Influence of GdVO₄:Eu³⁺ nanocrystals on growth, germination, root cell viability and oxidative stress of wheat (*Triticum aestivum* L.) seedlings

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Abstract

Increasing application of lanthanide-doped nanocrystals (LDNCs) entails a risk of a harmful impact on the natural environment. Therefore, in the presented study the influence of gadolinium orthovanadates doped with Eu^{3+} nanocrystals on wheat (*Triticum aestivum* L.), chosen as a model plant species, was investigated. The seeds were grown in Petri dishes filled with colloids of LDNCs at the concentrations of: 0, 10, 50 and 100 $\mu\text{g/ml}$. The plants' growth endpoints (number of roots, roots length, roots mass, hypocotyl length and hypocotyl mass) and germination rate were found to be not significantly changed after the exposure to $\text{GdVO}_4:\text{Eu}^{3+}$ nanocrystals at all used concentrations. The presence of LDNCs also had no effect on oxidative stress intensity determined on the basis of the amount of lipid peroxidation product (thiobarbituric acid reactive substances; TBARS) of the roots. Similarly, TTC (tetrazolium chloride) assay did not show any differences in cells' viability. However, root cells of the treated seedlings contained less amount of Evans Blue (EB) when compared to the control.

Keywords

nanotoxicology, lanthanide-doped nanoparticles, vascular plants, phytotoxicity, lipid peroxidation, cell viability

Introduction

Recently, a growing interest in the use of nanoparticles (NPs) has been observed. Their unique properties have been utilized in chemical and biological

sciences, medicine, as well as in industry and agriculture. However, along with many advantages, their use entails some risks. They can enter the environment and may have impact on the wildlife and human health (1–3). The effects of NPs may be completely different than those of their larger counterparts (bulk materials) (4–7). These differences result from higher surface area to volume ratio and higher surface reactivity of nanostructures. Moreover, due to smaller size NPs could enter organisms more easily as compared to bulk structures ⁴, although it is not always the case ⁵.

NPs are taken up by plants and they are subsequently spread and accumulated in the tissues, mainly in roots, stems and leaves (8–11). The NPs influence the contaminated plants in many ways (12–14). They may negatively affect germination, cells morphology, growth rate or final biomass of plants and induce oxidative stress (6,15–23). However, some authors have shown no negative consequences or even biostimulating effect of NPs on plants including oat (*Avena sativa* L.), corn (*Zea mays* L.), tomato (*Lycopersicon esculentum* L.), cress (*Lepidium sativum*), white mustard (*Sinapis alba*) sorghum (*Sorghum saccharatum*), lettuce (*Lactuca sativa* L.) and wheat (*Triticum aestivum* L.) (24–28). The positive effect occurred mainly at low concentrations and was reversed at higher doses of NPs (16,17). The improvement of growth may be caused by more effective uptake of water or nutrients in plants exposed to NPs (17,26,29), as well as interference with the toxic metals taken up from the medium (30,31). The overall effect of NPs on plants depends on numerous factors, such as time of the exposure, surface contamination, size, plants species, etc. (32–35).

Inorganic nanocrystals doped with lanthanide ions (lanthanide-doped nanocrystals, LDNCs) have been widely investigated. Their unique spectroscopic properties, such as the ability to luminescence, resistance to photobleaching and photochemical

degradation have been found very attractive for many potential applications (36,37). LDNCs are used in innovative technologies, optoelectronic industry (e.g. LCD), agriculture, as well as biological and medical sciences (e.g. drug delivery and bioimaging) (38–41). Among them, gadolinium-based LDNCs such as vanadates gadolinium doped with Eu^{3+} ions may be applied in magnetic resonance imaging, as catalysis, polarizers, laser hosts and luminescent materials (42).

Nanostructures used in the above-mentioned applications may be transferred to the environment. According to a study in California, the highest amount of gadolinium was observed in the water bodies close to hospitals and medical centres by the hospital and medical centres. This discovery suggests that the Gd-based contrast agents used for magnetic resonance imaging are the main source of contamination (43). Gadolinium may accumulate also in animal tissues, such as the human brain (44). A study with vertebrate cell lines has revealed that gadolinium may influence the vertebrate cells, e.g. cause apoptosis (45). Wysokińska et al. have reported that $\text{NaGdF}_4:\text{Yb}^{3+}, \text{Er}^{3+}$, NaGdF_4 or $\text{NaGdF}_4:\text{Eu}^{3+}$ NPs may negatively affect the viability of macrophages and fibroblast (39). Our previous study conducted on LDNCs similar to those used in this one has shown that $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{GdVO}_4:\text{Eu}^{3+}$ 5% core@shell type nanostructure had no effect on the erythrocyte sedimentation rate, morphology of red blood cells or their membrane permeability when present in concentrations up to 1 mg/mL (46).

Not many authors have been interested in the toxicity of LDNCs towards plants. According to literature data, LDNCs may be taken up by particular parts of plants and their presence may influence, similarly like other nanostructures, plant development (9,10,16–18,47,48). Moreover, most of the previously published data on phytotoxicity of LDNCs concerned fluorides, mainly NaYF_4 (9,10,16,17,49) or oxides (18). To the

best of our knowledge, no data have been published on the phytotoxicity of vanadates gadolinium doped with europium ions. In the presented study the impact of GdVO₄:Eu³⁺ NPs on wheat seedlings was investigated.

