Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2020.25244

186 (2020) 50–55
May

Ecotoxicity of selected nanoparticles in relation to micro-organisms in the water ecosystem

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@is.pw.edu.pl (M. Łebkowska)

Received 12 September 2019; Accepted 22 November 2019

ABSTRACT

In the literature, there are few ecotoxicological data concerning the effects of nanoparticles on reducers, which are an important element of the food chain in aquatic ecosystems. The work aimed to evaluate the influence of two types of engineering nanoparticles: aluminum oxide (nano-Al_2O_3) and zirconium oxide (nano-ZrO_2) on micro-organisms. In this work enzymatic assay (bioluminescence test) with *Vibrio fischeri* was performed as well as two growth tests: test with *Pseudomonas putida* and test microbial assay for toxic risk assessment with 10 species of bacteria and 1 species of fungi). In this study, the effect of the activity of nano-Al_2O_3 and nano-ZrO_2 on micro-organisms as compared to their bulk counterparts. The obtained values of concentrations of EC_{50} and no observed effect concentrations showed a different sensitivity of the organisms to the examined compounds. According to the European Union criteria, nano-Al_2O_3 was very toxic to *P. putida* (EC_{50} = 0.5 mg/L), while nano-ZrO_2 was harmful to *Pichia anomala* (EC_{50} = 89.80 mg/L) and *P. putida* (EC_{50} = 25.4 mg/L). Nanoparticles proved to be more toxic to tested micro-organisms 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; Aluminum oxide; Zirconium oxide; Micro-organisms; Ecotoxicity

1. Introduction

The effects of nanoparticles (NPs) on micro-organisms and antimicrobial mechanisms have not been revealed clearly. It was hypothesized in the literature that NPs can cause cell lysis or inhibit cell transduction. There are various mechanisms involved in cell lysis and growth inhibition [1].

Extensive use and increasing demand for NP will lead to their accumulation in the environment, especially in landfills and their water effluents. Many microbes have essential roles in the circulation of elements (carbon, sulfur, nitrogen, etc.), while others degrade pollutants and promote plant growth [2–4]. Therefore, effects on the populations of microbes that play beneficial roles in the environment could have negative consequences. On the other hand, control of pathogenic microbes using nanoparticles having antibacterial activity is a promising approach to defeat the multiresistant pathogens [2,5].

The toxicity of NPs against environmental microbes has been little studied. Datasheets of NPs most often include scarce toxicological data, and there are no results of ecotoxicity studies. Most of the available data relates to research on Ag NPs and usually includes a small set of test organisms, often insufficiently sensitive to nanoparticles. It is well known that Ag ions and Ag-based compounds are highly toxic to micro-organisms. Ecotoxicity tests carried out on 12 species of bacteria including *E. coli* [6,7] have shown their strong biocidal effects. Moreover, in the last years, Schiavo

* Corresponding author.

1944-3994/1944-3986 © 2020 Desalination Publications. All rights reserved.

et al. [8] reported the ecotoxicological effect of ZnO NPs on *V. fischeri* [8]. Thanks to these properties' Ag nanoparticles are being used as antimicrobial agents in many public places such as railway stations and elevators in China [1].

Particularly, there is a lack of information about chronic ecotoxicological effects caused by long-term and multigenerational exposure of aquatic organisms to nano compounds [9]. Moreover, most of the ecotoxicological data relate to animal studies [10]. While most of the research about the antimicrobial activity of nanoparticles concerns human pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli* [2]. Particularly limited is the information on the effects of NPs on the physiological processes of micro-organisms occurring in the aquatic and terrestrial ecosystem.

Nanoparticles are used for numerous physical, biological, and pharmaceutical applications. NPs of Ag, CuO, and ZnO being used industrially for several purposes including amendments to textiles, cosmetics, sprays, plastics, and paints also show antibacterial activity [11].

