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DNA changes in *Pseudomonas putida* induced by aluminum oxide nanoparticles using RAPD analysis

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ABSTRACT

This research has been conducted to determine the genotoxicity of nano-Al_2O_3 towards *Pseudomonas putida* bacteria using RAPD-PCR method. The results were compared with the impact of the *macro* form of the compound on DNA. On the basis of RAPD profiles using the primer OPA2, the research demonstrated a mutagenic action of the tested nanocompound. The obtained profiles of RAPD bands are different from the negative control by over 30–9.1%. For the products of PCR carried out using the primer OPA9, genetic similarity indexes relative to the negative control for nano-Al_2O_3 samples were 80% in all tested concentrations. Regarding the remaining primers, RAPD bands profiles for the samples with the nanocompound and the negative control varied only to a small extent. This study showed a decrease in the genetic stability of DNA (GTS, %) after treatment of the bacteria nano-Al_2O_3 as compared with the negative control. GTS value for the nano-Al_2O_3 was 65% (1,000 mg/L). In the remaining concentrations of nano-Al_2O_3, GTS remained in the range of 75–80%. The results showed that the nano-Al_2O_3 can induce modifications of the genetic material to a greater extent than the same compounds in the *macro* form.

Keywords: Aluminum oxide nanoparticles; RAPD-PCR; Genotoxicity

1. Introduction

Nanoparticles (NPS) are the products of nanotechnology, with dimensions smaller than 100 nm, the size of colloidal particles, usually smaller than bacterial cells and eukaryotic cells. Thanks to their unique chemical and physical properties such as: a high surface-to-volume ratio, chemical reactivity, ability to form aggregates, diffusivity, mechanical strength, and atoms dislocation; they have become an attractive material for commercial, technological, or therapeutic use [1–3]. These compounds are increasingly used in production processes of antibacterial agents, paints, cosmetics, food packaging, in medicine, microelectronics, water purification, as catalysts, or dietary supplements. It is expected that the rapidly growing nanomarket will reach an annual turnover of $2.6 trillion in 2014 [4].

The extent of the cytotoxic, teratogenic, and genotoxic interaction of nanoparticles may be much higher than the action of macro forms. Data provided by

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subject-related literature indicate that these compounds have the ability to accumulate in the respiratory system, penetrate through the skin, and accumulate in cellular organelles. They generate free radicals and inactivate enzymes, which contribute to cell apoptosis [3,5].

The Ames test based on the observation of reversion of mutation in a modified strain of *Salmonella typhimurium* recommended by the Organization for Economic Cooperation and Development (OECD) is used in order to determine the genotoxicity of chemical compounds, among others, showed mutagenicity of nanoparticles of titanium oxide [6]. Genotoxicity of titanium oxide nanoparticles is also confirmed by research conducted by Ghosh. The results of the comet assay demonstrated that they induced DNA damage in the nuclei of *A. cepa* roots and they exerted genotoxic effects in human lymphocytes even at the concentration of 0.25 mM [7].

Genotoxic properties were also demonstrated by silver nanoparticles. In Park and Choi research, it was found that the size of the silver nanoparticles of 50 nm caused some changes in the genetic material of *Daphnia magna*, even at concentrations of 1 and 1.5 μg/mL [8]. The research of silver nanoparticles genotoxicity conducted by Asghari et al. using fish *Danio rerio* showed that they accumulated in the cell nucleus and caused some damage to DNA [9]. Studies by Li et al. confirmed, on the other hand, antibacterial effects of silver nanoparticles. When bacteria cells were exposed to the concentration of 50 μg/mL Ag-NPs for 6 h, the cell DNA was condensed to a tension state and was likely to lose their replicating abilities. When *Staphylococcus aureus* cells were exposed to 50 μg/mL Ag-NPs for 12 h, the cell wall was lysed which resulted in the release of the cellular contents into the surrounding environments [10].

Studies by Lehar and Geffroy et al. using RAPD-PCR found some changes in the genome of fish *D. rerio* under the influence of cadmium sulfide and gold nanoparticles. In both cases, it was found that the number of hybridization of RAPD probes had been significantly modified after exposure of the fish to nanoparticles. In addition, the ability of compounds to accumulate in the animal tissues was observed and their capability of inducing some dysfunctions of many cellular organelles, including mitochondrial disorders was found [11,12].