Materials & Methods

Nanoparticles characterisation and elemental analysis

The NPs used for ecotoxicity investigation were based on inorganic matrix of orthovanadates doped with Eu³⁺ ions, GdVO₄: Eu³⁺, synthesized under hydrothermal conditions. Detailed description of the synthesis method and photophysical characterization of the NPs used are presented in the previous paper (50). After the synthesis of NPs, the concentration of the stock solution was 3.54 mg/ml as determined by inductively coupled plasma-optical emission spectrometry (Varian ICP-OES VISTA-MPX). Before the assays, transmission electron microscopy (TEM) images were recorded using an HRTEM JEOL ARM 200F transmission electron microscope at the accelerating voltage of 200 kV. Dynamic light scattering (DLS) and zeta potential measurements were performed using a Malvern Zetasizer Nano ZS instrument. Concentration of the colloid sample was 1 mg/mL. Zeta potential was measured at physiological pH.

In order to evaluate the uptake of LDNCs by the plant organisms, elementary analysis was used. After 3 days of incubation, the seedlings were collected, weighted, rinsed, and mineralized in a pressure reactor with HNO₃. Next, the samples were analysed using inductively coupled plasma optical emission spectroscopy (ICP OES).

Plant growth and treatment

Wheat (*Triticum aestivum* L.) seeds were kindly supplied by the Danko Company. The seeds were surface sterilised for 5 min. with 75% ethanol and another 10 min. with 1% sodium hyperchlorite according to (51). Afterwards, the seeds were washed with tap water for 30 min. and immersed in distilled water for 10 min.

Next, the seeds were incubated with the GdVO₄:Eu³⁺ NPs according to (32) with some modifications. The seeds were placed in Petri dishes (30 seeds per dish) and filled with colloids of LDNCs at the following concentrations: 0, 10, 50 or 100 µg/ml of the aqueous solution. The concentrations were determined on the basis of the results of previous studies of lanthanide-doped nanostructures. The colloids were sonicated before all assays. They were grown in the dark at a stable temperature of 22°C for 3 days.

Measurement of growth parameters

Germination rate, seedling growth (root and hypocotyl length), number of roots and fresh weight (root and hypocotyl mass) were measured. Six replicates (each consisting of 30 seedlings) were run per treatment.

Cell viability estimation

Cell viability was evaluated by two spectrophotometric methods: staining with Evans Blue (EB) and tetrazolium chloride (TTC). The first method is based on the accumulation of EB in cells with damaged membrane. The measurements were performed according to the procedure given in (52) with some modifications. Approximately 200 mg of roots were cut off on the ice and incubated with 0.25% Evans blue (Sigma, E-2129) for 20 min. at room temperature. Afterwards, the roots were washed twice for 15 min. with distilled water, homogenized in a mortar with the distaining solution (50 ml of ethanol, 49 ml of distilled water, and 1 ml of 10% SDS)

and incubated in the heating block for 15 min at 50°C. Next, the samples were centrifuged (12000 rpm, 20°C, 15 min.) and the absorbance of the supernatant was measured at 600 nm. Each experiment was repeated four times and all samples in each experiment were tested in duplicate.

The second method, consist in measurements of the amount of insoluble red formazan that is accumulated in living cells as a consequence of reduction of 2,3,5-triphenyltetrazolium chloride (TTC) with dehydrogenase (53). Seedling roots were cut off on ice and incubated with 1ml of 0.4% TTC (Serva; Heidelberg, Germany) for 24 hours at room temperature in the dark. Next, the roots were washed with distilled water, transferred to new tubes and flooded with 0.5 ml of 95% ethanol. The samples were homogenized with Tissue Lyser II (Qiagen) and incubated at 55°C for one hour. After that, another portion of 0.5 ml of 95% ethanol was added to the samples, which were next vortexed and centrifuged for 3 minutes at 10 000 rcf. The volumes of 800 µl of supernatant and 1400µl of 95% ethanol were mixed in spectrophotometer cuvette and the absorbance of the samples was measured at $\lambda=485$. Each experiment was repeated four times.

Lipid peroxidation determination

For assessment of lipid peroxidation in seedling roots, the amount of thiobarbituric acid-reactive substances (TBARS) was measured, according to the method described in (54) with some modifications. Whole roots of seedlings (approximately 200 mg) were cut off on ice and homogenised with 3 ml of 10% TCA (Sigma-Aldrich, TO699). Thereafter, the samples were centrifuged (12000 rpm, 4°C, 10 min.) and 1 ml of supernatant was transferred to glass tubes, filled with 4 ml of 0.5% TBA dissolved in 10% TCA. After incubation at 95°C for 30 min., the samples were cooled. Next, they were mixed by inversion and centrifuged (5000 rpm, 4°C, 2 min.).

The absorbance of the supernatant was measured at 532 nm and corrected for unspecific absorbance at 600 nm (0.5% TBA in 10% TCA was used as a blank). Each experiment was repeated four times and all samples in each experiment were tested in duplicate.

Statistics

Statistical analyses were performed using Statistica 13.3 for Windows (StatSoft). Differences in length and mass of the seedlings, as well germination rate between treatments were analysed with nonparametric Kruskal-Wallis test. For cell viability and lipid peroxidation quantification, analysis of variance (ANOVA) was used, followed by the Tukey post hoc test (if needed). Data were shown as a mean $\pm$ standard error (SE).

Results

Nanoparticles characterisation and elemental analysis

The NPs' structure and morphology were characterized on the basis of DLS measurements and TEM images. DLS analysis showed that the hydrodynamic diameter of NPs, including their agglomerates, varies between 140 and 180 nm (Fig 1a). Furthermore, TEM image analysis confirmed that NPs form agglomerates with the average grain size 70 ± 5 nm. Additionally, a negative zeta potential (-20.0 ± 4.29 mV), indicates that NPs colloids are stable at physiological pH and appropriate to use as water colloids (55). To present stability and transparency of $\text{GdVO}_4:\text{Eu}^{3+}$ NPs aqueous colloids at concentration of 10 mg/ml, pictures in a daylight were taken (Fig 1b, left

side). Furthermore, red luminescence under 254 nm UV excitation related to the presence of Eu^{3+} emitting ions was also performed (Fig 1b, right side).