Hence the purpose of this study was to assess the influence of aluminum and zirconium oxide nanoparticles on micro-organisms found in aquatic and terrestrial environments.

Aluminum oxide nanoparticles, 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 nanoparticles 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 and this way can influence among others the micro-organisms playing an important role in the processes of biological treatment of wastewater and water purification. [11,12].

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

2. Material and methods

2.1. Chemicals

Aluminum oxide nanoparticles (nano-Al_2O_3), nanopowder <50 nm with a specific surface area >40 m^2/g, zirconium oxide nanoparticles (nano-ZrO_2), nanopowder <100 nm with a specific surface area ≥25 m^2/g and aluminum and zirconium oxides of purity over 98% were obtained from Sigma-Aldrich. CAS no. of compounds containing Al_2O_3 is 1344-28-1 and ZrO_2 is 1314-23-4. The stock solutions of nano compounds and bulk counterparts with a concentration of 2,000 mg/L were prepared in deionized water. To avoid the formation of the aggregates, the stock dispersion was sonicated (0.4 kW and 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 1,000–0.2 mg/L.

2.2. Toxicological tests with the use of bacteria and fungi

Enzymatic and growth tests (acute and chronic - basic concepts in the global nomenclature of ecotoxicology) were performed with 12 species of bacteria and 1 species of fungi (yeast). Lyophilized strains of bacteria and fungi came from kits supplied by the manufacturers of the tests. *Pseudomonas putida* bacteria came from the own laboratory culture of the Department of Biology, Faculty of Building Services, Hydro, and Environmental Engineering, Warsaw University of Technology.

2.2.1. Bioluminescence test with Vibrio fischeri—LUMIStox

The bioluminescent bacteria test (acute test) is a bio test procedure. In this test, the cumulative effects of toxic substances in water can be measured without any knowledge of the exact composition or the ecotoxicity of the individual substances. The test is based on the fact that toxins can reduce the normal luminescence of the bacterium *Vibrio fischeri*.

LUMIStox test was performed in accordance with the methodology included in the implementing instruction provided by Dr. Lange (Germany) [13]. The assessment of the bioluminescence inhibition was conducted after 15 and 30 min of bacteria incubation with toxic substances. Calculation of the bioluminescence inhibition was performed using LUMISsoft II software.

2.2.2. Microbial assay for toxic risk assessment growth test

Microbial assay for toxic risk assessment (MARA) is bioassay (chronic test) performed with an array of microbial strains to assess the toxicity (inhibitory) or enhancement profile of the test substance (sample). The assay is based on the growth of the microbes (freeze-dried) employing a 96-well microplate format. The array is exposed to either a concentration gradient of the test sample or an undiluted sample. Growth of the micro-organisms in the array is measured by recording the reduction of a redox dye through the use of a flatbed scanner and image analysis software. Test with the use of lyophilized strains of bacteria and fungi (Table 1) was performed in accordance with the methodology included in the implementing instruction provided by NCIMB (the UK, 2008) [14]. The assessment of the micro-organism growth inhibition was conducted after 18 h of incubation with toxic substances. The assessment was based on the measurement of the surface area of the sludge, where

Table 1
List of species of bacteria and fungi used in MARA test

MARA No.	Species
1	*Microbacterium* sp.
2	*Brevundimonas diminuta*
3	*Citrobacter freundii*
4	*Comamonas testosteroni*
5	*Enterococcus casseliflavus*
6	*Delftia acidovorans*
7	*Kurthia gibsonii*
8	*Staphylococcus warneri*
9	*Pseudomonas aurantiaca*
10	*Serratia rubidaea*
11	*Pichia anomala*

the reduction of tetrazolium red contained in the culture medium was observed. Image analysis was performed with the use of MARA Software ver. 2.01.

2.2.3. Growth test with P. putida

Growth test with *P. putida* bacteria was performed in accordance with the methodology contained in ISO 107122-1994 [15]. The assessment of growth inhibition was conducted based on the measurement of the optical density of samples with λ = 610 nm at the beginning and at the end of the 16 h test.