The aim of this study was to assess DNA damage of gram (–) coccobacilli *Pseudomonas putida* by exposure to commercial aluminum oxide nanoparticles (nano-Al_2O_3 of size <50 nm) using the RAPD-PCR (random amplification of polymorphic DNA) technique. A random amplification of polymorphic DNA (RAPD) is called the "Fingerprinting" technique, based on amplification of genomic DNA using a single primer, or a combination of two or more primers, with the length of 10 to 20 bp, frequently of a random nucleotide sequence that renaturate to various regions of the genome simultaneously. Products obtained in the PCR reaction are separated electrophoretically on agarose/polyacrylamide gel give characteristic "genetic fingerprints" (DNA fingerprinting). The comparison of the profiles makes it possible to establish the level of similarity between the obtained profiles of DNA bands of the tested material with respect to the positive and negative control [13,14].

RAPD-PCR test has been used successfully in order to detect genetic damage in animals and plants [15–18]. However, this study is the first one relating to the use of RAPD-PCR technique with respect to bacteria and the influence of oxide aluminum nanoparticles on the genetic material of micro-organisms.

Little information is available on the destiny, transport, and effects of nanomaterials including metal-based particles such as nanosized Al_2O_3, in the environment. Nanosized aluminum is currently being used by the military and commercial industries in many applications including coatings, thermites, and propellants. The use of nanoaluminum in various applications may cause a release of the oxidized form, nano Al_2O_3, into the environment. As utilization of nanomaterials is on the rise, it is increasingly important to determine their potential environmental destiny and the effects [5,19].

This paper is a comparison between the effects on genetic material of bacteria *P. putida* by the nanoparticle form of aluminum oxide and the oxide of this compound.

2. Materials and methods

2.1. Chemicals

Aluminum oxide nanoparticles (nano-Al_2O_3), nanopowder <50 nm of specific surface of >40 m^2/g, aluminum oxide of purity of >98%, and methyl methanesulfonate (MMS) (positive control) were obtained from Sigma-Aldrich Company. Initial solutions of the tested compounds were prepared in deionised water. Because of the tendency of aggregate formation by the compounds, the obtained solutions of the compounds were sonicated for 30 min using an ultrasonic disintegrator of MDM-10 type (0.4 kW at a frequency of 20 kHz). These solutions of nano-Al_2O_3 and Al_2O_3 were diluted (using a medium according to ISO 107122-1994 test procedure for *P. putida*) in a decreasing series with the ratio of geometric progres-

sion of dilutions of $q = 10$ obtaining solutions in the concentration range 1,000–0.1 mg/L. MMS solution was prepared at 10 mg/L.

2.2. Bacterial strains

Heterotrophic gram-negative rods of *P. putida* were isolated from the activated sludge working in laboratory conditions, in Department of Biology, Faculty of Environmental Engineering, Warsaw University of Technology. Bacteria were isolated from the activated sludge working in laboratory conditions. An aseptic technique was used throughout the testing process. Constant bacterial cultures were maintained throughout the experimentation, and incubations for all tests were conducted for 16 h at 37˚C.

Bacterial cultures were then subjected to biochemical identification by API procedure. A Gram's stain was implemented to determine whether the bacteria were positive or negative. An oxidase test was performed by adding bacterial smear to the filter paper containing an oxidase reagent (a mixture of dimethyl-4-phenylenediamine hydrochloride and α-naphthol). Color development was observed within 1 min. A catalase test was performed by adding one drop of 30% hydrogen peroxide (Aflofarm, Pabianice, PL) to a slide that contained bacterial smear. A bubbling reaction was observed within 1 min. API 20 NE bacterial identification was performed according to the manufacturer's instruction (bioMérieux, Durham, NC).

The selection of strain was based upon the ability of these bacteria to degrade organic solvents. This ability has been put to use in bioremediation, or the use of micro-organisms to biodegrade oil [5,20,21]. The strains were multiplied in nutrient broth at a temperature of 26˚C for 18 h until the commencement of the logarithmic growth phase. Cultures of bacteria of optical density of 0.2 at $\lambda = 610$ nm were added to a liquid medium containing defined concentrations of the tested compounds. Samples were incubated in the dark for 16 h at 26˚C. Cultures obtained in this way were used for DNA isolation.

