Effect of AL_2O_3 and TiO_2 nanoparticles on aquatic organisms

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Abstract. Environmental toxicity of aqueous disperse systems of nanoparticles of binary compounds of titanium dioxides (with particle size Δ_{50}=5 nm, Δ_{50}=50 nm, Δ_{50}=90 nm), aluminum oxide alpha-forms (Δ_{50}=7 nm and Δ_{50}=70 nm) and macro forms (TiO_2 Δ_{50}=350 nm, Al_2O_3 Δ_{50}=4000 nm) were studied using biological testing methods. The bioassay was performed using a set of test organisms representing the major trophic levels. We found the dependence of the toxic effect concentration degree of $nTiO_2$ and nAl_2O_3 on the fluorescence of the bacterial biosensor "Ekolyum", the chemotactic response of ciliates *Paramecium caudatum*, the growth of unicellular algae *Chlorella vulgaris Beijer* and mortality of entomostracans *Daphnia magna Straus*. We revealed the selective dependence of $nTiO_2$ and nAl_2O_3 toxicity on the size, concentration and chemical nature of nanoparticles. The minimal concentration causing an organism's response on $nTiO_2$ and nAl_2O_3 effect depends on the type of the test-organism and the test reaction under study. We specified L(E)C_{50} and acute toxicity categories for all the studied nanoparticles. We determined that $nTiO_2$ (Δ_{50}=5 nm) belong to the category «Acute toxicity 1», $nTiO_2$ (Δ_{50}=90 nm) and nAl_2O_3 (Δ_{50}=70 nm) – to the category «Acute toxicity 2», nAl_2O_3 (Δ_{50}=7 nm) - to the category «Acute toxicity 3». No acute toxicity was registered for $nTiO_2$ (Δ_{50}=50 nm) and macro form TiO_2.

1. Introduction

Development and implementation of nanotechnologies in many industries increases the probability of harmful effects of nanoparticles (NPs) on the environment. Wide usage of NPs resulted in a great attention paid lately to NPs of metal oxides; titanium dioxide ($nTiO_2$) and aluminum oxide (nAl_2O_3) that are very popular among them.

$nTiO_2$ application is determined by its antibacterial and photocatalytic properties in the first place [1, 2]. nAl_2O_3 has a number of properties – thermal stability, high hardness, resistance and chemical inertness, which makes it possible to use it in various industries. The most wide-spread modification is α-Al_2O_3 used in optics and laser technologies [3].

It is common knowledge that NP size substances increase in their response ability, which is potentially hazardous for the environment. As there are various ways of polluting water bodies with nanoindustry products, it is necessary to assess NPs toxicity for water ecosystems. The requirement documents in Russia and abroad require this property of NPs to be determined.

The published data concerning the biological effects of $nTiO_2$ and nAl_2O_3 on aqueous environment are deficient, segmentary and discrepant. This is caused not only by the lack of necessary information about physical and chemical properties of the tested NPs, the method of their synthesis and experiment conditions, but also by the absence of a unified analytical system providing valid ecotoxicological assessment of the hazard the NPs cause to natural objects.

As a result of the programs and research grants implemented earlier (2008-2013) we developed a methodological approach to assessing ecological toxicity that helps to study test-reactions of a wide range of organisms with one algorithm.

2. Materials and methods

We tested $nTiO_2$ with mean particle size Δ_{50}=50 nm, Δ_{50}=90 nm, nAl_2O_3 (α-form) with particle size Δ_{50}=70 nm and macro forms TiO_2 Δ_{50}=350 nm and Al_2O_3 Δ_{50}=4000 nm obtained with the help of the pneumo circuital method («Mipor» Ltd, RF). The disperse systems $nTiO_2$ Δ_{50}=5 nm and nAl_2O_3 Δ_{50}=7 nm were obtained with the help of the method of laser ablation in distilled water, the initial concentration 50 mg/l [4].

