# Effects of $La_2O_3$ Nanoparticles and Bulk-$La_2O_3$ on the Development of *Pfaffia Glomerata* (Spreng.) Pedersen and Respective Nutrient Elements Concentration

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# Abstract

Nanoparticles (NPs) have been progressively applied in the last decades, which may impact the environment. Synthesis of pigments and nutrient elements uptake by plants can also be affected by NPs. The influence of lanthanum oxide nanoparticles ($La_2O_3$ NPs) on growth, pigments synthesis and nutrient elements uptake by *Pfaffia glomerata* (Spreng.) Pedersen, a medicinal plant native in South America, were evaluated in the present study. *P. glomerata* plantlets were cultivated for 28 days in the absence (control) and presence of 100, 200 and 400 mg $L^{-1}$ of $La_2O_3$ NPs or bulk-$La_2O_3$ (b-$La_2O_3$) at the same cultivation conditions. Root development, aerial part growth and pigments concentration in plants were affected by b-$La_2O_3$ and $La_2O_3$ NPs, mainly by $La_2O_3$ NPs. Calcium, Cu, Fe, K, La, Mg, Mn, Mo, P, S and Zn determination in stems and leaves revealed drastically and similar decrease of nutrient elements in plants cultivated in presence of 400 mg $L^{-1}$ of $La_2O_3$ NPs or b-$La_2O_3$. Alteration of nutrient elements concentration was also observed for the other treatments. Mapping of elements (using laser ablation inductively coupled plasma mass spectrometry) in leaves of plants submitted to treatment with 400 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs showed differences in the distribution of elements, indicating distinct effects of b-$La_2O_3$ and $La_2O_3$ NPs on *P. glomerata*. As such, this study demonstrated that $La_2O_3$ NPs may impact plants growth.


# Highlights

- Metal nanoparticles are of increasing use and are of environmental concern
- $La_2O_3$ affect growth, pigment synthesis and nutrient elements in *Pfaffiaglomerata*
- Changes of nutrient distribution is observed in leaves of *Pfaffiaglomerata*

# 1. Introduction

Plants need nutrient elements for growing and development. Elements such as Ca, K, Mg, N, P, S, B, Cl, Cu, Fe, Mn, Mo, Ni and Zn are required for chlorophyll production, cell structure integrity, enzymes synthesis, metabolic activities, energy storage and its transformation, regulation of the stomata aperture and ion transport (Aihemaiti et al. 2019; Gupta et al. 2014; Kötschau et al. 2013). On the other hand, toxic elements such as As, Cd and Pb can affect the absorption of essential elements by plants, causing oxidative stress, growth retardation, leaves necrosis and plants death (Aihemaiti et al. 2019; Gupta et al. 2013; Gupta et al. 2014; Li et al. 2018). In addition to the elements in ionic form, recent studies have shown positive and/or negative influence of metal nanoparticles (NPs) in plant development (Apodaca et al. 2017; García-Gómez et al. 2017; Iavicoli et al. 2017; Su et al. 2019).

Nanoparticles have been increasingly used in sensors, new materials, medicines and cosmetics, and as antimicrobial agent, catalyst, food additive, fertilizer (nanofertilizers) and pesticide (nanopesticides) (Ebbs et al. 2016; Iavicoli et al. 2017; Malhotra et al. 2020; Musial et al. 2020; Prasad et al. 2017). Nanoparticles of essentials elements, for example ZnO NPs, have been used as a controlled source of

nutrient for crop improvement (Montanha et al. 2020; Prasad et al. 2017). On the other hand, studies about uptake and toxicity of metal oxides NPs in plants revealed negative effect of NPs on seeds germination, length of root and shoots and dry biomass, besides alteration of nutrient elements concentration (Ebbs et al. 2016; Ma et al. 2010; Prasad et al. 2017).

Lanthanum, a lanthanide element (Ln), has not be considered a nutrient for plants. However, it has been observed that lanthanide elements (Lns) can influence seeds germination, roots size development, total biomass accumulation, production of secondary metabolite and absorption of minerals. Nevertheless, these effects can be positive or negative, depending on the Ln concentration (Hu et al. 2004; Khan et al. 2017; Zhang et al., 2013). Lanthanum oxide NPs ($La_2O_3$ NPs), commonly used in paint coatings, catalyst and luminescent materials, decreased the root length of cabbage, wheat, cucumber, radish, tomato, lettuce and rape when their seeds were soaked in 2000 mg $L^{-1}$ $La_2O_3$ NPs suspension before planting (Ma et al. 2010).

Uptake of $La_2O_3$ NPs by *Pfaffia glomerata* (Spreng.) Pedersen (also known as ginseng) has been investigated and the presence of $La_2O_3$ NPs into stems and leaves were observed (Neves et al. 2019). Ma et al. (2011) demonstrated that $La_2O_3$ NPs were taken by cucumber and transformed into needle-like $LaPO_4$ nanoclusters in intercellular regions in cucumber roots (Ma et al., 2011). However, a detailed study about $La_2O_3$ NPs effects on absorption of other elements by a plant and their distribution in the plant have not been investigated so far.

Mapping of elements in plants allows to understand changes in elements distribution due to external effects, elements transport pathways through plants and storage properties of cells that constitute or border the elemental transport corridor (Conn and Gilliham 2010; Kötschau et al. 2013).

Techniques such as micro-X-ray fluorescence (μ-XRF), secondary ion mass spectrometry (SIMS) and laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS) allow elements mapping in plants, with high resolution (μm to nm) (Ko et al. 2018; Köstchau et al. 2013; Neves et al. 2019; Pozebon et al. 2014). This possibility has opened new perspectives in studies about physiology, biochemical functions and metabolism in plants (Ko et al. 2018; Köstchau et al. 2013). In this contest, the use of LA-ICP-MS has increased due to the good spatial resolution achieved (in the μm range), sensitivity (limit of detection (LOD) in ng $g^{-1}$ to μg $g^{-1}$ range) and multi-element determination capability (Nunes et al. 2016; Pozebon et al. 2014).

Several calibration procedures have been proposed for quantitative elements mapping in plants, mainly calibration with certified reference materials (CRMs), matrix matched standards and/or internal standardization (IS) (Ko et al. 2018; Kötschau et al. 2013; Nunes et al. 2016).

Considering the increased use of Lns NPs and the scarce studies already conducted about effects of these NPs on plants development and nutrient elements uptake by plants, the effect of $La_2O_3$ NPs on *Pfaffia glomerata* (Spreng.) Pedersen were investigated in the present study. *Pfaffia glomerata* was

chosen for the study because it has medicinal properties and can be easily *in vitro* cultivated, which facilitates a controlled environment. To this end, plants were grown in the absence or presence of $La_2O_3$ NPs and nutrient elements determined in leaves and stems of the cultivated plants. In addition, plant development and pigments synthesis were evaluated. For comparison, plants were also grown in presence of bulk-$La_2O_3$ (b-$La_2O_3$).

## 2. Materials And Methods

# 2.1. Reagents and solutions

Ultrapure water (resistivity of 18.2 MΩ cm) was obtained from a Milli-Q system (Millipore Corp., USA) and used for solutions and samples preparation. Nitric acid (65% m $m^{-1}$, Merck, Germany), used for solutions preparation and samples decomposition, was purified by sub-boiling distillation (a Duopur Milistone, Italy, distiller was employed). Calibration solutions were prepared in 5% (v $v^{-1}$) $HNO_3$ by serial dilutions of 10 mg $L^{-1}$ multi-element reference solution (SCP 33 MS, SCP Science, Canada) and 1000 mg $L^{-1}$ (Certipur, Merck IV, Merck, Germany), mono-element reference solutions of S (Spex CertiPrep, USA) and P (Merck, Germany). For method accuracy evaluation, the certified reference materials NIST 1515 (apple leaves) from National Institute of Standards and Technology (NIST, USA) and BCR 670 (aquatic plant) from Community Bureau of Reference (Belgium) were analysed. The $La_2O_3$ NPs (purity of 99.99%, particles size ranging from 15 to 30 nm and with spherical morphology) used for plants treatment were acquired from Nanostrutured & Amorphous Materials Inc. (Houston, USA). The b-$La_2O_3$ (purity of 99.9%) was acquired from Neon Commercial (São Paulo, Brazil). Additional information about morphology, zeta potential and agglomeration of $La_2O_3$ NPs and b-$La_2O_3$ are given elsewhere (Neves et al. 2019).