2.3. Genomic DNA isolation

Genomic DNA was isolated from culture of *P. putida* using a kit of "Genomic Mini" in accordance with the manual enclosed by the manufacturer (A & A Biotechnology). The isolation process is based on the ability of binding DNA to silica deposits in high concentrations of chaotropic salt. The amount of DNA material, isolated from the bacterial culture was established by spectrophotometric measurements in the UV light at 260 nm wave length (nitric bases building nucleic acids absorb light at this wavelength). For this purpose, the researchers used camera "BioPhotometr" from Eppendorf Company. The condition of DNA amplification was optimized following the procedure of Conte et al. [22] with some modifications. The obtained DNA was stored for subsequent analysis in a refrigerator at 4˚C.

2.4. RAPD procedures

PCRs were performed in reaction mixtures of 25 μL containing approximately 25 ng of genomic DNA, 10 μM primer, 100 mM dNTPs (25 mM each), 10× reaction buffer, and 1U or 2U of Taq DNA polymerase. Sequences (5′→3′) from primers 1–4 were presented as follows:. TGCCGAGCTG (OPA 2); GTGATCGCAG (OPA 9); GGGTAACGCC (OPA 10); and AGGTGACCGT (OPA 18). Amplifications were performed in a DNA thermocycler (Mastercycler pro, Eppendorf) programmed for 2 min at 94˚C (the initial denaturing step), 35 consecutive cycles each consisting of 30 s at 94˚C (denaturing), 1 min at 31 or 33˚C (annealing), 2 min at 72˚C (extension), and followed by 1 cycle for 1 min at 72˚C (the final extension step). Control PCRs lacking genomic DNA were run with every set of samples. After amplification, RAPD reaction products were analyzed by electrophoresis on 1% agarose gels in 1xTAE (40 mM Tris base, 20 mM acetic acid and 1 mM EDTA) buffer at R.T. at 100 V for about 30–50 min. GeneRuler 1 kb DNA Ladder (Fermentas) was used as the molecular weight DNA standard. DNA bands were stained with ethidium bromide, visualized and photographed under UV light. The size of each amplification product was automatically estimated using the GelDoc-It Imaging System (Ultra-Violet Products Ltd) analyzer system. Digital processing of the images and computational analysis was performed using Gelix One 1-D Analysis Software (Biostep). Genetic similarity indexes for individual primers were established, and their mean and standard deviations were calculated.

2.5. Data analysis and processing

Genetic similarity index (S, %) between treated samples and control bacteria was calculated as the proportion of amplification products which were not polymorphic with respect to the total number of amplified products, 2× number of shared fragments/the total number of fragments [22,23]. Genomic template stability (GTS, %) was calculated as $100 - (100\ a/n)$, where a is RAPD polymorphic

profile detected in each sample treated and *n is* the number of the total bands in the control. Polymorphism observed in RAPD profiles included disappearance of a normal band and appearance of a new band in comparison to control RAPD profiles [24,25], and the average was then calculated for each experimental group exposed to different nano-Al_2O_3 and Al_2O_3 treatment. Changes in these values were calculated as a percentage of their control (set to 100%).

All amplifications were repeated twice, in order to confirm the reproducible amplification of scored fragments. Only reproducible and clear amplification bands were scored for the construction of the data matrix.

3. Results

Changes in the genetic material caused by the impact of aluminum oxide nanoparticles and aluminum oxide on DNA isolated from bacteria *P. putida* were evaluated using the RAPD-PCR technique. The PCR reaction used primers: OPA 2, OPA 10, OPA 9, and OPA 18. Photos of RAPD bands profiles are shown in Fig. 1. The obtained bands were used for the analysis of genetic stability of DNA (GTS, %) and the degree of similarity between bands profile (S, %) with relation to negative control, and the results are shown in Fig. 1 and in Tables 1–5.