Growth inhibition (I) was calculated according to the following equation:

$$I = \frac{B_c - B_n}{B_c - B_0} \times 100\% \quad (1)$$

where B_c is the optical density of suspension in control sample after time t; B_n is the optical density of suspension in the sample examined after time t; B_0 is the optical density of suspension in control in time 0.

2.3. Calculation of EC_{50} and no observed effect concentrations

Effective concentrations (EC_{50}) in acute and chronic tests were calculated using probit analysis, determining 95% confidence intervals [16].

No observed effect concentrations (NOEC) were determined using single-factor analysis of variance ($p < 0.05$) and Tukey's test [17].

2.4. Toxicity assessment of compounds

The assessment of toxicity of the test chemicals in relation to bioindicators was performed based on the European Union (EU) criteria – Directive 93/67/EEC (Table 2) [18–20].

3. Results and discussion

The obtained values of EC_{50} and NOEC showed different sensitivity of micro-organisms to the tested nanoparticles (Tables 3–6). In acute enzymatic tests, nano-Al_2O_3 and nano-ZrO_2 inhibited the process of bioluminescence by 36% and 30% respectively (in the highest tested concentration). EC_{50} in both cases was >200 mg/L. Aruoja et al. [21] in a standard acute test with *V. fischeri* obtained in the case of Al_2O_3 NPs EC_{50} > 100 mg/L [21].

Diverse reactions of micro-organisms to NPs were also observed in growth tests. Aluminium oxide nanoparticles inhibited growth of most of the bacteria. The highest sensitivity was shown in the case of *P. putida* – EC_{50} equalled 0.5 mg/L, while Fabrega et al. determined that the inhibition of *P. fluorescens* bacterial growth occurred only at 2000 mg/L [22].

The values EC_{50} obtained in the course of this study in the MARA test ranged from 100–902.6 mg/L, for *Citrobacter freundii* and *Pseudomonas aurantiaca*, respectively. The most resistant to the action of nanoparticles were *Staphylococcus warneri*, *Delftia acidovorans* and *P. anomala* (yeast). EC_{50} was: >1,000 mg/L. Jośko and Oleszczuk [23] examined different

Table 2
Assessment of toxicity of chemicals in relation to the criteria of their harmfulness to aquatic biocenoses according to EU

EC_{50} (mg/L)	Assessment of toxicity of chemical
<0.1	Extremely toxic
0.1–1	Very toxic
>1.0–10	Toxic
>10–100	Harmful
>100	Non-toxic

Table 3
Ecotoxicity of nano-Al_2O_3 in relation to micro-organisms

No.	Tested organisms	Test type	Test duration (h)	EC_{50} (mg/L) (95% confidence interval)	NOEC (mg/L)	Toxicity assessment
						UE Directive 93/67/EEC
1	*Pichia anomala*	Growth	18	>1,000	–	Non-toxic
2	*Delftia acidovorans*	Growth	18	>1,000	–	
3	*Staphylococcus warneri*	Growth	18	>1,000	25.00	
4	*Vibrio fischeri*	Enzymatic (bioluminescence)	15 min	>200	–	
5	*Vibrio fischeri*	Enzymatic (bioluminescence)	30 min	>200	–	
6	*Pseudomonas aurantiaca*	Growth	18	902.60 (900.48–134.59)	12.50	
7	*Kurthia gibsonii*	Growth	18	713.12 (609.54–931.92)	12.50	
8	*Brevundimonas diminuta*	Growth	18	623.68 (400.15–631.76)	12.50	
9	*Comamonas testosteroni*	Growth	18	494.79 (469.75–531.54)	1.90	
10	*Serratia rubidaea*	Growth	18	465.37 (442.63–528.66)	0.78	
11	*Microbacterium* sp.	Growth	18	351.94 (320.71–399.52)	0.50	
12	*Enterococcus casseliflavus*	Growth	18	266.41 (234.79–304.61)	0.39	
13	*Citrobacter freundii*	Growth	18	100.00 (87.54–121.84)	0.39	Harmful
14	*Pseudomonas putida*	Growth	16	0.50 (0.22–1.03)	0.19	Very toxic