Fig 1 a DLS analysis of the $\text{GdVO}_4\text{:Eu}^{3+}$ NPs. **b** Photographs presenting the colloidal $\text{GdVO}_4\text{:Eu}^{3+}$ NPs, in a daylight (left) and under 254 nm UV light excitation (right)

ICP-OES analysis revealed that the LDNCs were taken up by the wheat seedlings.

Growth and germination of the seedlings

Treatment with $\text{GdVO}_4\text{:Eu}^{3+}$ NPs had no significant impact on the morphology of wheat seedlings. The influence of the $\text{GdVO}_4\text{:Eu}^{3+}$ NPs on several growth endpoints, including the number of roots, roots length, hypocotyl length, roots mass and hypocotyl mass, was investigated. These parameters were not significantly changed as a result of the plants exposition for 3 days to 10, 50 and 100 $\mu\text{g/ml}$ of LDNCs, relative to the controls (Kruskal-Wallis test, $p>0.1$; Fig 2a-e). The studied LDNCs also did not affect the germination rate of the tested concentrations (Kruskal-Wallis test, $p>0.1$; Fig 2f).

Fig 2 Number of roots (a), roots length (b), hypocotyl length (c), roots mass (d), hypocotyl mass (e) and germination rate (f) of wheat, treated for 3 days with the $\text{GdVO}_4\text{:Eu}^{3+}$ NPs at concentrations: 0, 10, 50 and 100 $\mu\text{g/ml}$. The results are presented as mean $\pm$ SE

Oxidative stress

The degree of lipid peroxidation, estimated on the basis of the amount of TBARS did not change during the experiments. Treatments of the wheat with GdVO₄:Eu³⁺ NPs did not affect this factor, at all tested concentrations (ANOVA, p=0.36; Fig 3).

Fig 3 Lipid peroxidation of wheat cells, treated for 3 days with the GdVO₄:Eu³⁺ NPs at concentrations: 0, 10, 50 and 100 µg/ml. The results are presented as mean ± SE

Cell viability

The results of TTC staining showed that the exposure of the cells to GdVO₄:Eu³⁺ NPs had no impact on the amount of formazan level (ANOVA, p=0.83; Fig. 4a). However, BE uptake was significantly different in control root cells and the cells incubated with LDNCs (ANOVA, p<0.005). The roots growing in the medium without LDNCs contained significantly more BE dye than those growing in the medium with NPs, both at the 10 µg/ml (Tukey test, p<0.05) and 50 or 100 µg/ml (Tukey test, p<0.01) concentrations (Fig 4b).

Fig 4 Cell viability of wheat exposed for 3 days to GdVO₄:Eu³⁺ NPs at concentrations: 0, 10, 50 and 100 µg/ml, determined by TTC (a) and Blue Evans (b) staining assays. Asterisks (*) indicate statistically significant differences between the samples exposed to NPs and the control, Tukey test (*p<0.05 level, **p<0.01 level). The results are presented as mean ± SE

Discussion

In the presented study, wheat seeds were incubated with GdVO₄:Eu³⁺ NPs for 3 days. Even after this short time of treatment, the presence of LDNCs was detected inside the plants. However, results of the plants growth assays showed that GdVO₄:Eu³⁺ NPs had no significant effect on germination rate, seedlings elongation and biomass of plants, when used in all tested concentrations (Fig. 2). To the best of our knowledge, there were no hitherto studies on the phytotoxicity of GdVO₄: Eu³⁺ NPs. Although the LDNPs studied are poorly water soluble (56), some small amounts of the ions can be released to the solutions. Therefore, along with checking the influence of GdVO₄:Eu³⁺ NPs on wheat plants we have to consider the effect of their particular compounds (e.g. ions). Similarly as we have found for wheat, Blinova et al. have suggested that the oxides containing gadolinium (Ce_{0.9}Gd_{0.1}O₂, LaFeO₃, Gd_{0.97}CoO₃) were not toxic for duckweed *Lemna minor* even at 100 µg/ml (57). According to other authors gadolinium caused growth inhibition of Chlorophyta (18,29), whereas Romero-Freire has suggested that this effect was observed only when Gd³⁺ ions were mixed with Ce³⁺ and Lu³⁺ lanthanide ions. In another study, Gd₂O₃ NPs caused a decrease in roots growth of wheat *Triticum aestivum* and rape *Brassica napus* (58). This effect depended on the sequence of processes of soaking and incubating during treatments. In the same study, germination of wheat and rape seeds was not affected by the presence of gadolinium oxides NPs (58). However, Gd³⁺ ions decreased significantly shoot growth of maize *Zea mays* at 10 µg/ml (59). Ruíz-Herrera et al. have shown that GdCl₃ affected primary roots length in a dose-dependent manner (60). Unexpectedly, the same study has revealed that with increasing concentration of gadolinium ions, an increase in the total number of lateral roots per plant and lateral roots density increased (60). It has been reported that europium, another lanthanide component of the studied

GdVO₄:Eu³⁺ NPs, may be accumulated in macrophytes and alter the plant growth (61,62). The results obtained for one more component of the NPs studied, i.e. vanadium or vanadium oxide, which do not represent lanthanides, have been inconclusive. It has been suggested that plants incubated with high concentration of vanadium had shorter root length and poorer survival, whereas the low concentration of vanadium may improve the growth rate, accelerate flowering and biomass production (63), see the review in (64).