On the basis of RAPD profiles obtained for the PCR, it was found that the aluminum oxide nanoparticles can act genotoxically with *P. putida* bacteria. PCR products obtained with the use of the primer OPA 2 demonstrated the most mutagenic action of aluminum oxide nanoparticles. The obtained bands profiles were different from the negative control from over 33.3% (the concentration of 1,000 mg/L) to 9.1% (the concentration of 0.1 mg/L) (Table 1). Additionally, it was observed that the value of the degree of similarity (*S*) of the genetic RAPD profile obtained for nano-Al_2O_3 with the concentration of 1,000 mg/L using the primer OPA2 was largely similar to the positive control. The obtained bands profile differed from the result obtained for the positive control only by 12%. With other concentrations, differences were already much greater, within the limits of 28–36.4%. For the PCR products obtained with the use of the primer OPA 9, genetic similarity indexes with relation to the negative control for samples of nano-Al_2O_3 were 80% in all concentrations tested (Table 2). In the case of the primer OPA 10 and OPA 18, the mutagenic effect of aluminum oxide nanoparticles was the lowest. RAPD bands profiles for samples with nano-Al_2O_3were almost identical to bands profiles of the negative control (Tables 3 and 4). The degrees of similarity (*S*) of the obtained bands profiles for primers OPA 9, OPA 10, and OPA 18 were significantly different to the values obtained for the positive control. The differences

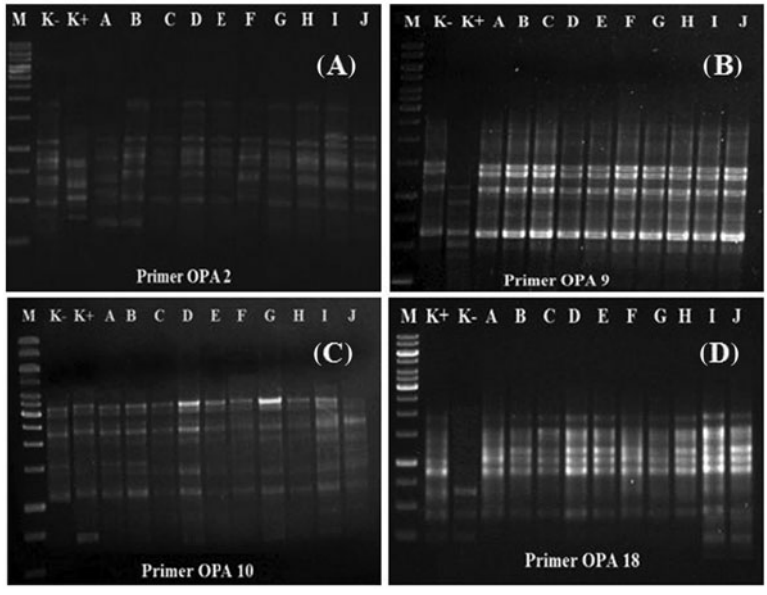

Fig. 1. RAPD profiles generated using primers OPA 2 (A), OPA 9 (B), OPA 10 (C), and OPA 18 (D) for genomic DNAs extracted from bacteria *P. putida* exposed to negative control (K−), positive control (K+), and various concentrations of nano-Al_2O_3 (1,000 mg/L—A, 100 mg/L—B, 10 mg/L—C, 1 mg/L—D, 0.1 mg/L—E) and Al_2O_3 (1,000 mg/L—F, 100 mg/L—G, 10 mg/L—H, 1 mg/L—I, 0.1 mg/L—J) for 16 h. M—GeneRuler 1 kb DNA ladder (10,000–250 kb).

Table 1
Degree of similarity of obtained profiles of *P. putida* bands in the presence of nano-Al_2O_3 and $Al_2O_3$for primer OPA 2

	Primer OPA 2	
Concentration [mg/L]	Al_2O_3 nanoparticles Genetic similarity index (*S*) after 16 h	Al_2O_3 Genetic similarity index (*S*) after 16 h
1,000	66.7	90.9
100	83.3	90.9
10	90.9	90.9
1	90.9	100
0.1	90.9	100
Positive control (MMS 10 mg/l)	54.5	

Table 2
Degree of similarity of obtained profiles of *P. putida* bands in the presence of nano-Al_2O_3 and $Al_2O_3$for primer OPA 9

	Primer OPA 9	
Concentration [mg/L]	Al_2O_3 nanoparticles Genetic similarity index (S) after 16 h	Al_2O_3 Genetic similarity index (S) after 16 h
1,000	80.0	80.0
100	80.0	80.0
10	80.0	80.0
1	80.0	80.0
0.1	80.0	80.0
Positive control (MMS 10 mg/l)	50.0	

Table 3
Degree of similarity of obtained profiles of *P. putida* bands in the presence of nano-Al_2O_3 and $Al_2O_3$for primer OPA 10

	Primer OPA 10	
Concentration [mg/L]	Al_2O_3 nanoparticles Genetic similarity index (S) after 16 h	Al_2O_3 Genetic similarity index (S) after 16 h
1,000	100	100
100	100	100
10	100	100
1	100	100
0.1	100	100
Positive control (MMS 10 mg/l)	44.4	

ranged from 30% for OPA 9 (at all concentrations tested) and OPA 18 (at the highest concentration tested) to 55.6% for the OPA 10 (at all concentrations tested).