Preparation of DS $nTiO_2$ and nAl_2O_3 on growth medium (GM) with the concentrations 0.000001, 0.00001, 0.0001, 0.001, 0.01, 0.1 and 1.0 mg/l was done in accordance with the technique [5] with further ultrasonic material dispersion (30 W/l, 5 minutes) just before biological testing**.**

The toxicity of $nTiO_2$ and nAl_2O_3 was studied by the change in luminescence of bacterial biosensor «Ekolyum» [6], the chemotactic response of ciliates *Paramecium caudatum* [6], the growth of unicellular algae *Chlorella vulgaris Beijer* [7] and mortality of entomostracans *Daphnia magna Straus* [8]. The assessment of toxicity of the analysed DS $nTiO_2$ and nAl_2O_3 with various particle sizes was done in accordance with the criteria given in certified methods' outlines [7, 8]. The research design is similar to the described in [9, 10].

The properties of $nTiO_2$ and nAl_2O_3 were verified with the help of transmission electronic microscop «Philips CM-30» (France), using the method of dynamic light scattering (DLS) with the help of the disperse particles sizes analyser «Zetasizer Nano ZS» (USA), the method of BET («TriStar 3000», USA) to measure specific surface and the method of X-ray structure analysis of the phase composition and structural characteristics (X-ray diffractometer Shimadzu XRD-6000, Japan). The research was conducted with the application of metrologically gauged instruments: Autolumat LB953 (Germany), spectrofluorometer «Shimadzu» RF-5301 PC (Japan), photoelectric colorimeter DMS-03 (Russia), concentration meter BIOTESTER-2 (Russia). The statistical processing of the obtained data was done with probit analysis.

3. Results

The results of our investigation showed that the dynamics of the fluorescence of bacterial biosensor «Ekolyum» in GM with $nTiO_2$ concentration did not depend on the size and concentration of NPs. The toxicity of particles of $nTiO_2$ (Δ_{50}=5 nm, Δ_{50}=50 nm and Δ_{50}=350 nm) practically for the whole tested concentration range (C) changed insignificantly (figure 1). The exception was the tested DS $nTiO_2$ Δ_{50}=5 nm with C=0.1 mg/l, the toxicity of which was above "permissible level" and, in accordance with the criteria of a standard method, was defined as a sample with "moderate" degree of toxicity (I=27.5±0.5 %). In DS TiO_2 (Δ_{50}=350 nm) we noted "suppression" of bio fluorescence within the interval of the tested concentrations. The fluorescence of the biosensor decreased with the increase in DS TiO_2 concentration, but the toxicity index value was above the "permissible level" by 1.0-2.0 % only.

Similar effects were registered during introducing in GM NPs Al_2O_3 Δ_{50}=7 nm and Δ_{50}=70 nm: no sufficient changes in the fluorescence of bacterial biosensor «Ekolyum» (figure 2) were found. In contrast to $nTiO_2$ Δ_{50}=5 nm the «moderate» degree of toxicity (I=23.5±8.5 %) was registered for DS with lower concentration of nAl_2O_3 Δ_{50}=70 nm (C=0.001 mg/l).

The macro form Al_2O_3 Δ_{50}=4000 nm unlike the nanoform Al_2O_3 of all the analysed concentrations of DS quenched the fluorescence of the biosensor. Within the interval C from 0.0001 mg/l to 1.0 mg/l the toxicity index exceeded the «permissible» level and changed within the range from I=20.5±0.5 % to I=27.9±0.4 %, which is defined as the sample with the «moderate» toxicity.

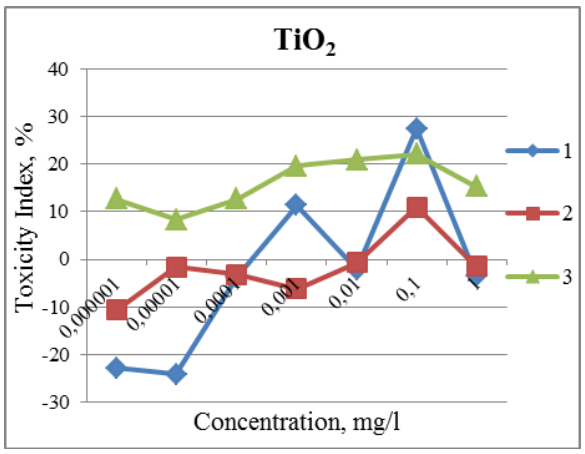

Figure 1. Toxicity index of DS TiO_2 for bacterial biosensor «Ekolyum»: 1 - Δ_{50} = 5 nm, 2 - Δ_{50} = 50 nm, 3 - Δ_{50} = 350 nm

