained for enzyme-treated CeO<sub>2</sub> NPs was in good agreement with the size distribution  
321 obtained for CeO<sub>2</sub> NPs stock suspension freshly prepared and hence the integrity of CeO<sub>2</sub> NPs is not  
322 affected by the enzyme used in the digestion procedure.

323 In addition, the influence of the plant matrix was investigated. Control leaves plus stems and  
324 control roots of radish were spiked with 5 mg L<sup>-1</sup> of the CeO<sub>2</sub> NPs suspension, submitted to the

325 enzymatic digestion procedure and the corresponding nanoparticle size distributions obtained by SP-  
326 ICP-MS. The size distributions obtained for both spiked tissues were in good agreement with those  
327 obtained for freshly enzyme-treated CeO<sub>2</sub> NPs (Fig. 2). These results showed that the procedure  
328 proposed can be used to extract CeO<sub>2</sub> NPs from plant tissues without causing their transformation.  
329



330

331 **Fig. 2.** Size distributions of: CeO<sub>2</sub> NPs stock suspension freshly prepared (blue); CeO<sub>2</sub> NPs after  
332 enzymatic digestion procedure (orange); control leaves plus stems (green) and roots (yellow) spiked  
333 with 5 mg L<sup>-1</sup> CeO<sub>2</sub> NPs suspension. Size distributions were adjusted to lognormal distributions (grey  
334 line) and the median diameter ± the standard deviation obtained.

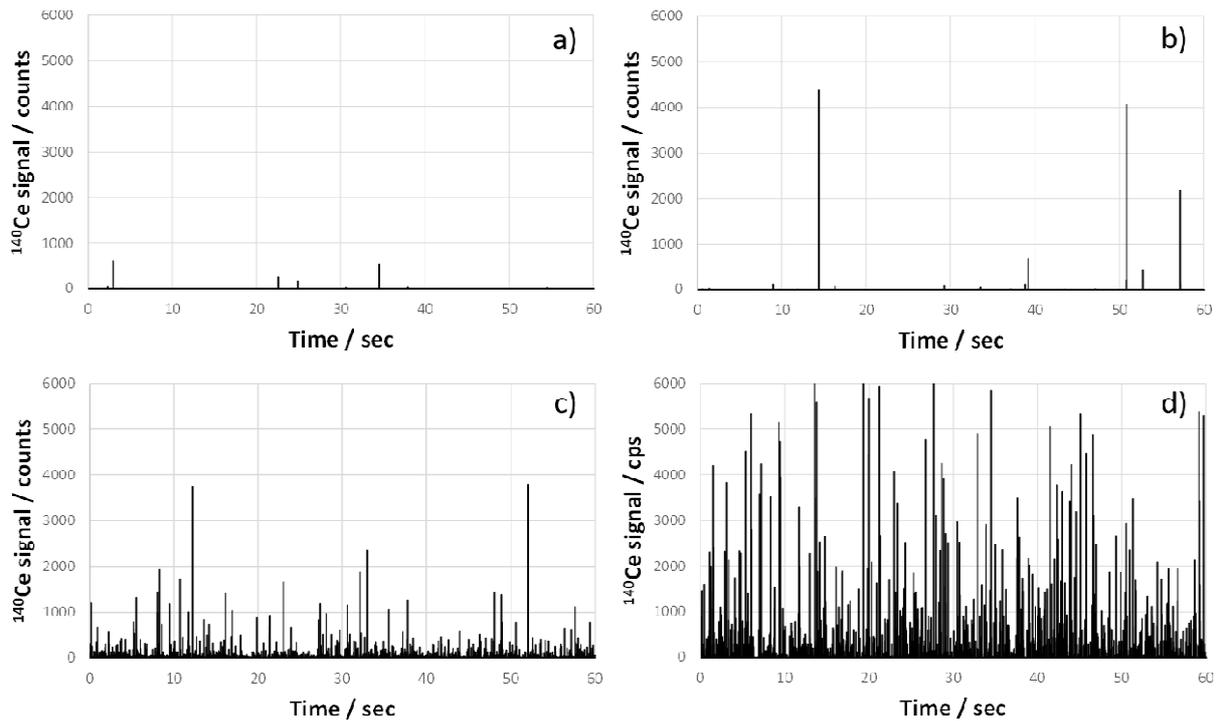
335

### 336 3.4. Single particle ICP-MS analysis of leaves plus stems and roots of radish

337 In order to identify the form of cerium inside the radish tissues cultivated from the stage of  
338 seeds, samples of leaves plus stems and samples of roots were subjected to the enzymatic procedure  
339 followed by SP-ICP-MS analysis. In a first step, roots and leaves plus stems of control plants were

