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Effects of selected nanoparticles on aquatic plants

Nina Doskocz*, Monika Załęska-Radziwiłł, Katarzyna Affek, Maria Łebkowska

Warsaw University of Technology, Faculty of Building Services, Hydro and Environmental Engineering, Nowowiejska 20, 00-653 Warsaw, Poland, emails: nina.doskocz@pw.edu.pl (N. Doskocz), monika.radziwill@pw.edu.pl (M. Załęska-Radziwiłł), katarzyna.affek@pw.edu.pl (K. Affek), maria.lebkowska@pw.edu.pl (M. Łebkowska)

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ABSTRACT

This paper presents results of ecotoxicological assessment of nano-Al_2O_3 and nano-ZrO_2 in relation to cyanobacteria, algae and higher plants. Bioindicators showed diverse sensitivity to nanocompounds. EC_{50} values were in the ranges: for nano-Al_2O_3 from 5.3 mg/L (*Desmodesmus quadricauda*) to 457.9 mg/L (*Scenedesmus obliquus*), for nano-ZrO_2 from 19.6 mg/L (*D. quadricauda*) to 277 mg/L (*Lemna minor*). According to EU criteria, nano-Al_2O_3 proved to be toxic to *D. quadricauda*, harmful to *Raphidocelis subcapitata* and *Microcystis aeruginosa* and non-toxic for other species of algae and *L. minor*. Nano-ZrO_2 was harmful to *D. quadricauda* and non-toxic to other tested species. Calculated predicted no effect concentration (PNEC) in the environment values were 0.0395 mg/L for nano-Al_2O_3 and 0.0416 mg/L for nano-ZrO_2. The risk assessment, conducted on the basis of the predicted environmental concentration (PEC)/PNEC quotient, showed a low risk in relation to aquatic plants caused by the presence of nano-Al_2O_3 when PEC were calculated using the statistical extrapolation model of Aldenberg–Jaworska. Nanoparticles proved to be more toxic to tested bioindicators than their bulk counterparts. This indicates that the nano-form of a given substance may pose a greater hazard for the environment than the same substance in the large form.

Keywords: Nanoparticles; Aluminium oxide; Zirconium oxide; Ecotoxicity; Aquatic plants; PNEC; Risk assessment

1. Introduction

Currently, the scope of use of nanomaterials is rapidly expanding. Nanoparticles (NPs) have become intensively used in agriculture, industrial products and in medicine [1–3]. They are added to cosmetics, paints (TiO_2, SiO_2 and ZnO), coatings (TiO_2, Al_2O_3 and ZnO) and as catalysts (CeO_2) in fuel [4]. Factors such as concentration, type, size, surface area, chemical composition, stability of NPs, and species sensitivity influence the uptake, translocation and accumulation of NPs in living organisms. The interaction of NPs with organisms can cause various physiological and biochemical changes, both positive and negative [5]. Given the rapid development of nanotechnology, there is an increased risk of exposure of humans and the environment to nanotechnology-based materials. There are some data on the ecotoxicity of NPs in relation to bacteria, fish or zooplankton, but little is known about the effects and toxic mechanisms of NPs on aquatic plants [6–9].

Plants are essential elements of all ecosystems acting as producers in a food chain. They are widespread and sensitive organisms characterized by high capacity of bioaccumulation due to their high surface of contact. Because of their properties they play a vital role in the fate and transport of NPs in the environment. Therefore, knowledge concerning the effects of NPs on physiological processes of plants is very important for assessing the safety of nanomaterials [10–12].

The purpose of this study was to assess the influence of aluminium and zirconium oxide NPs on cyanobacteria, algae and higher plants. Where possible, ecotoxicological effect estimators, including half maximal effective concentration (EC_{50}) and no observed effect concentrations (NOECs). Predicted no effect concentrations (PNECs) for the aquatic environment

* Corresponding author.