nanoparticles with the MARA test and noticed some growth of inhibition of sensitive strains of micro-organisms caused by Zn NPs and TiO_2 NPs [23]. The most sensitive species, otherwise than in this study, appeared to be *P. anomala* strain. *S. warneri* and *Serratia rubidaea* strains showed the lowest sensitivity (Table 3).

Nano-ZrO_2 proved to be less toxic to most micro-organisms than nano-Al_2O_3 (Table 4). The lowest value of concentration which inhibited bacterial growth by 50% compared to the control was obtained in the test with *P. putida* (EC_{50} = 25.4 mg/L). For six species of bacteria in MARA test EC_{50} were >1,000 mg/L (Table 4). In the MARA test, the most sensitive species to the nano-ZrO_2 was *P. anomala* and *Kurthia gibsonii*. The EC_{50} value was 89.8 and 212.26 mg/L, respectively. There is no data in the literature for nano-ZrO_2 toxicity in relation to micro-organisms.

Based on the UE criteria for acute toxicity nano-Al_2O_3 was non-toxic to eleven species and harmful to one species

Table 4
Ecotoxicity of nano-ZrO_2 in relation to micro-organisms

No.	Tested organisms	Test character	Test duration [h]	EC_{50} [mg/L] (95% confidence interval)	NOEC [mg/L]	Toxicity assessment
						UE Directive 93/67/EEC
1	*Staphylococcus warneri*	Growth	18	>1,000	31.20	Non-toxic
2	*Citrobacter freundii*	Growth	18	>1,000	–	
3	*Comamonas testosteroni*	Growth	18	>1,000	15.60	
4	*Delftia acidovorans*	Growth	18	>1,000	–	
5	*Pseudomonas aurantiaca*	Growth	18	>1,000	15.60	
6	*Serratia rubidaea*	Growth	18	>1,000	–	
7	*Vibrio fischeri*	Enzymatic (bioluminescence)	15 min	>200	–	
8	*Vibrio fischeri*	Enzymatic (bioluminescence)	30 min	>200	–	
9	*Enterococcus casseliflavus*	Growth	18	944.23 (812.09–994.86)	1.90	
10	*Brevundimonas diminuta*	Growth	18	263.55 (178.53–345.18)	–	
11	*Microbacterium* sp.	Growth	18	229.04 (221.73–297.34)	3.12	
12	*Kurthia gibsonii*	Growth	18	212.26 (158.55–246.76)	0.90	
13	*Pichia anomala*	Growth	18	89.80 (73.80–100.21)	0.50	Harmful
14	*Pseudomonas putida*	Growth	16	25.40 (20.12–32.66)	0.50	

Table 5
Ecotoxicity of Al_2O_3 in relation to micro-organisms

No.	Tested organisms	Test character	Test duration (h)	EC_{50} (mg/L_ (95% confidence interval)	NOEC (mg/L)	Toxicity assessment
						UE Directive 93/67/EEC
1	*Microbacterium* sp.	Growth	18	>1,000	–	Non-toxic
2	*Enterococcus casseliflavus*	Growth	18	>1,000	–	
3	*Delftia acidovorans*	Growth	18	>1,000	–	
4	*Kurthia gibsonii*	Growth	18	>1,000	–	
5	*Staphylococcus warneri*	Growth	18	>1,000	–	
6	*Pseudomonas aurantiaca*	Growth	18	>1,000	–	
7	*Serratia rubidaea*	Growth	18	>1,000	–	
8	*Pichia anomala*	Growth	18	>1,000	–	
9	*Vibrio fischeri*	Enzymatic (bioluminescence)	15 min	>200	–	
10	*Vibrio fischeri*	Enzymatic (bioluminescence)	30 min	>200	–	
11	*Citrobacter freundii*	Growth	18	471.00 (438.94–562.91)	15.60	Harmful
12	*Comamonas testosteroni*	Growth	18	344.60 (300.75–472.86)	6.30	
13	*Pseudomonas putida*	Growth	16	219.91 (143.21–241.22)	1.90	
14	*Brevundimonas diminuta*	Growth	18	91.95 (67.19–137.54)	3.12	