340 analyzed and the corresponding time scans obtained (Fig. 3a and 3b, respectively). As expected, only  
341 a few pulses above the background were observed for control plants. The presence of these pulses  
342 may be explained by a slight contamination of the sonication probe during the enzymatic digestion  
343 procedure, but their occurrence is not significant (less than 10 pulses out of 600,000 readings) and  
344 hence does not hamper the further analyses.

345



346

347 **Fig. 3.** Time scans obtained for samples of: a) roots, and b) leaves of control plants and for c) roots  
348 and d) leaves of plants treated with  $5\text{ mg L}^{-1}$  of  $\text{CeO}_2$  NPs.

349

350 Afterwards, the SP-ICP-MS analyses of  $\text{CeO}_2$  NPs treated samples were performed. Samples  
351 of roots were diluted 3000 times before analysis whereas samples of leaves plus stems were diluted  
352 200 times. Each tissue was analyzed by triplicate. Time scans obtained showed a significant number  
353 of pulses in both plants tissues (Fig. 3c and 3d), proving that radish is able to uptake  $\text{CeO}_2$  NPs and  
354 transports them into the aerial part of the plant. The presence of  $\text{CeO}_2$  NPs has been already reported  
355 in tomato (*S. lycopersicum L.*), cucumber (*C. sativus*), pumpkin (*Cucurbita pepo*), and soybean  
356 (*Glycine max*) shoots (Dan et al., 2016; Zhang et al., 2011; Zhang et al., 2017b), in *Arabidopsis*  
357 *thaliana* shoots (Yang et al., 2017) and in romain lettuce shoots (P. Zhang et al., 2017a). According to

358 the number of pulses detected, the nanoparticle number concentration in roots and in leaves plus  
359 stems was calculated as  $3.3 \times 10^{11}$  and  $2.4 \times 10^{10}$  NP L<sup>-1</sup>, respectively. The largest number of number  
360 concentration of nanoparticles observed in roots could be explained by a direct contact of roots with  
361 CeO<sub>2</sub> NPs suspension. Remarkably, the intensity of the pulses observed in the time scan obtained for  
362 leaves is significantly higher than for roots, which suggested the presence of bigger nanoparticles. In  
363 addition, no signal corresponding to dissolved cerium was observed for leaves, suggesting that CeO<sub>2</sub>  
364 NPs did not underwent dissolution during their transport, although small signal coming from  
365 dissolved form of cerium was observed for roots. This is in good agreement with other studies that  
366 have shown that the dissolution of CeO<sub>2</sub> NPs occur at the root surface rather than inside plants (P.  
367 Zhang et al., 2017b). For instance, Ma et al. (2015) shown that dissolution of CeO<sub>2</sub> NPs only occurred  
368 at root surfaces, whereas Ce (IV) was not reduced in the tissues in hydroponic cucumber plants. Other  
369 authors have reported that plants roots activity have an impact on the dissolution of CeO<sub>2</sub> in  
370 hydroponically grown wheat, pumpkin, and sunflower plants (Schwabe et al., 2015).