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were derived according to the Guidance document for the implementation of REACH [13,14]. Furthermore, in this paper the preliminary risk assessment aluminium oxide NPs were conducted by comparing the predicted environmental concentration (PEC) to the PNEC. The risks emanating from NP are determined by their potential hazards (such as toxicity), as well as by the extent the material will come into contact with an organism. The basis for a sound risk assessment of a possibly hazardous substance is thus a comparison between the exposure (concentration in the environment) and the toxic effects of the substance (concentration–response relationship). So far, no measurements of engineered NP in the environment have been available due to the absence of analytical methods able to quantify trace concentrations of NP [13,14]. Hence, in this work in order to carried out first assessment of the potential risk posed by Al_2O_3 NPs used data from statistical models available in literature [15].

Aluminium oxide NPs, an important kind of metal oxide NPs, are used by the military and commercial industries in many applications including coatings and propellants, whereas the zirconium oxide NPs are commonly used in dentistry and as drug carriers, such as insulin. With such wide applications, nano-Al_2O_3 and nano-ZrO_2 can be released into the environment and reach water bodies through wastewater and urban runoff. Considering this fact, understanding the hazards related to exposure of aquatic ecosystems to NPs seems to be essential in environmental risk assessment of these emerging contaminants.

In this study, the effect of activity of nano-Al_2O_3 and nano-ZrO_2 on aquatic plants was compared with their bulk counterparts (compounds of the macro-form – Al_2O_3 and ZrO_2).

2. Materials and methods

2.1. Chemicals

Aluminium oxide NPs (nano-Al_2O_3), nanopowder <50 nm with a specific surface area >40 m^2/g, zirconium oxide NPs (nano-ZrO_2), nanopowder <100 nm with a specific surface area ≥25 m^2/g and aluminium and zirconium oxides of purity over 98% were obtained from Sigma-Aldrich (Poland). CAS no. of compounds containing Al_2O_3 is 1344-28-1 and ZrO_2 is 1314-23-4. The stock solutions of nanocompounds and oxides with a concentration of 500 mg/L were prepared in deionized water. To avoid formation of the aggregates, the stock dispersion was sonicated (0.4 kW, 20 kHz) for 30 min before being diluted to the exposure concentrations. The stock solutions were diluted (using the medium with respect to the procedures of tests) in descending order with a geometric series of quotient $q = 2$ to obtain final concentrations of 500–0.19 mg/L.

2.2. Ecotoxicological tests

Growth tests were performed on cyanobacteria, algae and higher plants. *Microcystis aeruginosa* (CCALA 796), *Desmodesmus quadricauda* (CCALA 463) and *Raphidocelis subcapitata* (CCALA 433) were obtained from the Institute of Botany, Academy of Science in the Czech Republic. *Scenedesmus obliquus*, *Chlorella vulgaris* (green algae) and *Lemna minor* (higher plants) came from the laboratory culture owned by the Department of Biology, Faculty of Building Services, Hydro and Environmental Engineering, Warsaw University of Technology.

2.2.1. Growth test using cyanobacteria and algae

Growth tests with cyanobacteria and algae were performed according to PN-EN ISO 8692:2012 methodology [16]. The inhibition of growth of organisms was calculated on the basis of cell concentration measurement after 72 h of exposure to tested compounds in mineral medium.

2.2.2. Lemna minor growth inhibition test

The *L. minor* growth test was performed according to PN-EN ISO 20079:2004 methodology [17]. Growth inhibition assessments were performed on the basis of surface area measurement and number of leaves count at the beginning and at the end of a 7-d test. Measurements were made using UTHSCSA ImageTool digital image analysis software version 3.0.

2.3. Calculation procedures

2.3.1. Inhibition of growth of cyanobacteria, algae and higher plants

Growth rate of cyanobacteria, algae and higher plants was determined by the formula:

$$\mu = \frac{\ln N_n - \ln N_0}{t_n} \quad (1)$$

where μ is the growth rate; N_0 is the number of cells in 1 mL/number of leaves/leaf surface at time t_0; N_n is the number of cells in 1 mL/number of leaves/leaf surface at time t; t_n is the time ($t - t_0$).

Inhibition of growth was calculated according to the formula:

$$I\mu_i = \frac{\mu_c - \mu_i}{\mu_c} \cdot 100\ (\%) \quad (2)$$

where $I\mu_i$ is the percentage of inhibition; μ_i is the average growth rate of cyanobacteria, algae, plants in the test concentration; μ_c is the average growth rate of cyanobacteria, algae, plants in the control sample.