Table 6
Ecotoxicity of ZrO_2 in relation to micro-organisms

No.	Tested organisms	Test character	Test duration (h)	EC_{50} (mg/L) (95% confidence interval)	NOEC (mg/L)	Toxicity assessment
						UE Directive 93/67/EEC
1	*Microbacterium* sp.	Growth	18	>1,000	–	Non-toxic
2	*Kurthia gibsonii*	Growth	18	>1,000	–	
3	*Staphylococcus warneri*	Growth	18	>1,000	–	
4	*Pseudomonas aurantiaca*	Growth	18	>1,000	–	
5	*Serratia rubidaea*	Growth	18	>1,000	–	
6	*Pichia anomala*	Growth	18	>1,000	–	
7	*Vibrio fischeri*	Enzymatic (bioluminescence)	15 min	>200	–	
8	*Vibrio fischeri*	Enzymatic (bioluminescence)	30 min	>200	–	
9	*Citrobacter freundii*	Growth	18	792.00 (754.32–798.26)	62.50	
10	*Enterococcus casseliflavus*	Growth	18	700.00 (654.65–712.78)	62.50	
11	*Delftia acidovorans*	Growth	18	678.90 (671.54–721.43)	62.50	
12	*Brevundimonas diminuta*	Growth	18	528.80 (504.76–621.57)	15.60	
13	*Comamonas testosteroni*	Growth	18	338.18 (315.54–391.42)	6.25	
14	*Pseudomonas putida*	Growth	16	331.97 (276.94–348.65)	3.90	

of bacteria. High toxicity showed only in relation to *P. putida*. (Table 3). Nano-ZrO_2 was harmful to two species of bacteria (*P. anomala* and *P. putida*), while it was non-toxic to eleven species of bacteria (Table 4).

Both tested nanocompounds showed much higher impact on tested micro-organisms in chronic toxicity. The lowest NOEC values was 0.19 mg/L for Al_2O_3 NPs and 0.5 mg/L for ZrO_2 NPs (*P. putida*) (Tables 3 and 4).

The exact mechanism which NPs employ to cause antimicrobial effect is not known and is a debated topic. Literature data showed that NPs can anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell [1]. Studies of Lok et al. [24] reported that exposure of *E. coli* cells to nano-Ag destabilizes the outer membrane, collapses the plasma membrane potential. NPs actions may be due in part to their release of free ions. Ions can interact with the thiol groups of many vital enzymes and inactivate them [25]. Micro-organisms in contact with nanoparticles take in ions, which inhibit several functions in the cell and damage the cells [2]. Another mechanism by which the micro-organisms die is the formation of free radicals by the nanoparticles. They could damage the cell membrane and make it porous which can ultimately lead to cell death [6,26]. Our previous studies have shown that nano-Al_2O_3 and nano-ZrO_2 inhibit the activity of bacterial enzymes including dehydrogenase [27,28]. An experiment conducted by Nweke et al. [29] also showed inhibition of this enzyme by Zn NPs.