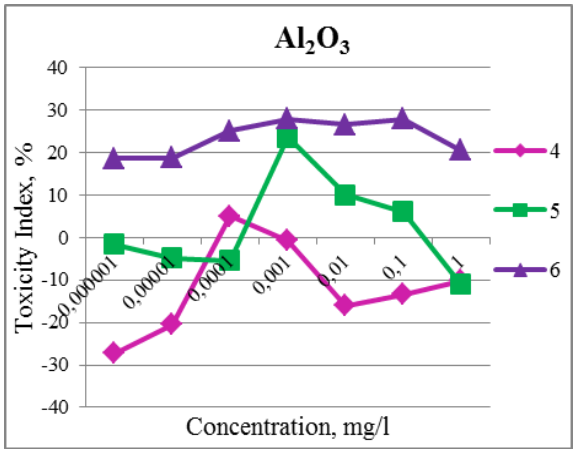

Figure 2. Toxicity index of DS Al_2O_3 for bacterial biosensor «Ekolyum»: 4 - Δ_{50} =7 nm, 5 - Δ_{50} = 70 nm, 6 - Δ_{50} = 4000 nm

On exposing *Paramecium caudatum* to DS $nTiO_2$ Δ_{50}=5 nm with C=0.000001 mg/l and C=0.00001 mg/l the toxicity index value corresponded to control values (figure 3). With the increase in the concentration of $nTiO_2$ Δ_{50}=5 nm in GM the percentage of ciliates outflow in DS was not monotonic, but decreased definitely, which demonstrated the NPs toxic effect. In DS with the concentrations 0.01, 0.1 and 1.0 mg/l the toxicity index changed within the range from 38.8±6.2 % to 69.9±16.8 % and according to the standard method [6] corresponded to the «moderate» degree of toxicity (0.40<I<0.70).

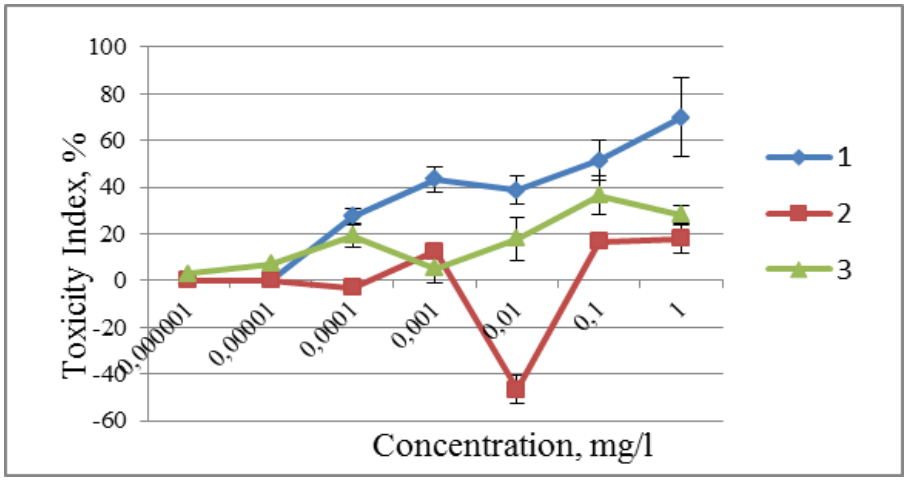

Figure 3. Toxicity index of DS $nTiO_2$ and nAl_2O_3 for *Paramecium caudatum:* 1 - $nTiO_2$ Δ_{50} = 5 nm, 2 - $nTiO_2$ Δ_{50} = 50 nm, 3 - nAl_2O_3 Δ_{50} = 70 nm

We registered the dependence of the chemotactic response of *Paramecium caudatum* on the particle size of TiO_2 in GM. The introduction of particles TiO_2 Δ_{50}=50 nm did not caused toxic impact on *Paramecium caudatum.* We found DS $nTiO_2$ Δ_{50}=50 nm with C=0.0001 mg/l and C=0.01 mg/l, in which the ciliates outflow exceeded the control values (figure 3). The highest stimulating effect was registered with C=0.01 mg/l (I= -46.6±9.4 %). We stated that the introduction of nAl_2O_3 Δ_{50}=70 nm to GM within the concentration range from 0.000001 to 1.0 mg/l resulted in the change in the

chemotactic response of *Paramecium caudatum* in the range of «permissible» values. The maximal toxicity index was registered with C=0.1 mg/l I=36.4 ±1.1 % (figure 3).