Results of the analysis of the genetic stability of DNA (GTS, %), which reflect changes in RAPD profiles, showed that the average value of GTS in all concentrations tested was smaller than in the negative control (Table 5). The largest decrease in genetic stability was found at the concentration of 1,000 mg/L—65%. GTS value for nano-Al_2O_3 at 100 mg/L was 73.4 and 77.5% with the concentration of 10 mg/L. In all other concentrations, the value of GTS remained at the same level of 80.3%. The data indicate that GTS, for the concentration of 1,000 mg/L, to a large extent is similar to the value obtained for the positive control which is 41.7%.

The results also indicate that aluminum oxide in the macro form is less mutagenic than the compound in the nanoparticle form (Tables 1–4). RAPD profiles

Table 4
Degree of similarity of obtained profiles of *P. putida* bands in the presence of nano-Al_2O_3 and $Al_2O_3$for primer OPA 18

	Primer OPA 18	
Concentration [mg/L]	Al_2O_3 nanoparticles Genetic similarity index (*S*) after 16 h	Al_2O_3 Genetic similarity index (*S*) after 16 h
1,000	80.0	87.7
100	80.0	87.5
10	80.0	87.5
1	87.7	100
0.1	87.5	100
Positive control (MMS 10 mg/l)	50.0	

Table 5
Genetic stability (GTS, %) of *P. putida* in the presence of various concentrations of nano-Al_2O_3 and Al_2O_3 after 16 h

			1,000 mg/L		100 mg/L		10 mg/L		1 mg/L		0,1 mg/L	
Primer	Negative control	Positive control (MMS 10 mg/l)	Nano-Al_2O_3	Al_2O_3	Nano-Al_2O_3	Al_2O_3	Nano-$A_{l2}O_3$	Al_2O_3	Nano-Al_2O_3	Al_2O_3	Nano-Al_2O_3	Al_2O_3
OPA 2	100	33.3	33.3	83.3	66.7	83.3	83.3	83.3	83.3	100	83.3	100
OPA 9	100	50.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
OPA 10	100	50.0	100	100	100	100	100	100	100	100	100	100
OPA 18	100	33.3	66.7	77.8	66.7	77.8	66.7	77.8	77.8	100	77.8	100
Mean	100	41.7	65.0	80.3	73.4	80.3	77.5	80.3	80.3	90.0	80.3	90.0

obtained from samples of Al_2O_3 showed greater similarity compared to the negative control than the profiles from samples with nano-Al_2O_3. In the presence of aluminum oxide, a smaller decrease in genetic stability in all concentrations tested as compared with the aluminum oxide nanoparticles was observed.

4. Discussion

The analysis of profiles obtained in RAPD-PCR reaction showed that the aluminum oxide nanoparticles have the potential to cause changes in the genetic material of bacteria. In the case of using nano-Al_2O_3 against *P. putida* with the primer OPA 2, the changes in the genetic material increased with the increasing concentration. The lowest genetic similarity indexes (Tables 1 and 4) as well as the greatest decrease in DNA genetic stability (Table 5) was obtained at the concentration of 1,000 mg/L of nano-Al_2O_3 as compared with the negative control, which indicates a high mutagenic action of the tested nanocompound in this test concentration. A similar effect was observed in studies conducted by Cenkci et al. on the influence of various kinds of chemical compounds on bean (*Phaseolus vulgaris* L.) seedlings. The study shows that DNA damage increased with an increase in concentrations of toxic chemicals [26], as well as indicates that the researched compound generates a similar number of mutations as MMS (positive control), whose mutagenic action was confirmed in numerous publications [14,27,28]. Modifications of RAPD patterns are possible due to one or a combination of the following events: DNA adducts, DNA breakage, and mutation (point mutations and large rearrangements [13,14,17,24,26,28–30]. In the case of samples using the remaining primers OPA 9, OPA10, and OPA 18, it was found that the degree of genetic similarity (S), and GTS do not differ much from the results obtained for the negative control. Some minor changes in the genome may indicate that the presence of aluminum oxide nanoparticles could lead to changes of a random nature [31]. Moreover, obtained in this study, RAPD profiles with a similar degree of genetic similarity to the negative control and a slight decrease in the genomic template stability may prove the induction of mechanisms responsible for DNA repair beyond a certain critical level of damage, which for aluminum oxide nanoparticles may be even less than the smallest used concentration of 0.1 mg/L [17,28]. The results also show that not every primer allows for obtaining reliable informa-