# 2.2. Instrumentation

An inductively coupled plasma optical emission spectrometer (ICP OES) (Optima 4300 DV, Perkin Elmer, Shelton, USA) was employed for Ca, Cu, Fe, K, Mn, Mg, P, S and Zn determination, whereas emission signals were collected in axially viewed plasma mode. The ICP OES instrument was equipped with a quartz torch with an alumina injector tube (2 mm i.d.), GemCone nebulizer, and cyclonic spray chamber. Lanthanum and Mo were determined by inductively coupled plasma-mass spectrometry (ICP-MS) using an ELAN DCR II instrument (from Perkin Elmer Sciex, Canada). The instrument was equipped with a quartz torch with a quartz injector tube (2 mm i.d.), concentric nebulizer and baffled cyclonic spray chamber. Argon with 99.998% of purity was used as plasma gas, auxiliary and nebulizer/carrier gas. A laser ablation system (LSX-266, Teledyne Photon Machines, Bozeman, MT, U.S.A.) with a frequency quadrupled Nd:YAG (266 nm) laser was connect to the ICP-MS instrument. The LA-ICP-MS system was employed for elements mapping in leaves. The operation parameters of ICP OES, ICP-MS and LA instruments are summarized in Table 1.

**Table 1**

## Instrumental parameters of the ICP OES, ICP-MS and LA instruments

| Parameter | ICP-MS | ICP OES |
|---|---|---|
| RF power, W | 1300 | 1400 |
| Plasma gas flow rate, L $min^{-1}$ | 15 | 15 |
| Auxiliary gas flow rate, L $min^{-1}$ | 1.3 | 0.20 |
| Nebulizer gas flow rate, L $min^{-1}$ | 1.00 | 0.70 |
| Sampler and skimmer cones | Pt | - |
| Sweeps per readings | 5 or 1* | - |
| Readings per replicate | 5 or variable* | - |
| Replicates | 3 or 1* | - |
| Dwell time, ms | 20 | - |
| Monitored isotopes, m/z | $^{13}C$*, $^{44}Ca$, $^{63}Cu$, $^{56}Fe$, $^{139}La$, $^{26}Mg$, $^{55}Mn$, $^{98}Mo$, $^{31}P$*, $^{34}S$* | - |
| Wavelength, nm<br><br>I – atomic line<br><br>II – ionic line | - | Ca II (317,933), Cu I (327,393), Fe II (238,204), K I (766,490), Mg I (285,213), Mn II (257,610), P I (213,617), S I (181,975), Zn II (206,200) |
| | **LA LSX-266** | |
| Fluency, J $cm^{-2}$ | 17.8 | |
| Pulse width, ns | 5 | |
| Repetition rate, Hz | 20 | |

| | |
|---|---|
| Spot size, µm | 100 |
| Scan speed, µm $s^{-1}$ | 100 |
| Ablation mode | line (standards); multiline (leaves) |
| Ablation cell volume, mL | 50 |
| Carrier gas flow rate (Ar), L $min^{-1}$ | 1.30 |

# 2.3. Plant cultivation

Murashige and Skoog (1962) culture medium was prepared and supplemented with equivalent amounts of 30 g $L^{-1}$ sucrose and 0.1 g $L^{-1}$ myo-inositol and the pH adjusted to 5.8. Suspensions containing 2000, 4000 or 8000 mg $L^{-1}$ of $La_2O_3$ NPs or b-$La_2O_3$ were prepared in ultrapure water and sonicated in an ultrasonic bath (Transsonic, Elma GmbH & Co., Germany) for 30 s to disperse the particles (Gomez-Garay et al. 2014). Then, 500 µL of each suspension were added to 9.5 mL of nutrient medium in glass tubes (2.5 cm i.d. x 16.0 cm height) to obtain cultivation media with 100, 200 and 400 mg $L^{-1}$ of $La_2O_3$ NPs or b-$La_2O_3$. Control cultivation medium was prepared by mixing 9.5 mL of nutrient medium and 500 µL of ultrapure water. All tubes containing nutrient medium were closed with an aluminium foil and autoclaved for 30 min. at 120°C and 1.0 atm.

*P. glomerata* plantlets 25-day-old *in vitro* cultivated were obtained from the Genetics Germplan Program, Federal University of Santa Maria, Brazil. Nodal segments (1.0 cm length) without leaves were inoculated in the cultivation medium, the flasks closed with an aluminium foil and then placed in a greenhouse where they were kept for 28 days at 25 ± 2°C, relative humidity between 50-60% and light density of 35 µmol $m^{-2}$ $s^{-1}$. The light was supplied by a cold white-fluorescent lamps during 16 h per day.

# 2.4. Root analysis

Roots were removed from cultivated plants, washed with distilled water and frozen (-5 $^{o}$C) during seven days. Subsequently, the roots were thawed at room temperature and scanned (Epson 11000 XL). The total length, surface area, total volume and average diameter of roots were determined using the WinRhizo Pro Software (Regent Intruments Canada Inc.). The roots were dried at 60°C until constant weight for determination of their dry mass.

# 2.5. Determination of pigments

Carotenoid and chlorophyll concentrations were determined following the method of Hiscox and Israelstam (1979) and estimated by means of the Lichtenthaler's formula (Hiscox and Israelstam 1979; Lichtenthaler 1987). Brie□y, 0.05 g of frozen leaves (-5 $^{o}$C) were incubated at 65°C in concentrated

dimethyl sulfoxide (DMSO) until the tissues were completely bleached. The DMSO solution absorbance was then measured at 470, 645 and 663 nm in a molecular absorption spectrometer (Model Bel Spectro S05, Italy) to determine the carotenoids, chlorophyll α and chlorophyll β concentrations, respectively. Total chlorophyll concentration was calculated by summing the chlorophyll α and chlorophyll β contents.

The anthocyanin concentration was determined according to Zhang and Quantick (1997). Anthocyanin was extracted from the leaves using 5 mL of methanol containing 1% v/v HCl and the absorbance of the extract measured at 600 and 530 nm. The anthocyanin concentration was expressed as the change of 0.1 unit of difference between absorbance measured at 530 nm and 600 nm.

# 2.6. Determination of nutrients elements and lanthanum

Plants were harvested after 28 days of planting and eighteen of them were divided into three groups (n = 3) with six plants each. Leaves and stems were segmented and dried at 60°C until constant weight. Then, approximately 50 mg of dried leaves or stem were exactly weighed and decomposed with 1.0 mL of $HNO_3$ in polypropylene flasks under heating in a water bath at 80 ºC for 2 h. Subsequently, the decomposed sample was analysed by ICP OES and ICP-MS. The CRMs NIST 1515 and BCR 670 were analysed in the same way as the plant samples to check the accuracy of the method. The CRMs analysis results are given in Supplementary Information.

# 2.7. Plant development analysis

The plants development was evaluated through plant length, number of leaves, sprouts and nodal segments and dry biomass. Plant length was measured using a measuring tape whereas the number of leaves, sprouts and nodal segments were visually counted one by one. The aerial part of plant was dried at 60°C until constant weight for dry matter (dry biomass) determination.