2.3.2. Calculation of EC_{50} and NOEC

Effective concentrations (EC_{50}) in acute and chronic tests were calculated using probit analysis, determining 95% confidence intervals [18]. NOECs were determined using single factor analysis of variance (ANOVA; $p < 0.05$) and Tukey's test [19].

2.4. Toxicity assessment of compounds

The assessment of toxicity of the test NPs and oxides in relation to aquatic bioindicators was performed on the basis of the European Union criteria—Directive 93/67/EEC [20].

2.5. Acute-to-chronic ratio determination

The relationship between acute and chronic toxicities is expressed as the ACR (acute-to-chronic ratio) and one of the aims of the study was to investigate whether experimental ACRs are close to 10 – a value accepted by the European Union (EU) and the organisation for economic co-operation and development (OECD). ACRs were obtained on the basis of chronic tests in which acute effects (expressed as EC_{50}) and chronic ecotoxicity (expressed as NOEC) were derived simultaneously, according to the formula:

$$ACR = \frac{EC_{50}}{NOEC} \quad (3)$$

where EC_{50} is the effective concentrations for 50% of the individuals of the species determined in the acute test; NOEC is the no observed effect concentration for individuals of the species determined in chronic tests.

2.6. Risk assessment

RQ was calculated for predicted or measured concentrations of PEC of aluminium oxide NPs according to the formula:

$$RQ = \frac{PEC}{PNEC} \quad (4)$$

where PEC is the predicted (or measured) environmental concentration; PNEC is the predicted no effect concentration in the environment.

The results were interpreted according to the following criteria: RQ 1 – high risk, RQ < 1 – low risk.

PNEC was calculated on the basis of chronic toxicity data (NOEC) using the statistical extrapolation model of Aldenberg–Jaworska [21].

The PEC (only for nano-Al_2O_3) values were chosen on the basis of full literature search, and derived from statistical models (Table 1).

Table 1
Predicted concentrations in surface water for aluminium oxide nanoparticles that are being used or could be used in cosmetics and personal care products and coatings

Compound	PEC in surface waters (mg/L)	Reference
Nano-Al_2O_3	0.0000002[a]	[15]
	0.0000012[b]	
	0.0000025[c]	

Note: Predictions are presented for market penetration factors of 0.1, 0.5 and 1.0.
[a]The situation where 10% of a product type contains the engineered nano-Al_2O_3 (0.1).
[b]The situation where half of a product type contains the engineered nano-Al_2O_3 (0.5).
[c]The situation where all of a product type contains the engineered nano-Al_2O_3 (1.0).

3. Results

The results of acute tests revealed diversified sensitivity of organisms to the tested compounds. Toxicity profiles are enriched with ACRs. In most of the cases, ACRs were higher than 10 – a value accepted by the EU and the OECD.

In the case of aluminium oxide NPs (Table 2) the EC_{50} values for cyanobacteria and algae ranged from 5.3 mg/L for *D. quadricauda* to 457.9 mg/L for *S. obliquus*. This compound also limited the growth of *L. minor* in relation to the surface area – EC_{50} after 7 d was 180 mg/L (Table 2).

Assessment of toxicity with respect to the acute effects according to EU criteria showed that the nano-Al_2O_3 were toxic to *D. quadricauda*, harmful to *R. subcapitata* and *M. aeruginosa*. NOEC (showing chronic effects) was 0.9 mg/L for these organisms. The test compound was non-toxic to other species of algae and *L. minor*. NOEC was 3.12 mg/L (*S. obliquus*), 1.56 mg/L (*C. vulgaris*), 6.24 mg/L (*L. minor* number of leaves) and 0.9 mg/L (*L. minor* – surface area; Table 2).

Zirconium oxide NPs were less harmful than aluminium oxide NPs for all bioindicators. EC_{50} obtained in the studies ranged from 19.60 mg/L for *D. quadricauda* to 277 mg/L for *L. minor* (surface area). For other bioindicators, the EC_{50} value was >500 mg/L (Table 3).

According to EU criteria, zirconium oxide NPs were harmful only to *D. quadricauda* and they were not toxic for other bioindicators (Table 3).