tion. The smallest degrees of genetic similarity were observed in RAPD profiles, which used the primer OPA 2. This allows one to conclude that this primer due to its sensitivity for detection and is the most effective of all the primers used since it captures the changes in the genetic material in a wide range of concentrations. The use of bigger number/combinations of primers in the study increases the chances of detecting mutations [28].

The research also proved that aluminum oxide leads to smaller changes in genetic material than aluminum oxide nanoparticles (Tables 4 and 5). Similar conclusions were obtained by Jośko and Oleszczuk. ZnO, TiO_2, and Ni nanoparticles showed a completely different toxicity from the same compounds in the traditional form in relation to plants. However, the research by Chrzanowska and Załęska-Radziwiłł demonstrated that the zirconium oxide and aluminum oxide have a much smaller impact on the bacteria *P. putida* and *Aeromonas hydrophila* in the planktonic form as well as in the biofilm form rather than the nanoparticle form of these compounds. The obtained data (and data from the subject-related literature) confirm that the nano form of a given substance may exhibit different properties and pose a much greater risk to the environment than the same substance in a large form [5,32,33].

The obtained results as well as data from literature confirm that RAPD-PCR has been successfully used to monitor the potential genotoxic effects of various nanoparticles [10–12,34]. Although RAPD has been shown to be a reliable, easy, quick, sensitive, and reproducible assay and it may be used to detect a wide range of DNA damage, it is recommended to optimize the reaction thoroughly (primer concentration, concentration of DNA template, polymerase and magnesium ions, and the parameters of the thermal cycler, or electrophoresis). RAPD-PCR is also a qualitative method. The impact of different kinds of DNA damage (e.g. strand breakage, modified bases, abasic sites, oxidized bases, and bulky adducts) on RAPD profiles can only be speculated unless amplicons are analyzed (e.g. sequencing). Therefore, some more specific methods are needed to obtain quantitative data [13,14,26,29,35].

5. Conclusions

The studies on the assessment of genotoxicity of aluminum oxide nanoparticles (nano-Al_2O_3) with respect to *P. putida* using RAPD-PCR allowed us to draw the following conclusions:

(1) The analysis of DNA bands indicated some potential mutagenic action of aluminum oxide nanoparticles;
(2) Of the four random primers used in research, the greatest changes in DNA were observed for the primer OPA 2. The lowest genetic similarity indexes (66.7%) with respect to the negative control were achieved at the highest concentration tested (1,000 mg/L) of the nanocompoud;
(3) These results were confirmed by the analysis of the genetic stability of DNA (GTS, %). The lowest mean value of GTS was obtained for the concentration of 1,000 mg/L;
(4) The genetic similarity indexes and DNA genetic stability (in the highest concentration tested) were similar to the results obtained for the positive control MMS. This allows us to judge that the tested compound generates a similar number of mutations as MMS;
(5) Genotoxicity of aluminum in its molecular form was much weaker than the compound in the nanoform;
(6) The RAPD method was successfully used in order to evaluate the damage to DNA of bacteria by the activity of aluminum oxide nanoparticles.

It has been one of the first studies related to the use of RAPD-PCR technique with respect to bacteria, and the influence of the aluminum oxide nanoparticles on the genetic material of micro-organisms. The results obtained and data from subject-related literature indicate that nanoparticles can induce modifications of the genetic material, which are quite often irreversible. This type of impact may be a threat not only to micro-organisms, but also to entire ecosystems. Hence, due to the extensive use of nanoparticles and their presence in the elements of the environment, there is a need for research at the molecular level on the effects of these compounds on organisms.