# 2.8. Analysis of leaves by LA-ICP-MS

Quantitative analysis of *P. glomerata* leaves by LA-ICP-MS was based on the method developed by Nunes et al. (2016). Calibration curves were prepared using filter paper discs (diameter of 17 mm), each one supporting 40 µL of calibration solution. Multielement calibration solutions (SCP 33 MS) with 0.25, 0.50, 1.25, 2.50, 5.00 and 10.0 mg $L^{-1}$ of Cu, Mn, Mo and Zn were added to filter paper discs, resulting in 0.50, 1.00, 2.50, 5.00, 10.0 and 20.0 µg $g^{-1}$ of these elements. Multi-element (Ca, Fe and Mg) and mono-element (P and S) solutions with 100, 250, 375, 500 and 1000 mg $L^{-1}$ of each element were also added, resulting in 0.20, 0.50, 0.75, 1.0 and 2.0 mg $g^{-1}$ of the five elements. A blank was prepared by deposition of 40 µL of 5% (v $v^{-1}$) $HNO_3$ on a filter paper disc. Then, the filter paper discs with the deposited solutions were dried under an infrared lamp at 80°C for 2 min. and fixed on quartz slides through polyvinyl acetate glue (PVA, Acrilex, Brazil). Three lines of approximately 10 mm length were ablated on each paper disc. To evaluate the LA-ICP-MS method accuracy, a pellet of certified apple leaves (NIST 1515) was prepared by pressing 350 mg of the powdered material with 5 tons (with a hydraulic press) for 2 min. and then ablated in the same way as the filter paper discs. The limit of quantification (LOQ) for each element was estimated by ablating 10 lines of the filter paper disc where the nitric acid solution had been deposited

(blank). The LOQ for each element was calculated following the IUPAC (International Union of Pure and Applied Chemistry) recommendations, using B + 10s, where B is the mean of element concentration of 10 determinations (10 lines ablated in the present case) and s is the respective standard derivation. In all LA-ICP-MS measurements, $^{13}C$ was used as IS. The CRM analysis results and LOQs for LA-ICP-MS are given in Supplementary Information. For elements mapping, leaves removed from *P. glomerata* plants cultivated in the absence (control plant) and presence of 400 mg $L^{-1}$ of $La_2O_3$ NPs or b-$La_2O_3$ were analysed. Prior analysis, the leaves were kept between two filter papers at 25°C and 60-70% humidity. Then, the dried leaves were fixed on quartz slides through a PVA glue and analysed by LA-ICP-MS at the same conditions used for the standards (paper disc where the reference solutions were deposited). The data obtained were exported in .xls format and images of element distribution generated using the MATLAB software (version 7.9.0).

## 2.9. Statistical analysis

The statistical analyses were performed by the Instat software. Data are presented as mean ± standard derivation (1SD) and were tested for statistical significance using analysis of variance (ANOVA) followed by Tukey's pairwise comparison. The default 95% confidence level was considered in all statistical analyses.

# 3. Results And Discussion

## 3.1. Root morphology

Concerning plants, water and elements absorption and synthesis of organic compounds take place in the plant roots. Roots also face contaminants in the soil, and they can change the roots growth and their physiology, which directly influence the plant development. Effects of Lns in plants were observed in previous studies (Hu et al. 2004; Khan et al. 2017; Ma et al. 2010; Zhang et al. 2013). In the present study, reduction of total length, dry mass, surface area and total volume of roots of plants cultivated in the presence of 100, 200 and 400 mg $L^{-1}$ of $La_2O_3$ NPs was observed (Fig. 1). Reduction of dry mass and total volume of roots of plants cultivated in presence of b-$La_2O_3$ was also observed for the three treatments. However, the total length and surface area of roots decreased only in presence of higher b-$La_2O_3$ concentration (400 mg $L^{-1}$).

The roots diameter increase is usually related to difficulties of root growth, possible due to toxicity of one or more substances present in the medium where the plant is cultivated (Bernardy et al. 2016). As can be observed in Fig. 1, the average diameter of roots of the plants cultivated in presence of 200 and 400 mg $L^{-1}$ of $La_2O_3$ NPs and 400 mg $L^{-1}$ of b-$La_2O_3$ increased.

Organic acids, amino acids and other substances are secreted by plants roots and produce Lns complexes that reach the xylem. Phosphorus in high concentration into xylem can precipitate Lns and block the passage of water and minerals into the roots (Ma et al. 2011; Migaszewski and Galuska 2015).

Physical obstruction caused by $La_2O_3$ NPs on the roots is also prone to occur; the size of cell wall pores of roots ranges from 2 to 20 nm, while the size of $La_2O_3$ NPs is in the range of 15 - 30 nm. Thus, part of $La_2O_3$ NPs could enter the cells and other part can be deposit and aggregate on cells wall and block the path of nutrients to plant cells, reducing roots growth (Chichiriccò and Poma 2015).

# 3.2. Pigments concentration

The photosynthetic pigments (chlorophyll and carotenoids) in leaves are responsible for the capture of light, which are essential to generate the energy necessary for plant development. Changes in the concentrations of these pigments are indicative of some stress caused by elements or substances (Li et al. 2018). Fig. 2 shows that the concentration of chlorophyll α, chlorophyll β, total chlorophyll and carotenoids increased in plants cultivated in presence of 100 mg $L^{-1}$ of $La_2O_3$ NPs or 200 mg $L^{-1}$ of b-$La_2O_3$. Such effect can be related to a catalytic action of La on the pigments production. The lower concentration of $La_2O_3$ NPs required to increase the pigments synthesis can be attributed to the greater activity of the NPs. Treatment with 400 mg $L^{-1}$ of $La_2O_3$ NPs or b-$La_2O_3$ decreased the concentration of these pigments remarkably. The chlorophyll and carotenoids concentrations decrease are related to the reduction of photosynthetic activity and dry matter (biomass), and oxidative stress (Khataee et al. 2017; Missaoui et al. 2017). Aggregation of $La_2O_3$ NPs on the surface of plants roots, which impairs water transport to leaves, may have also affected the pigments production in plants treated with 400 mg $L^{-1}$ of $La_2O_3$ NPs. A decrease of chlorophyll α, chlorophyll β and carotenoids in leaves was also observed when plants were grown in presence of $TiO_2$ NPs (Missaoui et al. 2017).

As can be seen in Fig. 2, the anthocyanins concentration increased in plants treated with b-$La_2O_3$ or $La_2O_3$ NPs, excepting plants treated with 100 mg $L^{-1}$ of b-$La_2O_3$. The increase in anthocyanins was naturally due to an antioxidant defense mechanism against overproduction of reactive oxygen species (ROS) that could damage lipids, proteins and DNA in plants under stress; anthocyanins actuate as a ROS scavenger, besides being hydrogen donor and metal chelator in plants. Additionally, the stress caused by b-$La_2O_3$ or $La_2O_3$ NPs could induce the production of $H_2O_2$, triggering anthocyanins biosynthesis by the plants. Changes in antioxidative enzyme activities (GPOX, APX and CAT) and membrane peroxidation damage was also observed in plants grown in presence of $TiO_2$ NPs (Missaoui et al. 2017).

# 3.3. Plants development

The plants length, number of segments, leaves, sprouts and dry mass of the aerial part of plants under study are illustrated in Fig. 3. According to this figure, plants cultivated in presence of 400 mg $L^{-1}$ of b-$La_2O_3$ were noteworthy affected, but the aerial part of plants treated with 100 and 200 mg $L^{-1}$ of b-$La_2O_3$ were not different of control plants. Plants treated with $La_2O_3$ NPs were more affected than those treated with b-$La_2O_3$. However, alike plants treated with b-$La_2O_3$, the number of sprouts did not decrease in plants treated with 400 mg $L^{-1}$ of $La_2O_3$ NPs. The plant aerial part development depends on root performance

and nutrients absorption. Thus, it can be said that $La_2O_3$ NPs affected nutrients absorption by plants, and the plant length, number of leaves and dry biomass were accordingly affected.