The obtained results also show that the bulk forms of tested compounds have a different impact on micro-organisms. Tested metal oxides influenced the enzymatic processes and bacterial growth to a much lesser extent (Tables 5 and 6). Assessment of toxicity according to EU criteria showed that the aluminum oxide was non-toxic to twelve species and harmful to two species of bacteria (*P. putida* and *Brevundimonas diminuta*). The lowest NOEC value was 1.9 mg/L (*P. putida*) (Table 5). In turn, zirconium oxide according to the UE criteria was non-toxic to all tested micro-organisms. The lowest NOEC was 3.9 mg/L (*P. putida*) (Table 6).

The literature data also indicate that nanoparticles may have other effects on the micro-organisms than the same compounds in their bulk forms. It might be a result of many different properties of nanoparticles such as high surface to volume ratio, high chemical reactivity, the ability to form aggregates, diffusivity, and mechanical strength. Moreover, nanoparticles due to their small size (1–100 nm) can penetrate the inside of an organism more easily than their bulk counterparts, where they can cause various types of dysfunction [6,27,28]. Previous our experiments also showed that bulk counterparts of Al_2O_3 and ZrO_2 do not affect micro-organisms [27,28]. No activities against bacteria have also been shown in studies of Gajjar et al. [2]. Bulk counterparts of nano-Ag, nano-CuO and nano-ZnO showed no bacteriostatic and bactericidal, indicating that particle size was determinant in the activity.

4. Conclusion

This research showed that tested nanoparticles might negatively impact on micro-organisms, which play a significant role in the distribution of organic matter in the aquatic ecosystem. It indicates that the release of NPs into the environment may be harmful to the efficacy of beneficial microbes that function in the circulation of elements, pollutant degradation, and plant growth. It was also found that nanoforms of metal oxides influence-microorganisms in a different way than their bulk counterparts. Therefore, available ecotoxicity data about compounds in bulk forms cannot be used to assess the harmfulness of their nano form. Due to specific features that are characteristic of nano compounds,

current guidelines are not sufficient to protect the biodiversity of natural microbial communities. In order to protect them, much broader studies should be conducted on individual strains of micro-organisms. Moreover, research should include not only the conventional tests but also the molecular ones, which would provide an explanation of the mechanisms behind nanoparticles impact on micro-organisms.

References

[1] S. Prabhu, E.K. Poulose, Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects, Int. Nano Lett., 2 (2012) 1–10.

[2] P. Gajjar, B. Pettee, D.W. Britt, W. Huang, W.P. Johnson, A.J. Anderson, Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida* KT2440, J. Biol. Eng., 3 (2009) 1–13.

[3] G.H. Osler, M. Sommerkorn, Toward a complete soil C and N cycle: incorporating the soil fauna, Ecology, 88 (2007) 1611–1621.

[4] A.G. Khan, Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation, J. Trace Elem. Med. Biol., 18 (2005) 355–364.

[5] N. Jones, B. Ray, K.T. Ranjit, A.C. Manna, Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms, FEMS Microbiol. Lett., 1 (2008) 71–76.

[6] J.S. Kim, E. Kuk, K.N. Yu, J.-H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.-Y. Hwang, Y.-K. Kim, Y.-S. Lee, D.H. Jeong, M.-H. Cho, Antimicrobial effects of silver nanoparticles, Nanomedicine, 3 (2007) 95–101.

[7] G. Zhao Jr., S.E. Stevens, Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the silver ion, Biometals, 11 (1998) 27–32.

[8] S. Schiavo, M. Oliviero, J. Li, S. Manzo, Testing ZnO nanoparticle ecotoxicity: linking time variable exposure to effects on different marine model organisms, Environ. Sci. Pollut. Res. Int., 25 (2018) 4871–4880.

[9] A. Gupta, M. Gopal, K.V. Tilak, Mechanism of plant growth promotion by rhizobacteria, Indian J. Exp. Biol., 39 (2000) 856–862.

[10] M. Załęska-Radziwiłł, N. Doskocz, Ecotoxicity of zirconium oxide nanoparticles in relation to aquatic invertebrates, Desal. Wat. Treat., 57 (2016) 1443–1450.