It has been demonstrated that modulated nutrient content in the tested plants may have impact on the growth rate. Garcia-Jimenez et al have reported that a solution of NH₄VO₃ added in a concentration of 5 mM stimulated the uptake of nutrients by the plants, when the concentration of this compound was increased to 15 mM, the stimulating effect was noticeable but reduced. Exposure to NH₄VO₃ at 5 µM has been found to cause an increase in the level of amino acids and sugars in leaves and roots (63). Other studies have revealed that the presence of europium caused a decrease in the concentration of macroelements in *Lathyrus sativus* roots (61). In the same paper it has been reported that europium activated proteolytic enzymes, and therefore increased the concentration of amino acids in the cells, causing plants to be less vulnerable to an arid environment (61). Saatz et al. have demonstrated significant correlations between the concentration of gadolinium in the nutrient solution and the concentration of Ca, Mg and P in the root tissues (59). However, it is unlikely that in the present study exposure to GdVO₄:Eu³⁺ NPs could have impact on seedlings mineral composition, as the seedlings were grown in water (or colloids with LDNCs). The differences between literature data and our results on growth and development of wheat may be related to several factors, such as differences in solubility and structures

of NPs, as well as assays' duration. Moreover, the toxicity may depend on the presence of compounds other than the tested one (30,65).

The effect of NPs on lipid peroxidation (19,65–67), see the review in (13) and cell viability (8,32,66,68) has been studied, however, according to the present state of knowledge, the influence of LDNPs on the cells' membrane damage and lipid peroxidation in plants has not been studied as yet. As mentioned above, the reported effects may be caused not only by LDNCs themselves but also by the ions released by these compounds. It has been shown that vanadium ions when present at the concentration 50 mg V in 1 kg of soil caused cell death, reduction of membrane integrity and activation of antioxidant enzymes in chickpea *Cicer arietinum* L. (69). In our study, the oxidative stress of the seedlings was evaluated by measuring the amount of lipid peroxidation product (thiobarbituric acid reactive substances, TBARS) as a biomarker. The assay showed that the $GdVO_4:Eu^{3+}$ NPs did not cause oxidation of the membrane lipid of the wheat root cells when used in all tested concentrations (Fig. 3).

The effect of the NPs presence on the cells viability was assessed by two methods: TTC and EB assays. They provided different results. According to the TTC results, the presence of LDNCs had no impact on the amount of formazan level, when used in all tested concentrations. It suggested that LDNCs did not cause cells death during the treatment. However, the results of the EB assay, revealed that incubation of the seedlings with $GdVO_4:Eu^{3+}$ NPs induced significant alterations in cells staining by EB dye. It has been proved that the presence of EB dye in cells is correlated with the loss of integrity of the plasma membrane of the cells and their mortality (52). Unexpectedly, in the presented study the control cells had higher absorbance than the treated ones (Fig. 4b). The mechanism of the observed phenomenon is not known, but some explanation can be proposed. First of all, the obtained results might be caused by

decreased uptake of EB dye by the cells incubated with the LDNCs than the control. The LDNCs may create aggregates and therefore block pores of the cells, which prevents the access of the dye to the inside of the cells. It has been indeed shown that NPs might adhere to cell membranes and thus may alert intracellular transport (70). Alternatively, the LDNCs may interact with ions or structures responsible for pumping EB dye out.

Conclusions

Summarising, there is no evidence for toxic effect of $\text{GdVO}_4\text{:Eu}^{3+}$ NPs on vascular plants, when used in concentrations of 100 $\mu\text{g/ml}$ and lower. On the other hand, the observed decrease in the Evans Blue level in the treated seedlings indicated that $\text{GdVO}_4\text{:Eu}^{3+}$ NPs affect membrane functioning. Thus, it would be interesting to get more detailed insights into the effect of these NPs on plants physiology.

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References

1. Judy JD, Bertsch PM. Bioavailability, Toxicity, and Fate of Manufactured Nanomaterials in

- 328 Terrestrial Ecosystems [Internet]. 1st ed. Vol. 123, Advances in Agronomy. Elsevier Inc.; 2014.
329 1–64 p. Available from: <http://dx.doi.org/10.1016/B978-0-12-420225-2.00001-7>
- 330 2. Bystrzejewska-Piotrowska G, Golimowski J, Urban PL. Nanoparticles: Their potential toxicity,
331 waste and environmental management. Waste Manag [Internet]. 2009;29(9):2587–95.
332 Available from: <http://dx.doi.org/10.1016/j.wasman.2009.04.001>
- 333 3. Peralta-Videa JR, Zhao L, Lopez-Moreno ML, de la Rosa G, Hong J, Gardea-Torresdey JL.
334 Nanomaterials and the environment: A review for the biennium 2008–2010. J Hazard Mater
335 [Internet]. 2011 Feb;186(1):1–15. Available from:
336 <http://dx.doi.org/10.1016/j.jhazmat.2010.11.020>
- 337 4. Hawthorne J, De La Torre Roche R, Xing B, Newman LA, Ma X, Majumdar S, et al. Particle-size
338 dependent accumulation and trophic transfer of cerium oxide through a terrestrial food chain.
339 Environ Sci Technol. 2014;48(22):13102–9.
- 340 5. De la Torre Roche R, Servin A, Hawthorne J, Xing B, Newman LA, Ma X, et al. Terrestrial
341 Trophic Transfer of Bulk and Nanoparticle La₂O₃ Does Not Depend on Particle Size. Environ
342 Sci Technol [Internet]. 2015 Oct 6;49(19):11866–74. Available from:
343 <http://pubsdc3.acs.org/doi/10.1021/acs.est.5b02583>
- 344 6. Modlitbová P, Novotný K, Pořízka P, Klus J, Lubal P, Zlámalová-Gargošová H, et al.
345 Comparative investigation of toxicity and bioaccumulation of Cd-based quantum dots and Cd
346 salt in freshwater plant *Lemna minor* L. Ecotoxicol Environ Saf [Internet]. 2018 Jan;147(August
347 2017):334–41. Available from:
348 <http://linkinghub.elsevier.com/retrieve/pii/S0147651317305523>
- 349 7. Lead JR, Batley GE, Alvarez PJJ, Croteau M-N, Handy RD, McLaughlin MJ, et al. Nanomaterials
350 in the environment: Behavior, fate, bioavailability, and effects-An updated review. Environ
351 Toxicol Chem. 2018 Aug;37(8):2029–63.