The risk assessment was applied the risk quotient (PEC/PNEC), when PEC was calculated using the statistical extrapolation model of Aldenberg–Jaworska and was 0.0395 and 0.0416 mg/L for nano-Al_2O_3 and for nano-ZrO_2, respectively (Table 4). Risk in this work was performed only for aluminium oxide NPs, because in the literature there are no data about PEC in water for nano-ZrO_2 and their bulk counterparts. The preliminary risk assessment due to the presence of the investigated nano-Al_2O_3 in relation to aquatic plants revealed a low risk, when PNEC was calculated according to the Aldenberg–Jaworska model with assumptions of 95% protected species and 95% probability (Table 4).

4. Discussion

Results obtained in this work as well as literature data show that producers in aquatic ecosystems are sensitive to Al_2O_3 NPs. There is, however, not many reports concerning potential harmfulness of zirconium oxide NPs in relation to aquatic autotrophs. The EC_{50} values of Al_2O_3 NPs for *R. subcapitata* are consistent with the results of Aruoja et al. [22]. Depending on the size of the Al_2O_3 NPs the EC_{50} was in the range of 10–100 mg/L. In contrast, in research of Griffitt et al. [23] EC_{50} was lower and equalled 8.3 mg/L. Sadiq et al. [24] reported the inhibitory effects of aluminium oxide NPs on *Chlorella* sp. and *Scenedesmus* sp. The EC_{50} values were 45.4 and 39.35 mg/L, respectively, and were higher than those obtained in this work. There are no data in the literature on the influence of the aluminium oxide NPs on cyanobacteria. The inhibition of *M. aeruginosa* growth was observed only in the study of Wang et al. [25] in the presence of nano-CuO, where a decrease in chlorophyll contents (a, b) and carotenoids were additionally observed. Among all producers' representatives, *L. minor* was the least sensitive to nano-Al_2O_3

Table 2
Toxicity profile for nano-Al_2O_3 and Al_2O_3

Bioindicators	Nano-Al_2O_3				Al_2O_3			
	EC_{50} (95% confidence intervals; mg/L)	NOEC (mg/L)	ACR	Toxicity assessment UE Directive 93/67/EEC	EC_{50} (95% confidence intervals; mg/L)	NOEC (mg/L)	ACR	Toxicity assessment UE Directive 93/67/EEC
L. minor	>500	6.24	–	Non-toxic	>500	–	nd	Non-toxic
S. obliquus	457.90 (355.62–529.69)	3.12	146.7		>500	–	nd	
C. vulgaris	233.30 (142.76–331.64)	1.56	149.5		>500	–	nd	
L. minor	180.0 (123.68–254.71)	0.9	200.0		>500	–	nd	
R. subcapitata	96.30 (77.29–151.01)	0.9	107.0	Harmful	>500	1.9	–	
M. aeruginosa	30.40 (22.73–36.77)	0.9	33.7		>500	1.9	–	
D. quadricauda	5.30 (2.68–7.98)	0.9	5.8	Toxic	>500	1.9	–	

nd, Not defined.

Table 3
Toxicity profile for nano-ZrO_2 and ZrO_2

Bioindicators	Nano-ZrO_2				ZrO_2			
	EC_{50} (95% confidence intervals; mg/L)	NOEC (mg/L)	ACR	Toxicity assessment UE Directive 93/67/EEC	EC_{50} (95% confidence intervals; mg/L)	NOEC (mg/L)	ACR	Toxicity assessment UE Directive 93/67/EEC
L. minor	>500	–	nd	Non-toxic	>500	–	nd	Non-toxic
S. obliquus	>500	–	nd		>500	–	nd	
C. vulgaris	>500	–	nd		>500	–	nd	
L. minor	>500	–	nd		>500	–	nd	
R. subcapitata	>500	1.9	–		>500	–	nd	
M. aeruginosa	277.00 (244.54–364.77)	1.9	145.8		>500	–	Nd	
D. quadricauda	19.60 (16.44–19.83)	1.9	10.3	Harmful	>500	6.25	–	

nd, Not defined.