[11] J.A. Rodríguez, M. Fernández-García, Synthesis, Properties, and Applications of Oxide Nanomaterials, John Wiley and Sons, New Jersey, 2008.

[12] A. Kaur, U. Gupta, A review on applications of nanoparticles for the preconcentration of environmental pollutants, J. Mater. Chem., 19 (2009) 8279–8289.

[13] LUMIStox, Bedienungsanleitung Manual, Dr. Lange, Hach Lange, Dusseldorf, 1994.

[14] NCIMB, Manual for MARA Microplates, National Collection of Industrial, Food and Marine Bacteria, Aberdeen, 2008.

[15] ISO 107122-1994 Water Quality—*Pseudomonas putida* Growth Inhibition Test (*Pseudomonas* Cell Multiplication Test).

[16] E. Weber, Grundniss der biologischenStatistokfür Naturwissenschaftler, Landwirte und Medizimer, Veb Fischer Verlag, Jena, 1972.

[17] P.M. Berthouex, L.C. Brown, Statistic for Environmental Engineers, Lewis Publishers/C.R.C. Press, Boca Raton, FL, 1994.

[18] Commission Directive 93/67/EEC of 20 July 1993 Laying Down the Principles for Assessment of Risks to Man and the Environment of Substances Notified in Accordance with Council Directive 67/548/EEC.

[19] Commission of the European Communities, Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for Existing Substances, Part II-Environmental Risk Assessment, Brusssels, Belgium, 1996.

[20] M. Załęska-Radziwiłł, K. Affek, J. Rybak, Ecotoxicity of chosen pharmaceuticals in relation to micro-organisms—risk assessment, Desal. Wat. Treat., 52 (2014) 3908–3917.

[21] V. Aruoja, S. Pokhrel, M. Sihtmäe, M. Mortimer, L. Mädlerb, A. Kahru, Toxicity of 12 metal-based nanoparticles to algae, bacteria and protozoa, Environ. Sci. Nano., 2 (2015) 630–644.

[22] J. Fabrega, The Impacts of Silver Nanoparticles on Planktonic and Biofilm Bacteria, Dissertation Presented to the University of Birmingham, Birmingham, 2009.

[23] I. Jośko, P. Oleszczuk, The influence of ZnO and TiO_2 nanoparticles on the toxicity of sewage sludges, Environ. Sci. Processes Impacts, 15 (2013) 296–306.

[24] C.N. Lok, C.M. Ho, R. Chen, Q.Y. He, W.Y. Yu, H. Sun, P.K. Tam, J.F. Chiu, C.M. Che, Proteomic analysis of the mode of antibacterial action of silver nanoparticles, J. Proteome Res., 5 (2006) 916–924.

[25] Y. Matsumura, K. Yoshikata, S. Kunisaki, T. Tsuchido, Mode of bacterial action of silver zeolite and its comparison with that of silver nitrate, Appl. Environ. Microbiol., 69 (2003) 4278–4281.

[26] M. Danilcauk, A. Lund, J. Saldo, H. Yamada, J. Michalik, Conduction electron spin resonance of small silver particles, Spectrochim. Acta, Part A, 63 (2006) 189–191.

[27] N. Doskocz, K. Affek, M. Załęska-Radziwiłł, Effect of aluminium oxide nanoparticles on the enzymatic activity on microorganisms of activated sludge, E3S Web of Conferences, 44 (2018) 1–9.

[28] N. Doskocz, K. Affek, M. Załęska-Radziwiłł, The effect of zirconium oxide nanoparticles on dehydrogenase and hydrolytic activity of activated sludge microorganisms, E3S Web of Conferences, 44 (2018) 1–8.

[29] C.O. Nweke, C.S. Alisi, J.C. Okolo, C.E. Nwanyanwu, Toxicity of Zinc to heterotrophic bacteria from a tropical river sediment, Appl. Ecol. Environ. Res., 5 (2007) 123–132.