8. Ma Y, Zhang P, Zhang Z, He X, Li Y, Zhang J, et al. Origin of the different phytotoxicity and biotransformation of cerium and lanthanum oxide nanoparticles in cucumber. *Nanotoxicology* [Internet]. 2014;5390:1–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24877678>
9. Nordmann J, Buczka S, Voss B, Haase M, Mummenhoff K. In vivo analysis of the size- and time-dependent uptake of NaYF₄:Yb,Er upconversion nanocrystals by pumpkin seedlings. *J Mater Chem B* [Internet]. 2015;3(1):144–50. Available from: <http://xlink.rsc.org/?DOI=C4TB01515K>
10. Hirschmüller A, Nordmann J, Ptacek P, Mummenhoff K, Haase M. In-Vivo Imaging of the Uptake of Upconversion Nanoparticles by Plant Roots. *J Biomed Nanotechnol* [Internet]. 2009 Jun 1;5(3):278–84. Available from: <http://openurl.ingenta.com/content/xref?genre=article&issn=1550-7033&volume=5&issue=3&spage=278>
11. Soil C. Root System Architecture, Copper Uptake and Tissue Distribution in Soybean (*Glycine max* (L.) Merr.) Grown in Copper Oxide Nanoparticle (CuONP)-Amended Soil and Implications for Human Nutrition. *Plants*. 2020;9(1326):1–24.
12. Ma X, Geiser-Lee J, Deng Y, Kolmakov A. Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. *Sci Total Environ* [Internet]. 2010 Jul 15;408(16):3053–61. Available from: <http://dx.doi.org/10.1016/j.scitotenv.2010.03.031>
13. Ma C, White JC, Dhankher OP, Xing B. Metal-Based Nanotoxicity and Detoxification Pathways in Higher Plants. *Env Sci Technol*. 2015;49(12):7109–22.
14. Kulacki KJ, Cardinale BJ. Effects of Nano-Titanium Dioxide on Freshwater Algal Population Dynamics. Bansal V, editor. *PLoS One* [Internet]. 2012 Oct 10;7(10):e47130. Available from: <http://dx.plos.org/10.1371/journal.pone.0047130>
15. Hao Y, Ma C, Zhang Z, Song Y, Cao W, Guo J, et al. Carbon nanomaterials alter plant physiology and soil bacterial community composition in a rice-soil-bacterial ecosystem. *Environ Pollut*

- 376 [Internet]. 2018 Jan;232:123–36. Available from:
377 <https://doi.org/10.1016/j.envpol.2017.09.024>
- 378 16. Yin W, Zhou L, Ma Y, Tian G, Zhao J, Yan L, et al. Phytotoxicity, Translocation, and
379 Biotransformation of NaYF₄ Upconversion Nanoparticles in a Soybean Plant. *Small* [Internet].
380 2015 Sep;11(36):4774–84. Available from: <http://doi.wiley.com/10.1002/sml.201500701>
- 381 17. Peng J, Sun Y, Liu Q, Yang Y, Zhou J, Feng W, et al. Upconversion nanoparticles dramatically
382 promote plant growth without toxicity. *Nano Res* [Internet]. 2012 Nov 2;5(11):770–82.
383 Available from: <http://link.springer.com/10.1007/s12274-012-0261-y>
- 384 18. Joonas E, Aruoja V, Olli K, Syvertsen-Wiig G, Vija H, Kahru A. Potency of (doped) rare earth
385 oxide particles and their constituent metals to inhibit algal growth and induce direct toxic
386 effects. *Sci Total Environ* [Internet]. 2017 Sep;593–594:478–86. Available from:
387 <http://dx.doi.org/10.1016/j.scitotenv.2017.03.184>
- 388 19. Ma Y, Xie C, He X, Zhang B, Yang J, Sun M, et al. Effects of Ceria Nanoparticles and CeCl₃ on
389 Plant Growth , Biological and Physiological Parameters , and Nutritional Value of Soil Grown
390 Common Bean (*Phaseolus vulgaris*). *Small*. 2020;1907435:1–9.
- 391 20. Lee W-M, An Y-J, Yoon H, Kweon H-S. TOXICITY AND BIOAVAILABILITY OF COPPER
392 NANOPARTICLES TO THE TERRESTRIAL PLANTS MUNG BEAN (*PHASEOLUS RADIATUS*) AND
393 WHEAT (*TRITICUM AESTIVUM*): PLANT AGAR TEST FOR WATER-INSOLUBLE NANOPARTICLES.
394 *Environ Toxicol Chem* [Internet]. 2008;27(9):1915. Available from:
395 <http://doi.wiley.com/10.1897/07-481.1>
- 396 21. Oukarroum A, Barhoumi L, Pirastru L, Dewez D. Silver nanoparticle toxicity effect on growth
397 and cellular viability of the aquatic plant *Lemna gibba*. *Environ Toxicol Chem*. 2013
398 Apr;32(4):902–7.
- 399 22. Andersen CP, King G, Plocher M, Storm M, Pokhrel LR, Johnson MG, et al. Germination and

early plant development of ten plant species exposed to titanium dioxide and cerium oxide nanoparticles. *Environ Toxicol Chem*. 2016 Sep;35(9):2223–9.