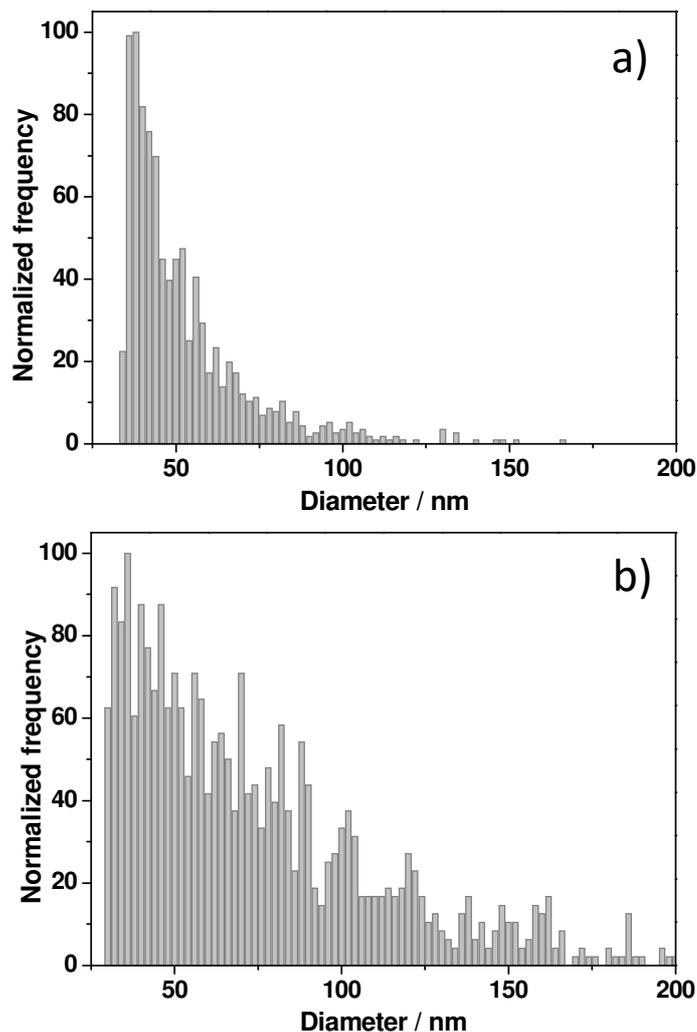
371 Due to the small signal coming from dissolved form of cerium observed after analysis of roots  
372 by SP-ICP-MS, a speciation study was performed by means of size exclusion chromatography (SEC)  
373 coupled to ICP-MS. The chromatogram obtained (Fig. S1) showed just one signal coming from high  
374 molecular weight cerium compounds which could be explained by the ability of metal ions to create  
375 agglomerates even with low molecular weight ligands. However, the extraction efficiency of cerium  
376 from radish roots (procedure explained in Supplementary Information), was determined at only 0.19%  
377 and therefore, due to negligible presence of cerium compounds, the speciation study of cerium was  
378 not continued.

379 From time scans and by applying the ionization efficiency factor calculated before, the size  
380 distributions of CeO<sub>2</sub> NPs in radish tissues were obtained. As it can be observed, the particle size  
381 distribution obtained for radish roots was narrower (Fig. 4a), with a median diameter of 42.7 nm,  
382 suggesting a preferential uptake of NPs at smaller sizes and that the agglomerates present in growth  
383 medium already after one day are not taken up by the plant. The finding that plant uptake of CeO<sub>2</sub>  
384 NPs depends on the particle size and smaller particles are more readily taken up by plants has been  
385 previously suggested (Zhang et al., 2011). However, the particle size distribution obtained for leaves

386 plus stems (Fig. 4b), showed a significant number of nanoparticles at higher diameters than in the case  
387 of roots (median diameter: 60.3 nm). Since it was found that plants are not able to take up big  
388 nanoparticles this finding may be explained by an agglomeration process that takes place at the  
389 endpoint of transport of CeO<sub>2</sub> NPs from roots to above ground organs. Once CeO<sub>2</sub> NPs have entered  
390 the plant, they are translocated up to above-ground organs through the xylem system, driven by the  
391 transpiration stream (Zhao et al., 2013). The endpoint of this transport pathway is the leaves (Zhao et  
392 al., 2013), where nutrients and water arrive through the veins. At this point, nanoparticles will be  
393 locally more concentrated and hence agglomeration by contact is more likely to occur. Similarly,  
394 particle aggregates have been found in leaves of *Arabidopsis thaliana* treated with CeO<sub>2</sub> NPs (Yang et  
395 al., 2017).