Figure 4 shows images of plants that were cultivated absence and in presence of b-$La_2O_3$ or $La_2O_3$ NPs to illustrate the root and aerial part of plants. It can be seen in Figure 4 that both the aerial part and roots of plants treated with $La_2O_3$ NPs were more affected than plants treated with b-$La_2O_3$.

# 3.4. Determination of nutrient elements and La

Water-soluble Lns are easily taken by plant roots through complexation with organic acids, which allows the elements uptake and their transport to aerial parts of the plant (Khan et al. 2017; Ma et al. 2011). Ma et al. (2011) reported biotransformation of $La_2O_3$ NPs into $La^{3+}$ and $LaPO_4$ in cucumber roots. However, in another study reported, $La_2O_3$ NPs were found in stems and leaves of *P. glomerata* cultivated in presence of 400 mg $L^{-1}$ of $La_2O_3$ NPs (Neves et al. 2019). Therefore, different interactions of NPs occur in plants and distinct effects are possible due to differentiated physicochemical properties of NPs (Ma et al. 2011). Consequently, the presence of NPs in plants may also influence the uptake and transport of other elements in plants.

Figure 5 shows the concentration of nutrient elements in stems and leaves of *P. glomerata* plants cultivated in absence (control) and presence of 100, 200 e 400 mg $L^{-1}$ b-$La_2O_3$ or $La_2O_3$ NPs. In general, the nutrient elements concentration in stems and leaves of plants cultivated in presence of $La_2O_3$ NPs changed more than in presence of b-$La_2O_3$ when compared to control plants. Iron and Mn were the most affected elements whose concentrations reduced significantly for all treatments with b-$La_2O_3$ or $La_2O_3$ NPs. Other elements very affected were Mg and S whose concentrations decreased significantly in stems and leaves. Nevertheless, in stems of plants cultivated in presence of 100 mg $L^{-1}$ of b-$La_2O_3$ the Mg and S concentrations did not change significantly when compared to control plants. The Ca and Zn concentrations decreased significantly in leaves, differently of the stems where the Ca concentration decrease was not significant. Zinc concentration reduced only in stem of plants cultivated in presence of 400 mg $L^{-1}$ $La_2O_3$ NPs. However, the Zn concentration in leaves reduced for all treatments with b-$La_2O_3$ or $La_2O_3$ NPs, differently of the Ca concentration. The Ca concentration did not decrease significantly, except in the leaves of plants cultivated in presence of 100 mg $L^{-1}$ of b-$La_2O_3$.

In Fig. 5 it is seem that in some cases the element concentration increased when plants were treated with b-$La_2O_3$ or $La_2O_3$ NPs. The reason might be the beneficial effects of La for plants, which depends on La concentration (Rim 2016; Thomas et al. 2014; Zhang et al. 2013). For example, the Cu, Mo and P concentrations increased significantly in stems of plants cultivated in presence of 100 mg $L^{-1}$ of $La_2O_3$ NPs. Copper concentration increase is also observed in stems of plants treated with 200 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs. On the other hand, in stems of plants cultivated in presence of 400 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs the Cu concentration decreased significantly. The Cu concentration in leaves reduced significantly for 400 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs, differently of what is observed for the other two

treatments. The P concentration increased in the stem of plants treated with 100 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs, whereas the element concentration in stems of plants cultivated in presence of 200 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs did not change significantly. Contrarily, in stem of plants treated with 400 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs the concentration of P reduced significantly. A decrease of P concentration in leaves were observed for treatments with 200 and 400 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs. The Mo concentration in leaves and stems of treated plants decreased, except in stems of plants cultivated in presence of 100 mg $L^{-1}$ of $La_2O_3$ NPs, where the Mo concentration increased significantly. The K concentration did not change in stems of plants cultivated in presence of b-$La_2O_3$ or $La_2O_3$ NPs. However, the K concentration increased significantly in leaves of plants cultivated in presence of 400 mg $L^{-1}$ of b-$La_2O_3$.

The increase or decrease of nutrient elements concentration in stems and leaves of the treated plants could be associated with La absorption by the plants (see Fig. 6). Remarkable and similar decrease of investigated elements occurred in stems and leaves of plants treated with 400 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs. However, the La concentration found in plants cultivated in presence of b-$La_2O_3$ was seven-fold higher in their leaves and four-fold higher in their stems when compared to plants grown in presence of $La_2O_3$ NPs. This demonstrates that the plants absorbed more La when it was added in the form of b-$La_2O_3$ to plants. As previously discussed, the size of part of the $La_2O_3$ NPs is lower than the cell wall pores where the NPs can obstruct the channels instead of entering the cells and thus reduce the absorption of nanoparticles. The agglomeration of nanoparticles, as shown in Neves et al. (2019), can also influence their absorption by plants.

However, roots of plants grown in periodically waterlogged soil or nutrient solution have the lacunate cortex where there are large intercellular spaces, making possible NPs penetration. Moreover, the endodermis has passage cells that can act as a pathway for water and dissolved solutes. Such cells can be the route for NPs transport from endodermis to xylem in the root. The NPs can move along the xylem together with bulk water/sap and achieve the superior parts of the plant (Su et al. 2019); the presence of $La_2O_3$ NPs in stems and leaves has been observed in previous study (Neves et al. 2019). However, the high ionic strength of the sap (due to the abundant presence of divalent cations) may cause partial NPs aggregation and obstruct the pathway to nutrients in the plant. Therefore, NPs not only interact within the plant vascular system, but also impair the absorption of nutrient elements due to physical obstruction (Martínez-Fernández et al. 2016).

According to Fig. 6, for treatment with 100 mg $L^{-1}$ of $La_2O_3$ NPs or b-$La_2O_3$, the La amount transported to stems and leaves was higher in plants cultivated in presence of $La_2O_3$ NPs (34.7 ± 1.6 μg $g^{-1}$ in stem and 81.2 ± 8.8 μg $g^{-1}$ in leaves) than in plants cultivated in presence of b-$La_2O_3$ (25.1 ± 0.4 μg $g^{-1}$ in stem and 22.4 ± 0.2 μg $g^{-1}$ in leaves). These results demonstrated that when present in low concentration (100 mg $L^{-1}$ in the present case) the La in $La_2O_3$ NPs is more easily taken by the plant. It should be cited that

NPs agglomeration is lower when present in lower concentration as already discussed. In addition, the higher reactivity of $La_2O_3$ NPs in comparison to b-$La_2O_3$ can increase the bioavailability of La species for plants (Khan et al. 2017). Nevertheless, an opposite effect is observed for stems and leaves of plants grown in presence of 400 mg $L^{-1}$ of b-$La_2O_3$. The La concentration increased in stems and decreased in leaves of plants cultivated in presence of 100 or 200 mg $L^{-1}$ of $La_2O_3$ NPs, possibly due to aggregation of NPs in the sap (Su et al. 2019).

Effects of Lns on stabilization and functionalization of cytoplasm membrane, changing of membrane characteristics and membrane cells fluidity were reported (Hu et al. 2004). Lanthanum could influence several reactions in cells, decreasing the permeability of cells membrane. Therefore, La could influence ionic fluxes and affect several plants process, including nutrients uptake. These processes are based on complex mechanisms that are not understood and well described yet (Hu et al. 2004; Khan et al. 2017).