References

[1] S.K. Brar, V. Mausam, R.D. Tyagi, R.Y. Surampalli, Engineered nanoparticles in wastewater and wastewater sludge—Evidence and impacts, Waste Manage. 30 (2008) 504–520.
[2] G. Bystrzejewska-Piotrowska, J. Golimowski, P.L. Urban, Nanoparticles: Their potential toxicity, waste and environmental management, Waste Manage. 29 (2009) 2587–2595.
[3] M. Łebkowska, M. Załęska-Radziwiłł, Ecotoxicity of nanoparticles, Och. Śr. 33 (2011) 23–26.

[4] A. Gajewicz, B. Rasulev, T.C. Dinadayalane, P. Urbaszek, T. Puzyn, D. Leszczynska, J. Leszczynski, Advancing risk assessment of engineered nanomaterials: Application of computational approaches, Adv. Drug. Deliv. Rev. 64 (2012) 1663–1693.
[5] N. Chrzanowska, M. Załęska-Radziwiłł, The impacts of aluminum and zirconium nano-oxides on planktonic and biofilm bacteria, Desalin. Water Treat. 52 (2014) 3680–3689.
[6] S. Jomini, J. Labille, P. Bauda, C. Pagnout, Modifications of the bacterial reverse mutation test reveals mutagenicity of TiO_2 nanoparticles and byproducts from a sunscreen TiO_2-based nanocomposite, Toxicol. Lett. 215 (2012) 54–61.
[7] M. Ghosh, M. Bandyopadhyay, A. Mukherjee, Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: Plant and human lymphocytes, Chemosphere 81 (2010) 1253–1262.
[8] S.Y. Park, J. Choi, Geno and ecotoxicity evaluation of silver nanoparticles in freshwater crustacean *Daphnia magna*, Environ. Eng. Res. 15 (2010) 23–27.
[9] S. Asghari, S.A. Johari, J.H. Lee, Y.S. Kim, Y.B. Jeon, H.J. Choi, M.C. Moon, I.J. Yu, Toxicity of various silver nanoparticles compared to silver ions in *Daphnia magna*, J. Nanobiotechnol. 2 (2012) 10–14.
[10] W.R. Li, X.B. Xie, Q.S. Shi, S.S. Duan, Y.S. Ouyang, Y.B. Chen, Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*, BioMetals 24 (2011) 135–141.
[11] C. Ladhar, B. Geffroy, S. Cambier, Impact of dietary cadmium sulphide nanoparticles on Danio rerio zebrafish at very low contamination pressure, Nanotoxicology 8 (2014) 676–685.
[12] B. Geffroy, C. Ladhar, S. Cambier, M. Treguer-Delapierre, D. Brèthes, J.P. Bourdineaud, Impact of dietary gold nanoparticles in zebrafish at very low contamination pressure: The role of size, concentration and exposure time, Nanotoxicology 6 (2012) 144–160.
[13] W. Liu, Y.S. Yang, P.J. Li, Q.X. Zhou, L.J. Xie, Y.P. Han, Risk assessment of cadmium-contaminated soil on plant DNA damage using RAPD and physiological indices, J. Hazard. Mater. 161 (2009) 878–883.
[14] S. Cenkci, M. Yıldız, I.H. Ciğerci, A. Bozdağ, H. Terzi, E.S. Terzi, Evaluation of 2,4-D and Dicamba genotoxicity in bean seedlings using comet and RAPD assays, Ecotoxicol. Environ. Saf. 7 (2010) 1558–1564.
[15] C.W. Theodorakis, Integration of genotoxic and population genetic endpoints in biomonitoring and risk assessment, Ecotoxicology 10 (2001) 245–256.
[16] J.A. Hagger, F.A. Atienzar, A.N. Jha, Genotoxic, cytotoxic, developmental and survival effects of tritiated water in the early life stages of the marine mollusc, Mytilus edulis, Aquatic Toxicol. 74 (2005) 205–217.
[17] F.A. Atienzar, A.N. Jha, The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: A critical review, Mutat. Res. 613 (2006) 76–102.
[18] A. Mengoni, C. Gonnelli, F. Galardi, R. Gabbrielli, Genetic diversity and heavy metal tolerance in populations of Silene paradoxa L. (Caryophyllaceae): A random amplified polymorphic DNA analysis, Mol. Ecol. 9 (2000) 1319–1324.