Table 4
Risk assessment for tested compounds in relation to algae, cyanobacteria and higher plants

Compounds	PNEC (mg/L)	PEC in surface waters (mg/L)	RQ (PEC/PNEC)	Risk assessment
Nano-Al_2O_3	0.0395	0.0000002[a]	0.00000506	Low risk
		0.0000012[b]	0.00003037	
		0.0000025[c]	0.00000632	
Al_2O_3	0.113	–	–	–
Nano-ZrO_2	0.0416	–	–	–
ZrO_2	0.258	–	–	–

[a]The situation where 10% of a product type contains the engineered aluminium oxide nanoparticles.
[b]The situation where half of a product type contains the engineered aluminium oxide nanoparticles.
[c]The situation where all of a product type contains the engineered aluminium oxide nanoparticles.

(EC_{50}-168h > 500 mg/L – number of leaves). Additionally, Al_2O_3-NPs stimulated the growth of the plant. Similar results were obtained by Lee et al. [26]. They observed that the concentration of 2,000 mg/L aluminium oxide NPs did not inhibit the growth of the root of *Arabidopsis thaliana*. Likewise, our previous study on NPs' influence the growth of *Sorgo saccharatum*, *Lepidium sativum*, *Sinapis alba* showed inhibitory effects of nano-Al_2O_3 and nano-ZrO_2 in higher concentrations and stimulation of the growth in lower concentrations [27].

In order to assess the risk, in this study standard test conditions were applied, despite the recent concerns raised about the relevance of these methods for assessing the risks of NPs

Table 5
Predicted concentrations (PEC) for a range of engineered nanoparticles and calculation of the risk quotient (PEC/PNEC) for water, soil and air

Particle type	PEC			RQ (PEC/PNEC)			References
	Water (μg/L)	Soil (μg/kg)	Air (μg/m^3)	Water	Soil	Air	
Nano-CeO_2	<0.0001	<0.01	–	–	–	–	[15]
Nano-Au	0.14	5.99	–	–	–	–	[15]
Nano-SiO_2	0.007	0.03	–	–	–	–	[15]
Nano-ZnO	76	3,194	–	–	–	–	[15]
CNT	0.005	0.01	0.0015	0.005	nd	0.000015	[28]
Nano-Ag	0.03	0.02	0.0017	0.0008	nd	nd	[28]
Nano-TiO_2	0.70	0.40	0.0015	>0.7	nd	0.0015	[28]

nd, Not determined due to lack of ecotoxicological data.

[14]. The preliminary assessment of the potential risk posed by the nano-Al_2O_3 was carried out for water by comparison with the PEC (derived from statistical models available in literature) to the PNEC. Special algorithms were used to estimate potential concentrations of a range of NPs in water arising from use. These assessments focused on cosmetics and personal care products and paints as data were available on concentrations of a range of ENPs in these products. As only limited data were available on the fraction of each market comprised of ENP-containing products, three hypothetical scenarios were modelled: (1) the situation where 10% of a product type contains the engineered NP, (2) the situation where half of a product type contains the engineered NP and (3) the situation where all of a product type contains the engineered NP [15]. Based on this data, in this work we have shown that the RQ factor for tested NPs was 0.00000506, 0.00003037 and 0.00000632 mg/L, respectively.

Data concerning PEC (and also PNEC) of NPs are hardly found in literature. This is due to the fact that measurement or prediction of environmental concentrations of engineered NPs is still hampered by many difficulties, including detection, differentiation between natural forms of the material and engineered or nanoscale formats [14] which currently impedes progress in understanding the relevant exposure pattern. Furthermore, no data are available on the relevance of the extrapolation factors used for calculating PNECs. Current knowledge on NPs' specific interactions with the aqueous environment is limited. The PNEC and PEC results obtained in this paper can be considered as preliminary [15]. All data about PEC and RQ available in the literature are derived from statistical modelling (Table 5) [15,28].

The results of our own research have allowed us to pre-estimate the risks posed by the presence of certain NPs in the environment. However, this assessment must be critically analyzed and should only be the initial stage of the whole procedure. The risk quotients obtained in this work and in other authors' work not necessarily mean that the environment is risk-free. The values of PEC, PNEC and RQ available in the literature for different NPs vary considerably. This is primarily due to the difference in the method used and the material used. Therefore, many studies should not be compared. It is also necessary to accurately characterize the NPs used, each type of particle should be analyzed separately [29].