## 3.5. Mapping of nutrient elements in leaves

Figure 7 shows the nutrient elements distribution in leaves of *P. glomerata* plants cultivated in presence of 400 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs and control plants. Leaves of these plants were chosen for elements mapping for better visualization of the effects of b-$La_2O_3$ and $La_2O_3$ NPs. According to Fig. 5, the Cu, Fe and Mg concentrations decreased in leaves of plants treated with 400 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs in relation to control pants. In Figure 7 it is possible to observe that the distribution of these elements was differently affected; Fe was more concentrated around the main vein in leaves of control plants and in those of plants treated with b-$La_2O_3$ or $La_2O_3$ NPs; Mg distribution was similar in leaves of plants treated with b-$La_2O_3$ or $La_2O_3$ NPs, whereas the element was more homogeneously distributed when compared to leaves of control plants. Cooper concentration was practically homogenous in leaves of control plant or cultivated in presence of $La_2O_3$ NPs, but the element was more concentrated in the tip of leaves of plants treated with b-$La_2O_3$. The Mo, P and S distribution in leaves of control plants was homogeneous, but more concentrated in the tip of leaves of plants treated with b-$La_2O_3$; Mo and S were more concentrated in the centre of leaves of plants treated with $La_2O_3$ NPs while P was homogeneously distributed in these leaves. The Zn and Mn concentrations decreased in leaves of plants cultivated in presence of b-$La_2O_3$ or $La_2O_3$ NPs in relation to control plants leaves where Zn was more concentrated in the main vein. Manganese was more concentrated in the main vein in leaves of plants treated with b-$La_2O_3$, in the tip of leaves of control plants and more homogenously distributed in leaves of plants treated with $La_2O_3$ NPs. Calcium is not affected by both species of lanthanum, however some differences in its distribution can be observed mainly for plant treated with $La_2O_3$ NPs. Therefore, the images in Figure 7 make clear the alteration of nutrient elements distribution in leaves of *P. glomerata* treated with 400 mg $L^{-1}$ of $La_2O_3$ NPs or b-$La_2O_3$. The nutrient elements mapping also revealed distinct effects caused by $La_2O_3$ NPs and b-$La_2O_3$.

# 4. Conclusion

It can be concluded that both $La_2O_3$ NPs and b-$La_2O_3$ added to plants can affect their development, pigments synthesis and nutrient elements uptake. However, the $La_2O_3$ NPs and b-$La_2O_3$ effects are dissimilar. The reduction of nutrient elements concentrations in leaves and stems was dependent on the $La_2O_3$ form, whereas $La_2O_3$ NPs affected more than b-$La_2O_3$. Plants treated with the latter were visible better, denoting that $La_2O_3$ NPs was more toxic to plants than was b-$La_2O_3$. The worst root and aerial part development and changes in pigments concentration revealed the more severe effect of $La_2O_3$ NPs. Although the nutrient elements concentrations were similar in stems and leaves of plants treated with 400 mg $L^{-1}$ of b-$La_2O_3$ or $La_2O_3$ NPs, the elements mapping showed significant difference of their distribution in leaves. The results obtained in the present study demonstrated that $La_2O_3$ NPs can exert a negative effect on plants, depending on the $La_2O_3$ NPs concentration. The effect of $La_2O_3$ NPs at concentration lower than 100 mg $L^{-1}$ is the objective of future studies, to check if their effects would be positive or different of those observed for higher $La_2O_3$ NPs concentration.

# Declarations


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### EthicalApproval

Notapplicable.

### ConsenttoParticipate

All authors confirm consent to participate in this journal.

### Consent to Publish

All authors accept to publishing.

### Authors Contributions

Vinicius Machado Neves was responsible for the analysis by LA-ICP-MS and text organization.Graciela Marini Heidrich was responsible for the analysis by ICP-MS.Camila Cavalheiro da Costa was responsible for the plant cultivation.Julia Gomes Farias was responsible for the plant cultivation.Fernando Teixeira Nicoloso was responsible for the plant cultivation and text revision.DircePozebon was responsible for text organization and revision.ValderiLuiz Dressler was responsible for supervising the data and text organization.


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### Competinginterests

The authors declare no competing interests.

### Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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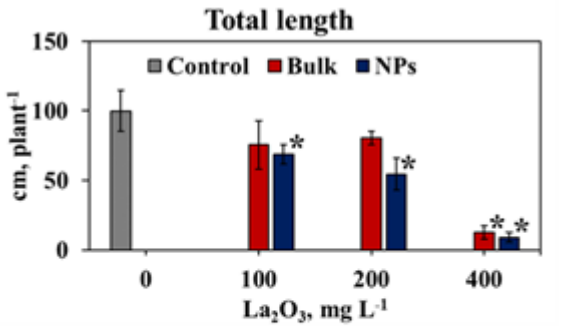
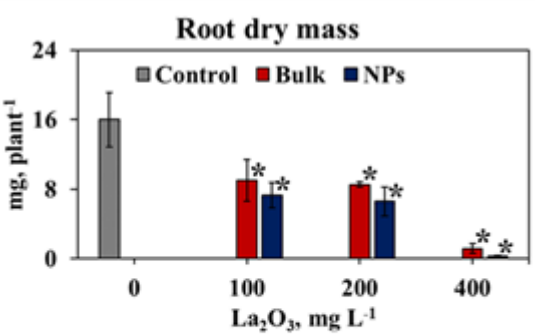
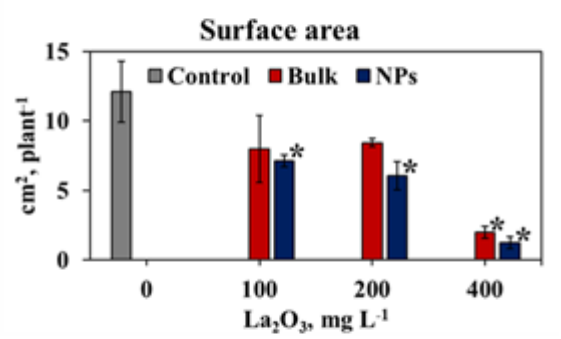
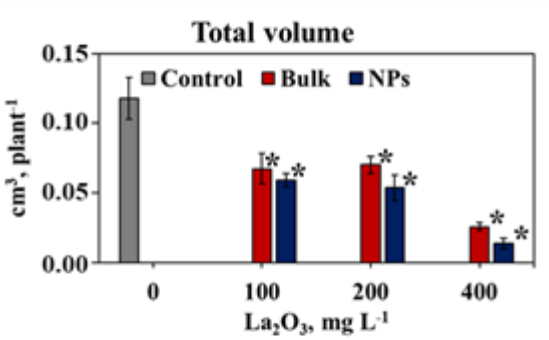
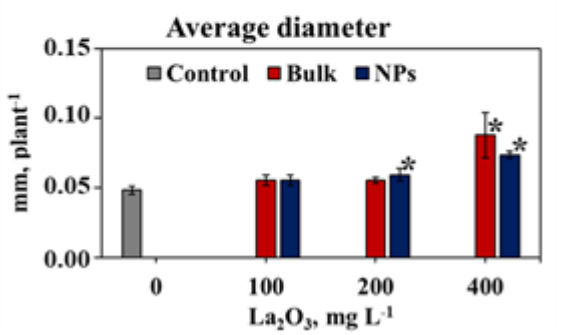
# Figures