23. Shu L, Yan Q, He Z, Xu K. Effects of Titanium Dioxide Nanoparticles on Photosynthetic and Antioxidative Processes of *Scenedesmus obliquus*. *Plants*. 2020;9(1748):1–13.

24. Libralato G, Costa Devoti A, Zanella M, Sabbioni E, Mičetić I, Manodori L, et al. Phytotoxicity of ionic, micro- and nano-sized iron in three plant species. *Ecotoxicol Environ Saf* [Internet]. 2016 Jan;123(2008):81–8. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0147651315300312>

25. Gavina A, Bouguerra S, Lopes I, Marques CR, Rasteiro MG, Antunes F, et al. Impact of organic nano-vesicles in soil: The case of sodium dodecyl sulphate/didodecyl dimethylammonium bromide. *Sci Total Environ* [Internet]. 2016 Mar;547:413–21. Available from: <http://dx.doi.org/10.1016/j.scitotenv.2015.12.163>

26. Khodakovskaya M, Dervishi E, Mahmood M, Xu Y, Li Z, Watanabe F, et al. Carbon Nanotubes Are Able To Penetrate Plant Seed Coat and Dramatically Affect Seed Germination and Plant Growth. *ACS Nano* [Internet]. 2009 Oct 27;3(10):3221–7. Available from: <https://pubs.acs.org/doi/10.1021/nn900887m>

27. Lian J, Wu J, Xiong H, Zeb A, Yang T, Su X, et al. Impact of polystyrene nanoplastics (PSNPs) on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *J Hazard Mater* [Internet]. 2020 Mar;385:121620. Available from: <https://doi.org/10.1016/j.jhazmat.2019.121620>

28. Hayes KL, Mui J, Song B, Sani ES, Eisenman SW, Sheffield JB, et al. Effects, uptake, and translocation of aluminum oxide nanoparticles in lettuce: A comparison study to phytotoxic aluminum ions. *Sci Total Environ* [Internet]. 2020 Jun;719:137393. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0048969720309037>

29. Romero-Freire A, Joonas E, Muna M, Cossu-Leguille C, Vignati DAL, Giamberini L. Assessment of the toxic effects of mixtures of three lanthanides (Ce, Gd, Lu) to aquatic biota. *Sci Total Environ* [Internet]. 2019 Apr;661:276–84. Available from: <https://doi.org/10.1016/j.scitotenv.2019.01.155>
30. Mohmood I, Lopes CB, Lopes I, Tavares DS, Soares AMVM, Duarte AC, et al. Remediation of mercury contaminated saltwater with functionalized silica coated magnetite nanoparticles. *Sci Total Environ* [Internet]. 2016;557–558:712–21. Available from: <http://www.sciencedirect.com/science/article/pii/S0048969716304995>
31. Hu C, Liu L, Li X, Xu Y, Ge Z, Zhao Y. Effect of graphene oxide on copper stress in *Lemna minor* L.: evaluating growth, biochemical responses, and nutrient uptake. *J Hazard Mater* [Internet]. 2018 Jan;341:168–76. Available from: <http://dx.doi.org/10.1016/j.jhazmat.2017.07.061>
32. Silva S, Oliveira H, Craveiro SC, Calado AJ, Santos C. Pure anatase and rutile + anatase nanoparticles differently affect wheat seedlings. *Chemosphere* [Internet]. 2016 May;151:68–75. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0045653516301990>
33. Monteiro C, Daniel-da-Silva AL, Venâncio C, Soares SF, Soares AMVM, Trindade T, et al. Effects of long-term exposure to colloidal gold nanorods on freshwater microalgae. *Sci Total Environ* [Internet]. 2019 Sep;682:70–9. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0048969719320637>
34. Thiagarajan V, M. P, S. A, R. S, N. C, G.K. S, et al. Diminishing bioavailability and toxicity of P25 TiO₂ NPs during continuous exposure to marine algae *Chlorella* sp. *Chemosphere* [Internet]. 2019 Oct;233:363–72. Available from: <https://doi.org/10.1016/j.chemosphere.2019.05.270>
35. Silva S, Oliveira H, Silva AMS, Santos C. The cytotoxic targets of anatase or rutile + anatase nanoparticles depend on the plant species. *Biol Plant* [Internet]. 2017 Dec 3;61(4):717–25. Available from: <http://link.springer.com/10.1007/s10535-017-0733-8>