The obtained results also showed that the studied compounds in bulk counterparts are less toxic than the same compounds in the NP forms (Tables 2–4). This fact confirms the reports from the literature that the nano forms of a given substance may constitute a far greater danger to the environment than the same substances in the large form. Song et al. [3] proved that CuO NPs are more harmful to *L. minor* in comparison with the effects of bulk CuO. Also, the research of Załęska-Radziwiłł and Doskocz [30,31] proved that zirconium oxide and aluminium oxide have significantly smaller influence on *P. putida* and *A. hydrophila* and aquatic invertebrates than the NP form of these compounds.

Greater toxic effect of NPs on plants, it might be a result of many different properties of these compounds such as high surface to volume ratio, high chemical reactivity, the ability to form aggregates, diffusivity and mechanical strength. NPs have greater specific surface area, and therefore greater reactivity and potential for generating reactive oxygen species than their bulk counterparts, and thus their expected inhibition of activity should be greater. Moreover, NPs due to their small size (1–100 nm) can penetrate into the inside of an organism more easily than their bulk counterparts, where they can cause various types of dysfunction [32–33].

5. Conclusions

The conducted studies concerning the ecotoxicity of aluminium and zirconium oxide NPs and aluminium and zirconium oxides towards *M. aeruginosa*, *D. quadricauda*, *R. subcapitata*, *S. obliquus* and *L. minor* made it possible to formulate the following conclusions:

- Aluminium and zirconium oxide NPs triggered harmful effects in aquatic cyanobacteria, algae and plants; nano-Al_2O_3 proved to be more toxic.
- *D. quadricauda* was most sensitive to the influence of the tested NPs.
- NOEC values calculated from chronic tests for nano-Al_2O_3 and nano-ZrO_2 were significantly lower than EC_{50} values. Therefore, extrapolation of NOEC values with EC_{50} is impossible with the use of commonly accepted ACR factor = 10.

- Calculated PNEC values, parameters essential for risk assessment, of tested NPs in relation to the producers in aquatic ecosystems equalled 0.0395 mg/L for nano-Al_2O_3 and 0.0416 mg/L for nano-ZrO_2.
- The conducted assessment revealed low risk to aquatic plants.
- Toxicity of Al_2O_3 and ZrO_2 in macro forms was definitely lower than in the case of the nano forms.

This work confirms literature data and proves that the presence of NPs in aquatic ecosystems may adversely affect the aquatic flora. Therefore, there is a necessity for ecotoxicity studies of NPs in relation to producers. The study also shows that the currently available ecotoxicity data concerning compounds in bulk counterparts cannot be used to assess the harmfulness of their nano-form counterparts. REACH regulation for chemical compounds seems to be insufficient to assess the potential danger and the risk to aquatic environment caused by NPs [34].

Increasing production of NPs leads to their accumulation in the environment, for example, in bottom sediments. If released, they may trigger effects in aquatic plants that play beneficial role in the environment. Negative consequences may concern plant growth inhibition or stimulation [25]. Furthermore, NPs may accumulate and move in plant tissues, and therefore, the plants as the major all ecosystems must be considered in the overall assessment of the fate and transport and exposure of the NPs in the environment. There is also a constant need for further studies, including not only conventional but also multispecies, chronic and molecular tests in order to explain the mechanisms of NPs' influence on physiological processes of plants. It seems necessary to develop novel methods of risk assessment dealing with the presence of NPs in aquatic ecosystems. So far assessment of the risk due to the presence of active substances in surface waters in relation to aquatic organisms was based on the PEC/PNEC ratio, where PEC stands for predicted or measured environmental concentration, but due to their specific properties, the main challenge is to accurately determine the exposure of ecosystems to nanocompounds. Currently, there are no analytical methods suitable for detection of trace concentrations of NPs. Therefore, expected concentrations have to be predicted by extrapolations and analogies [28,35]. It is only by collecting all these data that quantitative environmental risk assessment of NPs can be made.

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