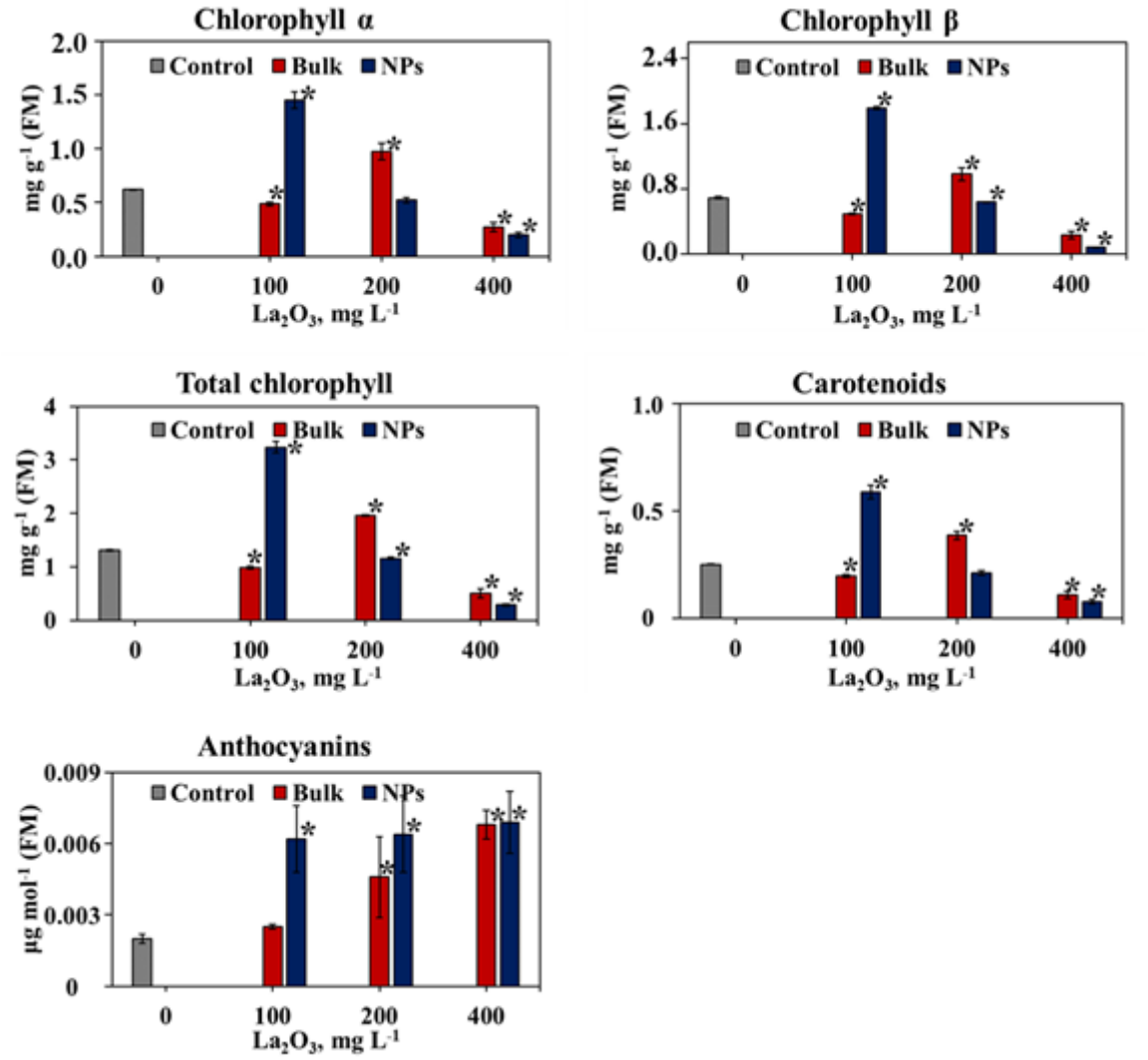



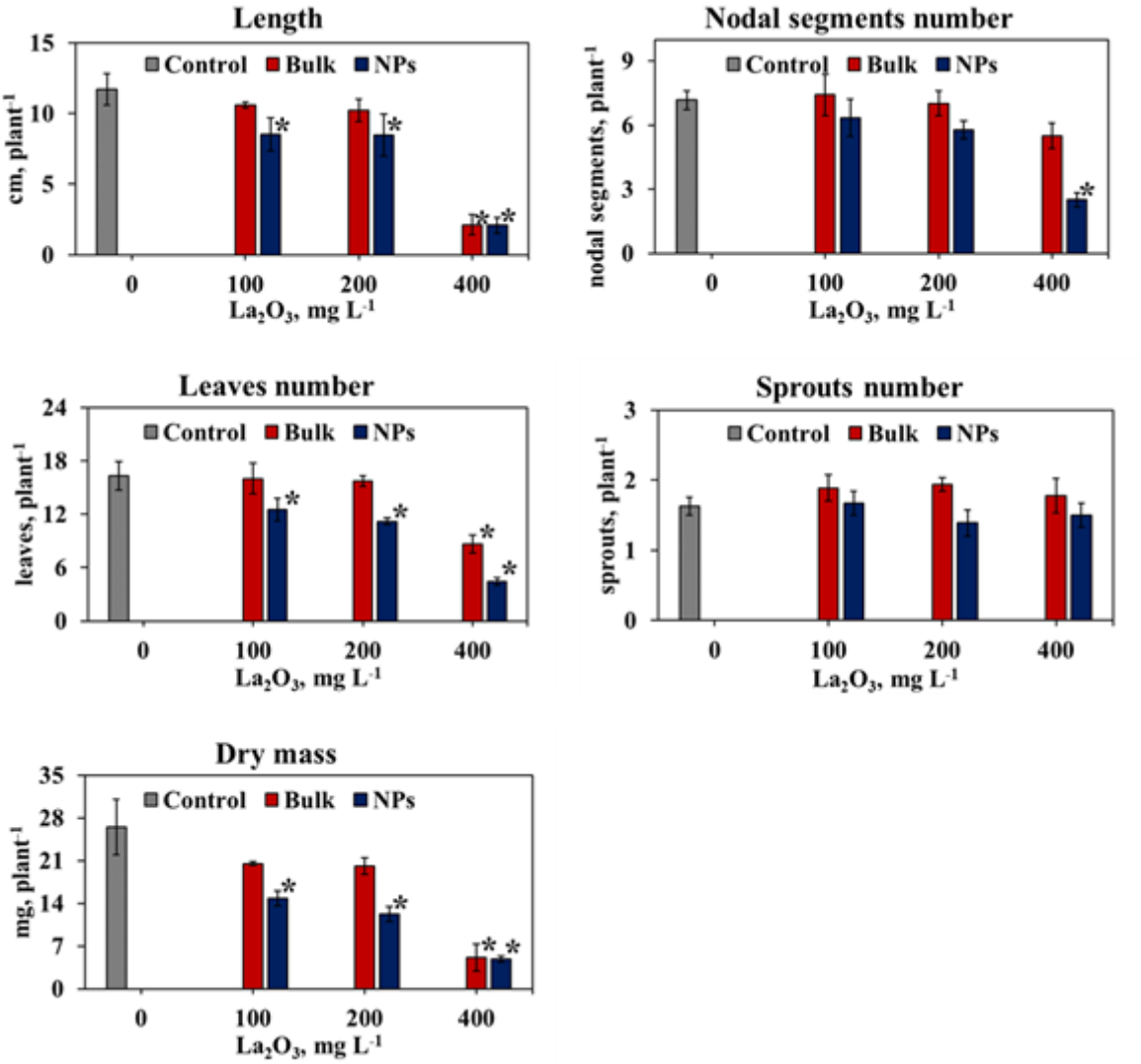
## Figure 1

Effect of b-La2O3 or La2O3 NPs on root morphology of P. glomerata cultivated in vitro. Results are expressed as the average and standard deviation of three determinations (three groups with six plants, each). Bars marked with * denote values significantly different ($\rho < 0.05$) of control plants.