[19] J.G. Coleman, D.R. Johnson, J.K. Stanley, A.J. Bednar, C.A. Weiss Jr., R.E. Boyd, J.A. Steevens, Assessing the fate and effects of nano aluminum oxide in the terrestrial earthworm, *Eisenia fetida*, Environ. Toxicol. Chem. 7 (2010) 1575–1580.
[20] A. Ruiz-Manzano, L. Yuste, F. Rojo, Levels and activity of the *Pseudomonas putida* global regulatory protein Crc vary according to growth conditions, J. Bacteriol. 187 (2005) 3678–3686.
[21] N.C.M. Gomes, I.A. Kosheleva, W.R. Abraham, K. Smalla, Effects of the inoculant strain Pseudomonas putida KT2442 (pNF142) and of naphthalene contamination on the soil bacterial community, FEMS Microbiol. Ecol. 54 (2005) 21–33.
[22] C. Conte, I. Mutti, P. Puglisi, DNA fingerprinting analysis by a PCR based method for monitoring the genotoxic effects of heavy metals pollution, Chemosphere 37 (1998) 2739–2749.
[23] M. Nei, W.H. Li, Mathematical model for studying genetic variation in terms of restriction endonucleases, Proc. Natl. Acad. Sci. USA 76 (1979) 5269–5273.
[24] F. Atienzar, M. Conradi, A. Evenden, A. Jha, M. Depledge, Qualitative assessment of genotoxicity using RAPD: Comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo (a) pyrene, Environ. Toxicol. Chem. 18 (1999) 2275–2282.
[25] C. Luceri, C. De Filippo, G. Caderni, L. Gambacciani, M. Salvadori, A. Giannini, P. Dolara, Detection of somatic DNA alterations in azoxymethane-induced F344 rat colon tumors by random amplified polymorphic DNA analysis, Carcinogenesis 21 (2000) 1753–1756.
[26] S. Cenkci, M. Yıldız, I.H. Cigˇerci, M. Konuk, A. Bozdag, Toxic chemicals-induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris* L.) seedlings, Chemosphere 76 (2009) 900–906.
[27] C.W.O.H. Veld, J. Jansen, M.Z. Zdzienicka, H. Vrieling, A.A. van- Zeeland, Methyl methane sulfonate-induced hprt mutation spectra in the Chinese hamster cell line CH09 and its xrcc1-deficient derivative EM-C11, Mutat. Res-Fundam. Mol. Mech. Mutagenes 398 (1998) 83–92.
[28] A. Zgórska, Application of bioindicative methods to assess the changes in waste water of genotoxicity under disinfection processes, Silesian University of Technology, Faculty of Energy and Environmental Engineering—Dissertation, Gliwice, 2013.
[29] W. Liu, P.J. Li, X.M. Qi, Q.X. Zhou, L. Zheng, T.H. Sun, Y.S. Yang, DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis, Chemosphere 61 (2005) 158–167.
[30] F.A. Atienzar, A.N. Jha, The random amplified polymorphic DNA (RAPD) assay to determine DNA alterations, repair and transgenerational effects in B(a)P exposed Daphnia magna, Mutat. Res. 552 (2004) 125–140.
[31] S.H.M. Qari, DNA-RAPD fingerprinting and cytogenetic screening of genotoxic and antigenotoxic effects of aqueous extracts of costus speciosus (Koen.), JKAU: Sci 22 (2010) 133–152.

[32] I. Jośko, P. Oleszczuk, Influence of soil type and environmental conditions on ZnO, TiO_2 and Ni nanoparticles phytotoxicity, Chemosphere 92 (2013) 91–99.
[33] T. Snopczyński, K. Góralczyk, K. Czaja, P. Struciński, A. Hernik, W. Korcz, J.K. Ludwicki, Nanotechnology, Opportunities and risks, Roczn. Pzh. 2 (2009) 101–111.
[34] A. Dedeh, A. Ciutat, M. Treguer-Delapierre, J.P. Bourdineaud, Impact of gold nanoparticles on zebrafish exposed to a spiked sediment, Nanotoxicology 21 (2014), doi: 10.3109/17435390.2014.889238.
[35] H.D. Wolf, R. Blust, T. Backeljau, The use of RAPD in ecotoxicology, Mutat. Res. 566 (2004) 249–262.