396

397



398 **Fig. 4.** Size distributions obtained for a) roots, and b) leaves plus stems of radish

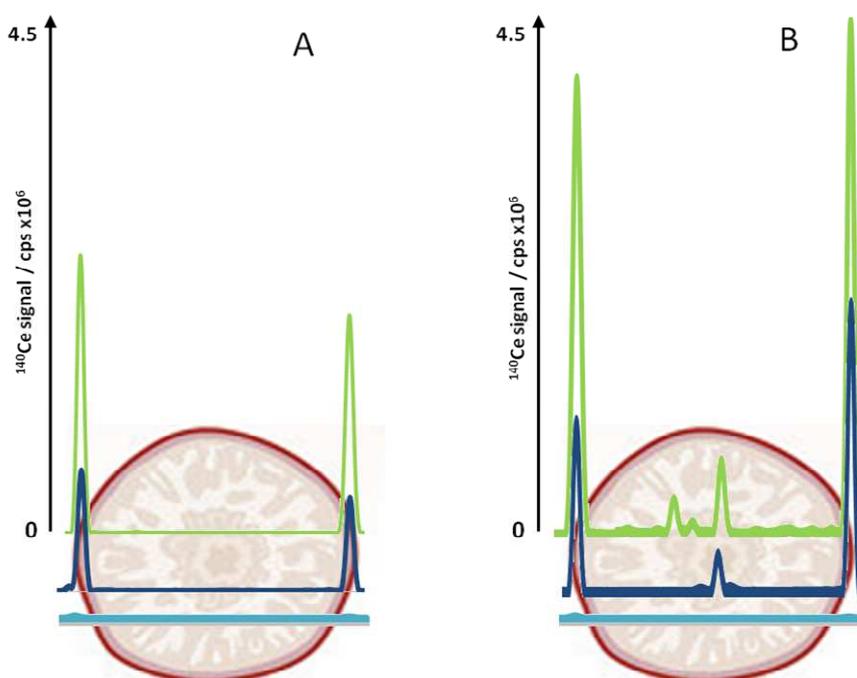
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400 The biotransformation of CeO<sub>2</sub> NPs inside plants remains a controversial issue. Some authors  
401 have suggested the uptake of Ce(III) followed by re-precipitation as pathway in CeO<sub>2</sub> NPs uptake by  
402 plants (Schwabe et al., 2015). However, our study showed that the dissolution of cerium is not  
403 significant and thus this mechanism can be discarded. On the other hand, cerium has been shown to be  
404 present in the shoots of cucumber and lettuce plants as CeO<sub>2</sub> and cerium carboxylates (Zhang et al.,  
405 2012; Zhang et al., 2017a), although the majority of studies have shown that cerium mostly remained  
406 as CeO<sub>2</sub> NP in plants tissues (Majumdar et al., 2014; Zhang et al., 2017b). For instance, in a study  
407 conducted with romaine lettuce, the amount of cerium carboxylates in leaves was reported at 3.5% (P.  
408 Zhang et al., 2017a). These previous findings make us state that the cerium signal observed in radish  
409 leaves plus stems is due to CeO<sub>2</sub> NPs and/or aggregates.

#### 410 *3.5. Study of spatial distribution of CeO<sub>2</sub> NPs in radish roots by LA-ICP-MS*

411 The results obtained by SP-ICP-MS analysis showed that the great majority of CeO<sub>2</sub> NPs  
412 remain in the form of nanoparticles both in roots and leaves. Therefore, in order to check if CeO<sub>2</sub> NPs  
413 have the ability to enter and be accumulated inside the radish tissues, the localization of CeO<sub>2</sub> NPs in  
414 the radish roots, as edible part of the plant, was determined by LA-ICP-MS. Analyses were performed  
415 after cultivation of radish carried out on adult plants. It is worth to mention that radish belongs to the  
416 group of plants with root system in type of taproot, with one central and dominant root. Additionally,  
417 taproot of radish is in the shape of fusiform root – The primary root of the system is the widest in the  
418 middle with secondary root tapers towards the bottom. Optimisation of a LA-ICP-MS analysis in  
419 terms of sensitivity and S/N ratio allowed to choose optimum scan speed of 100 μm s<sup>-1</sup> and spot size  
420 of 250 μm; increase in the laser beam spot size resulted in a considerable gain in sensitivity and  
421 homogeneity of the signal as more material is introduced into the plasma per time unit. After analysis  
422 of control plants cultivated for 2 days, no signal corresponding to cerium was observed (Fig. 5). The  
423 results obtained for radish samples treated with 5 mg L<sup>-1</sup> of CeO<sub>2</sub> NPs (Fig. 5A) showed peaks from  
424 cerium only at the external part of the analysed slices. Nanoparticles were found at a depth of about