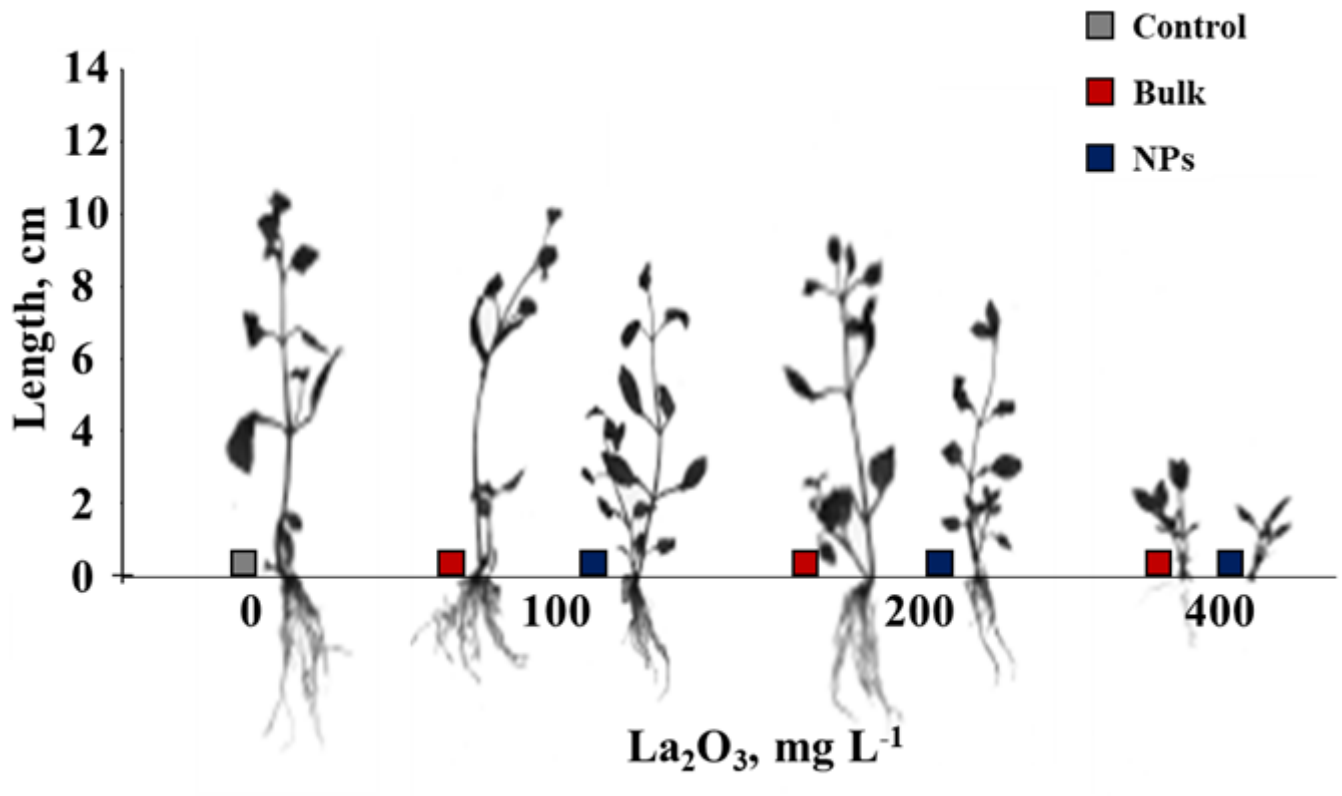

## Figure 2

Effect of b-La2O3 or La2O3 NPs on pigments concentration in P. glomerata cultivated in vitro. Results in fresh matter (FM) are expressed as the average and standard deviation of three determinations (three plants were analysed). Bars marked with * denote values significantly different ($\rho < 0.05$) of control plants.



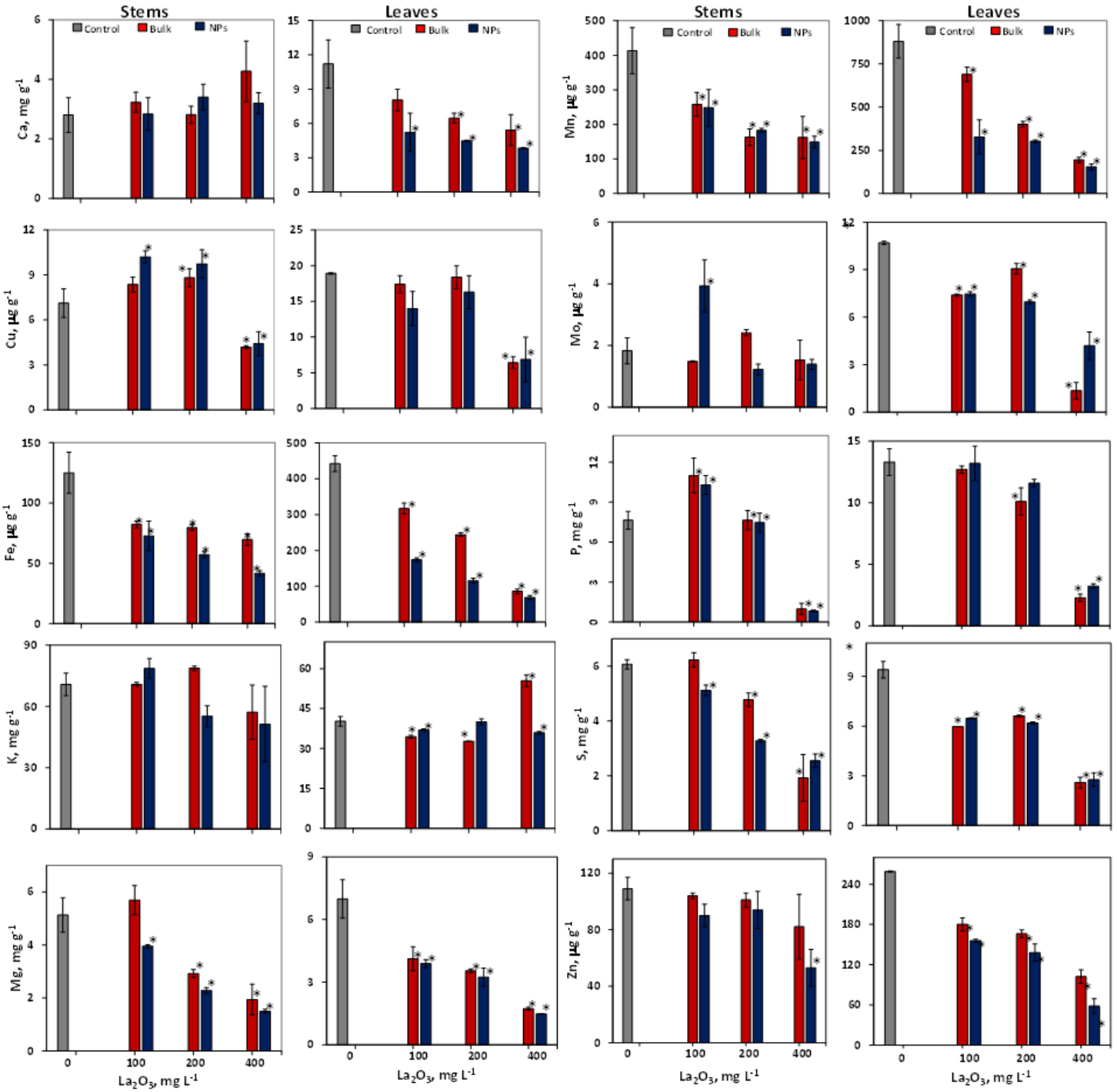

## Figure 3

Effect of b-La2O3 or La2O3 NPs on the aerial part of P. glomerata cultivated in vitro. Results are expressed as the average and standard deviation of three determinations (three groups with six plants each). Bars marked with * denote values significantly different ($\rho < 0.05$) of control plants.