- 448 36. Mazierski P, Sowik J, Miodyńska M, Trykowski G, Mikołajczyk A, Klimczuk T, et al. Shape-
449 controllable synthesis of GdVO₄ photocatalysts and their tunable properties in photocatalytic
450 hydrogen generation. *Dalt Trans.* 2019;48(5):1662–71.
- 451 37. Wang D, Zhao C, Gao G, Xu L, Wang G, Zhu P. Multifunctional NaLnF₄@MOF-In
452 nanocomposites with dual-mode luminescence for drug delivery and cell imaging.
453 *Nanomaterials.* 2019;9(9).
- 454 38. Sun N, Ding Y, Tao Z, You H, Hua X, Wang M. Development of an upconversion fluorescence
455 DNA probe for the detection of acetamiprid by magnetic nanoparticles separation. *Food*
456 *Chem [Internet].* 2018 Aug;257:289–94. Available from:
457 <http://linkinghub.elsevier.com/retrieve/pii/S0308814618303960>
- 458 39. Wysokińska E, Cichos J, Kowalczyk A, Karbowiak M, Strzdała L, Bednarkiewicz A, et al. Toxicity
459 mechanism of low doses of NaGdF₄:Yb³⁺,Er³⁺ upconverting nanoparticles in activated
460 macrophage cell lines. *Biomolecules.* 2019;9(1).
- 461 40. Dong R, Li Y, Li W, Zhang H, Liu Y, Ma L, et al. Recent developments in luminescent
462 nanoparticles for plant imaging and photosynthesis. *J Rare Earths [Internet].* 2019
463 Sep;37(9):903–15. Available from:
464 <https://linkinghub.elsevier.com/retrieve/pii/S100207211830930X>
- 465 41. Szczeszak A, Grzyb T, Nowaczyk G, Ekner-Grzyb A. Emission colour changes in the CaF₂ sub-
466 microspheres doped with Yb³⁺, Er³⁺ and Mn²⁺ ions. *J Alloys Compd [Internet].* 2020
467 Mar;817:152718. Available from: <https://doi.org/10.1016/j.jallcom.2019.152718>
- 468 42. Szczeszak A, Grzyb T, Śniadecki Z, Andrzejewska N, Lis S, Matczak M, et al. Structural,
469 Spectroscopic, and Magnetic Properties of Eu³⁺-Doped GdVO₄ Nanocrystals Synthesized by
470 a Hydrothermal Method. *Inorg Chem.* 2014 Dec;53(23):12243–52.
- 471 43. Hatje V, Bruland KW, Flegal AR. Increases in Anthropogenic Gadolinium Anomalies and Rare

- Earth Element Concentrations in San Francisco Bay over a 20 Year Record. *Environ Sci Technol* [Internet]. 2016 Apr 19;50(8):4159–68. Available from: <https://pubs.acs.org/doi/10.1021/acs.est.5b04322>
44. Kanda T, Fukusato T, Matsuda M, Toyoda K, Oba H, Kotoku J, et al. Gadolinium-based Contrast Agent Accumulates in the Brain Even in Subjects without Severe Renal Dysfunction: Evaluation of Autopsy Brain Specimens with Inductively Coupled Plasma Mass Spectroscopy. *Radiology* [Internet]. 2015 Jul;276(1):228–32. Available from: <http://pubs.rsna.org/doi/10.1148/radiol.2015142690>
45. Mizgerd JP, Molina RM, Stearns RC, Brain JD, Warner AE. Gadolinium induces macrophage apoptosis. *J Leukoc Biol* [Internet]. 1996 Feb;59(2):189–95. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8603991>
46. Szczeszak A, Ekner-Grzyb A, Runowski M, Mrówczyńska L, Grzyb T, Lis S. Synthesis, photophysical analysis, and in vitro cytotoxicity assessment of the multifunctional (magnetic and luminescent) core@shell nanomaterial based on lanthanide-doped orthovanadates. *J Nanoparticle Res* [Internet]. 2015 Mar 17;17(3):143. Available from: <http://link.springer.com/10.1007/s11051-015-2950-4>
47. Modlitbová P, Hlaváček A, Švestková T, Pořízka P, Šimoníková L, Novotný K, et al. The effects of photon-upconversion nanoparticles on the growth of radish and duckweed: Bioaccumulation, imaging, and spectroscopic studies. *Chemosphere* [Internet]. 2019 Jun;225:723–34. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0045653519305107>
48. Blinova I, Muna M, Heinlaan M, Lukjanova A, Kahru A. Potential Hazard of Lanthanides and Lanthanide-Based Nanoparticles to Aquatic Ecosystems: Data Gaps, Challenges and Future Research Needs Derived from Bibliometric Analysis. *Nanomaterials* [Internet]. 2020 Feb 14;10(2):328. Available from: <https://www.mdpi.com/2079-4991/10/2/328>

- 497 49. Hu S, Wu X, Chen Z, Hu P, Yan H, Tang Z, et al. Uniform NaLuF₄ nanoparticles with strong
498 upconversion luminescence for background-free imaging of plant cells and ultralow power
499 detecting of trace organic dyes. Mater Res Bull [Internet]. 2016 Jan;73:6–13. Available from:
500 <http://dx.doi.org/10.1016/j.materresbull.2015.08.020>
- 501 50. Szczeszak A, Grzyb T, Śniadecki Z, Andrzejewska N, Lis S, Matczak M, et al. Structural,
502 Spectroscopic, and Magnetic Properties of Eu³⁺-Doped GdVO₄ Nanocrystals Synthesized by a
503 Hydrothermal Method. Inorg Chem [Internet]. 2014;53(23):12243–52. Available from:
504 <http://pubs.acs.org/doi/abs/10.1021/ic500354t>
- 505 51. Chmielowska-Bąk J, Holubek R, Frontasyeva M, Zinicovscaia I, İşidoğru S, Deckert J. Tough
506 Sprouting – Impact of Cadmium on Physiological State and Germination Rate of Soybean
507 Seeds. Acta Soc Bot Pol [Internet]. 2020 Jun 24;89(2):1–10. Available from:
508 <https://pbsociety.org.pl/journals/index.php/asbp/article/view/asbp.8923>
- 509 52. Jacyn Baker C, Mock NM. An improved method for monitoring cell death in cell suspension
510 and leaf disc assays using evans blue. Plant Cell Tissue Organ Cult [Internet]. 1994
511 Oct;39(1):7–12. Available from: <http://link.springer.com/10.1007/BF00037585>
- 512 53. Towill LE, Mazur P. Studies on the reduction of 2,3,5-triphenyltetrazolium chloride as a
513 viability assay for plant tissue cultures. Can J Bot [Internet]. 1975 Jun 1;53(11):1097–102.
514 Available from: <http://www.nrcresearchpress.com/doi/10.1139/b75-129>
- 515 54. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. Arch Biochem Biophys
516 [Internet]. 1968 Apr;125(1):189–98. Available from:
517 <https://linkinghub.elsevier.com/retrieve/pii/0003986168906541>
- 518 55. Nuñez NO, Rivera S, Alcantara D, de la Fuente JM, García-Sevillano J, Ocaña M. Surface
519 modified Eu:GdVO₄ nanocrystals for optical and MRI imaging. Dalton Trans [Internet]. 2013
520 Aug 14 [cited 2014 Apr 30];42(30):10725–34. Available from:

<http://www.ncbi.nlm.nih.gov/pubmed/23770723>

56. Gnach A, Lipinski T, Bednarkiewicz A, Rybka J, Capobianco J a. Upconverting nanoparticles: assessing the toxicity. *Chem Soc Rev* [Internet]. 2015 Sep 1 [cited 2014 Oct 7];44(6):1561–84. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25176037>
57. Blinova I, Vija H, Lukjanova A, Muna M, Syvertsen-Wiig G, Kahru A. Assessment of the hazard of nine (doped) lanthanides-based ceramic oxides to four aquatic species. *Sci Total Environ* [Internet]. 2018 Jan;612:1171–6. Available from: <https://doi.org/10.1016/j.scitotenv.2017.08.274>
58. Ma Y, Kuang L, He X, Bai W, Ding Y, Zhang Z, et al. Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere* [Internet]. 2010 Jan;78(3):273–9. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0045653509012764>
59. Saatz J, Vetterlein D, Mattusch J, Otto M, Daus B. The influence of gadolinium and yttrium on biomass production and nutrient balance of maize plants. *Environ Pollut* [Internet]. 2015 Sep;204:32–8. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0269749115001839>
60. Ruíz-Herrera LF, Sánchez-Calderón L, Herrera-Estrella L, López-Bucio J. Rare earth elements lanthanum and gadolinium induce phosphate-deficiency responses in *Arabidopsis thaliana* seedlings. *Plant Soil* [Internet]. 2012 Apr 11;353(1–2):231–47. Available from: <http://link.springer.com/10.1007/s11104-011-1026-1>
61. Tian HE, Gao YS, Li FM, Zeng F. Effects of Europium Ions (Eu³⁺) on the Distribution and Related Biological Activities of Elements in *Lathyrus sativus* L. Roots. *Biol Trace Elem Res* [Internet]. 2003;93(1–3):257–70. Available from: <http://link.springer.com/10.1385/BTER:93:1-3:257>
62. Shtangeeva I. Europium and Cerium Accumulation in Wheat and Rye Seedlings. *Water, Air,*

- 545 Soil Pollut [Internet]. 2014 Jun 27;225(6):1964. Available from:
546 <http://link.springer.com/10.1007/s11270-014-1964-3>
- 547 63. García-Jiménez A, Trejo-Téllez LI, Guillén-Sánchez D, Gómez-Merino FC. Vanadium stimulates
548 pepper plant growth and flowering, increases concentrations of amino acids, sugars and
549 chlorophylls, and modifies nutrient concentrations. Niedz RP, editor. PLoS One [Internet].
550 2018 Aug 9;13(8):e0201908. Available from:
551 <https://dx.plos.org/10.1371/journal.pone.0201908>
- 552 64. Aihemaiti A, Gao Y, Meng Y, Chen X, Liu J, Xiang H, et al. Review of plant-vanadium
553 physiological interactions, bioaccumulation, and bioremediation of vanadium-contaminated
554 sites. Sci Total Environ [Internet]. 2020 Apr;712:135637. Available from:
555 <https://linkinghub.elsevier.com/retrieve/pii/S0048969719356323>
- 556 65. Noman M, Shahid M, Ahmed T, Tahir M, Naqqash T, Muhammad S, et al. Green copper
557 nanoparticles from a native Klebsiella pneumoniae strain alleviated oxidative stress
558 impairment of wheat plants by reducing the chromium bioavailability and increasing the
559 growth. Ecotoxicol Environ Saf. 2020;192(December 2019).
- 560 66. Silva S, Craveiro SC, Oliveira H, Calado AJ, Pinto RJB, Silva AMS, et al. Wheat chronic exposure
561 to TiO₂-nanoparticles: Cyto- and genotoxic approach. Plant Physiol Biochem [Internet]. 2017
562 Dec;121(October):89–98. Available from:
563 <https://linkinghub.elsevier.com/retrieve/pii/S0981942817303406>
- 564 67. Yusefi-Tanha E, Fallah S, Rostamnejadi A, Pokhrel LR. Particle size and concentration
565 dependent toxicity of copper oxide nanoparticles (CuONPs) on seed yield and antioxidant
566 defense system in soil grown soybean (Glycine max cv. Kowsar). Sci Total Environ [Internet].
567 2020 May;715:136994. Available from: <https://doi.org/10.1016/j.scitotenv.2020.136994>
- 568 68. Soares C, Branco-Neves S, de Sousa A, Pereira R, Fidalgo F. Ecotoxicological relevance of nano-

NiO and acetaminophen to *Hordeum vulgare* L.: Combining standardized procedures and physiological endpoints. *Chemosphere* [Internet]. 2016;165:442–52. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0045653516312437>

69. Imtiaz M, Ashraf M, Rizwan MS, Nawaz MA, Rizwan M, Mehmood S, et al. Vanadium toxicity in chickpea (*Cicer arietinum* L.) grown in red soil: Effects on cell death, ROS and antioxidative systems. *Ecotoxicol Environ Saf* [Internet]. 2018 Aug;158:139–44. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0147651318303154>

70. Contini C, Schneemilch M, Gaisford S, Quirke N. Nanoparticle–membrane interactions. *J Exp Nanosci* [Internet]. 2018 Jan 1;13(1):62–81. Available from: <https://doi.org/10.1080/17458080.2017.1413253>

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