425 1.2 mm leading to the conclusion that CeO<sub>2</sub> NPs have the ability to enter and be accumulated into the  
426 radish tissues, which is in good agreement with another study performed with radish in soil (W.  
427 Zhang et al., 2017b). Similar situation was observed in the case of radish samples treated with 50 mg  
428 L<sup>-1</sup> CeO<sub>2</sub> NPs (Fig. 5B). Signals from cerium were registered just below the skin surface, with higher  
429 intensity than those observed for samples treated with 5 mg L<sup>-1</sup> CeO<sub>2</sub> NPs. However, one additional  
430 signal was observed in the central part of samples after 1 day and 2 days of treatment, which suggest  
431 that cerium is accumulated and transported by secondary roots from bottom towards the central part of  
432 radish roots, not only through the surface. Accumulation of the metals in the taproot of plants was  
433 already observed after a LA-ICP-MS analysis of another root vegetables such as carrot (Yudasari et  
434 al., 2018). In addition, two additional signals were observed for samples treated with CeO<sub>2</sub> NPs for  
435 two days, suggesting that after accumulation, nanoparticles can be translocated within analysed radish  
436 tissues. Moreover, it was observed that the intensity of cerium signals increased with time for both  
437 treatments, showing that a higher content of cerium can be accumulated in radish roots with a longer  
438 exposure time; a relevant information in terms of food safety taking into account the persistence of  
439 CeO<sub>2</sub> NPs in the environment. A good reproducibility of the cerium spatial distribution was achieved  
440 after comparing results of the different slices of the same radish root or from different radish roots  
441 with the same exposure concentration and time.



442

443 **Fig. 5.** LA-ICP-MS analysis of radish roots treated with A) 5 mg L<sup>-1</sup> CeO<sub>2</sub> NPs B) 50 mg L<sup>-1</sup> CeO<sub>2</sub>  
444 NPs. Light blue line: control plant; dark blue line: plants treated for 1 day; green line: plants treated  
445 for 2 days

446

#### 447 **4. Conclusions**

448 The uptake, bioaccumulation, translocation, physico-chemical characterization and  
449 localization of CeO<sub>2</sub> NPs in radish was investigated by using different mass spectrometry based  
450 techniques. Our results showed that after cultivation, the majority of cerium remain in roots, with a  
451 low transportation up to leaves and stems (TF 0.017) as it has been observed in other studies. In order  
452 to get a correct particle size distribution, the behavior of CeO<sub>2</sub> NPs in plasma was investigated,  
453 showing that ICP-MS sensitivity depends on the physicochemical form of cerium and thus the  
454 ionization efficiency must be taken into account. Although CeO<sub>2</sub> NPs underwent agglomeration in  
455 hydroponic medium after 1 day, the SP-ICP-MS analysis indicated that radish only takes up small  
456 nanoparticles, since the presence of bigger nanoparticles and/or aggregates was not observed. The  
457 studies performed discarded interaction with plant matrix or effect of the enzymatic digestion  
458 procedure that affected the size of the nanoparticles. However, the analysis of leaves plus stems  
459 showed the presence of nanoparticles with bigger sizes, which suggests that nanoparticles undergo  
460 agglomeration at the endpoint of their transportation. Finally, the analysis by LA-ICP-MS in radish  
461 roots showed that the accumulation of CeO<sub>2</sub> NPs occurs mainly below the skin surface but after  
462 accumulation they have the ability to enter and be translocated within the tissue. The results obtained  
463 in this work can be consider meaningful from the point of view of food safety.

464

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470 for preparation of samples for LA-ICP-MS analysis.

471

## 472 **Appendix A. Supplementary data**

473 Electronic supplementary information (ESI) available: procedure of extraction and fractionation of Ce  
474 species from radish samples by SEC-ICP-MS; size distributions of CeO<sub>2</sub> NPs in grow medium at  
475 different incubation times; SEC-ICP-MS chromatogram of ammonium acetate extract of radish roots.

476

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