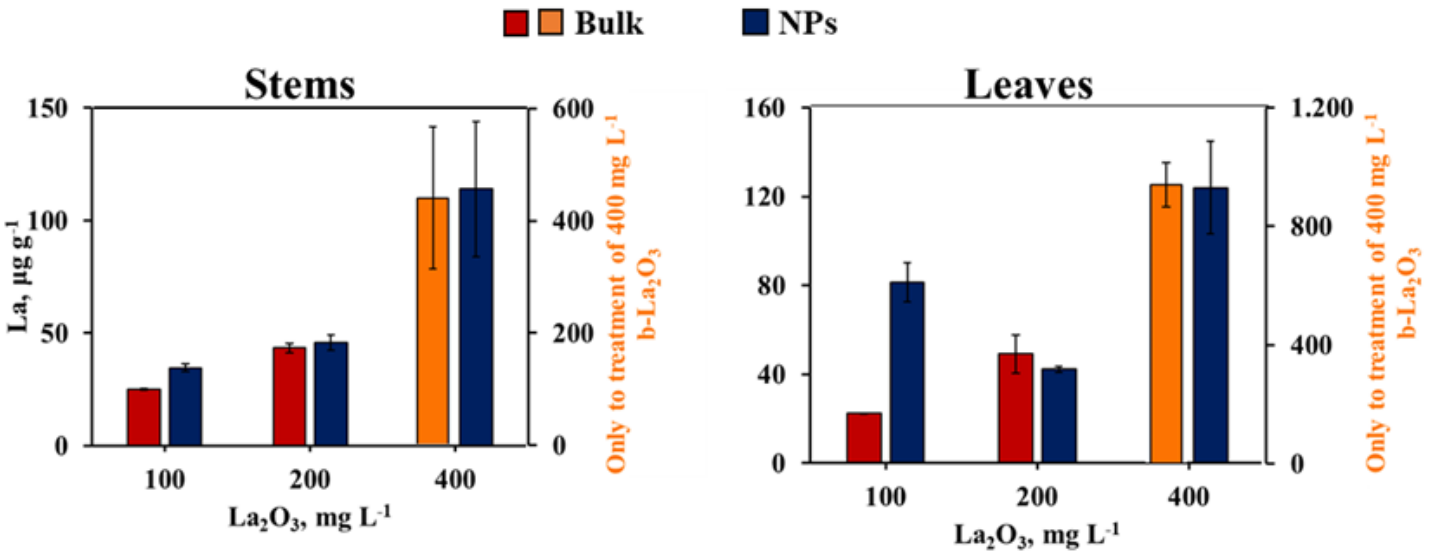

## Figure 4

Images of scanned P. glomerata plants cultivated in absence (control) and presence of b-La2O3 or La2O3 NPs.



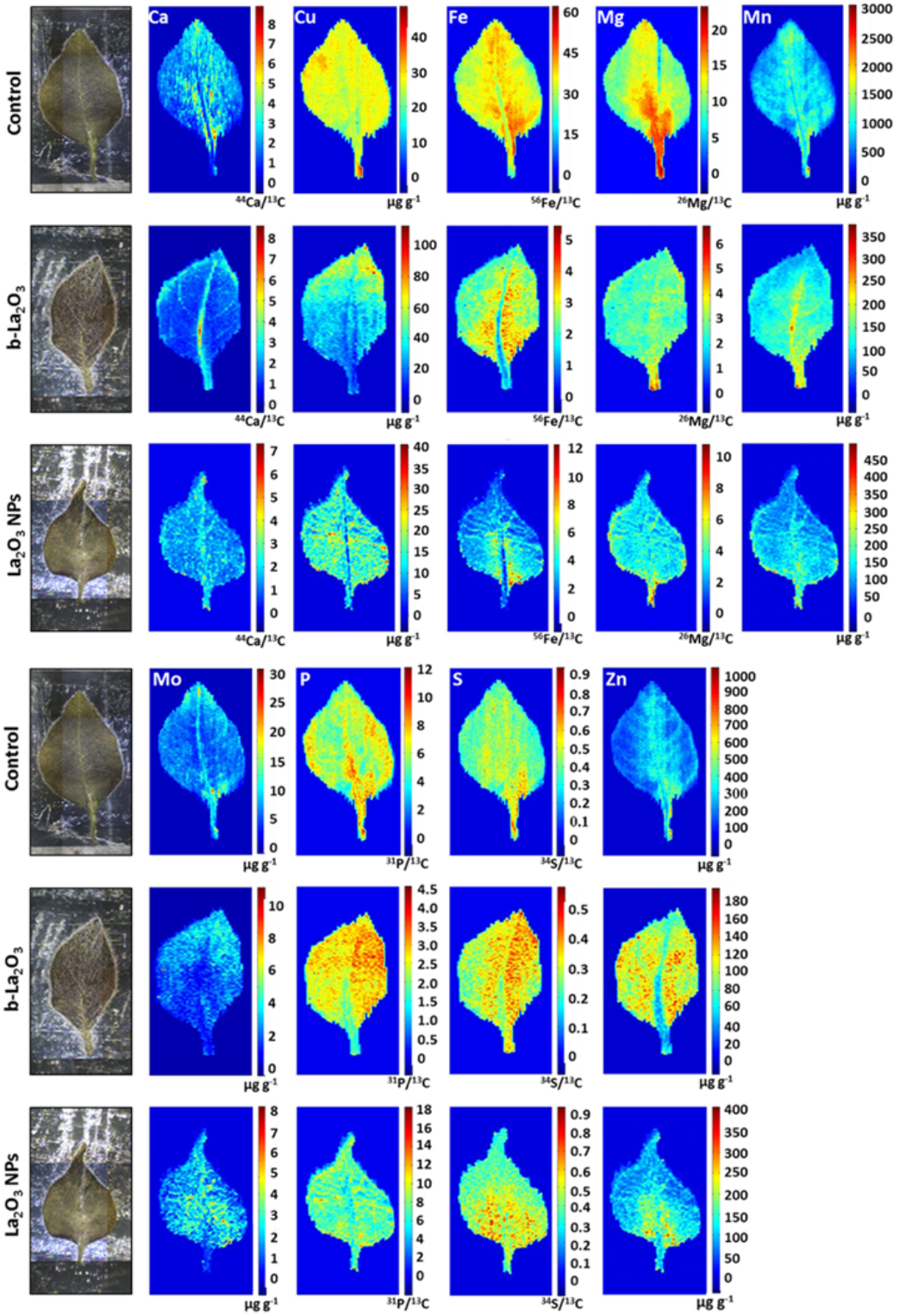

## Figure 5

Effect of b-La2O3 or La2O3 NPs on nutrient elements concentration in stems and leaves of P. glomerata cultivated in vitro. Concentrations are expressed as the mean and standard deviation of three determinations by means of ICP OES (Ca, Cu, Fe, K, Mg, Mn, P, S and Zn) or ICP-MS (Mo) after sample decomposition. Bars marked with * denote concentrations significantly different ($p < 0.05$) of those found in control plants.




## Figure 6

Lanthanum concentration in stems and leaves of P. glomerata cultivated in vitro. Concentration values are mean and standard derivation of three replicate determinations. Lanthanum was not detected in control plant (results not shown in the figures).




## Figure 7

Distribution of nutrient elements in P. glomerata cultivated in absence (control) and presence of b-La2O3 or La2O3 NPs. Images on the left are photographs of the leaves fixed on quartz slides. The other images were generated from data obtained in LA-ICP-MS analysis. Copper, Mn, Mo and Zn (could be quantified, and images are for their concentrations. The ratio element signal/13C signal (internal standard) images are shown for Ca, Fe, Mg, P and S.

# Supplementary Files

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- SupplementaryInformation.docx