Our investigations resulted in revealing the dependence of $nTiO_2$ effect on the particle size. The particles $nTiO_2$ Δ_{50}=5 nm and Δ_{50}=90 nm inhibited the growth of *Chlorella vulgaris Beijer*, with $nTiO_2$ Δ_{50}=5 nm in contrast to $nTiO_2$ Δ_{50}=90 nm producing a pronounced toxic effect (figure 4).

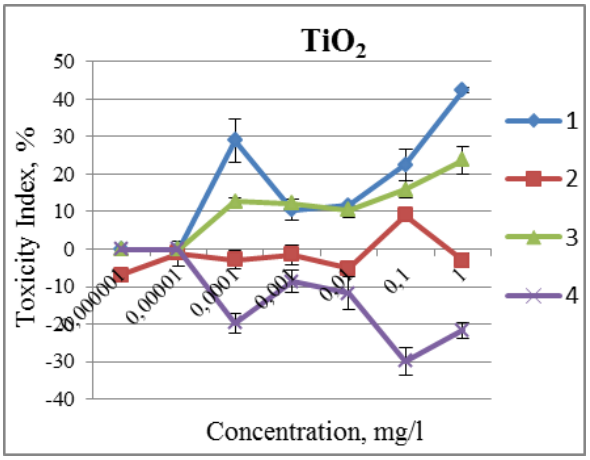

Figure 4. Toxicity index of DS TiO_2 for *Chlorella vulgaris Beijer:* 1 - Δ_{50}=5 nm, 2 - Δ_{50} = 50 nm, 3 - Δ_{50} = 90 nm, 4 - Δ_{50} =350 nm

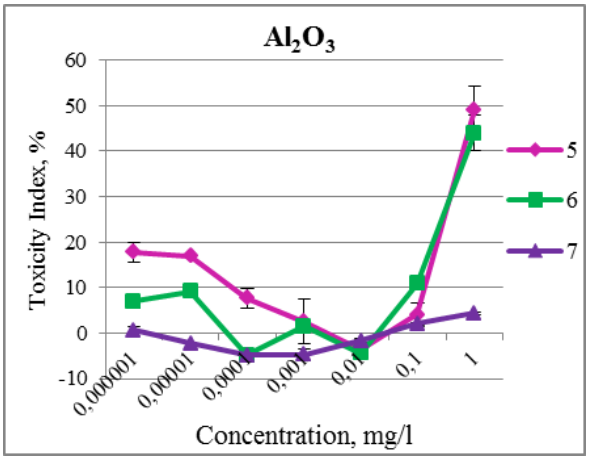

Figure 5. Toxicity index of DS Al_2O_3 for *Chlorella vulgaris Beijer:* 5 - Δ_{50}=7 nm, 6 - Δ_{50}=70 nm, 7 - Δ_{50}=4000 nm

The introduction into GM of the algae DS $nTiO_2$ Δ_{50}=50 nm caused insignificant stimulation of *Chlorella vulgaris Beijer* cells' growth. We registered a non-monotonic decrease in the growth of alga cells while cultivating *Chlorella vulgaris Beijer* in the analysed DS $nTiO_2$ Δ_{50}=5 nm. The toxic effect of $nTiO_2$ Δ_{50}=5 nm showed itself with C=0.0001 mg/l (I=29.0±5.9 %) already. With further increase in the concentration the toxicity of DS decreased (I=10.4±2.8 % with C=0.001 mg/l and I=11.7±0.5 % with C=0.01 mg/l), and then increased again to gain the maximal effect with C=1.0 mg/l. The growth of *Chlorella vulgaris Beijer* decreased by 33.8 % compared to the control values. The introduction into GM of the particles TiO_2 Δ_{50}=350 nm in macro form had a stimulating effect on *Chlorella vulgaris Beijer* cells' growth in concentration dependence. The maximal response was registered during introducing particles into C=0.1 mg/l. The toxicity of DS TiO_2 Δ_{50}=350 nm was close to significant manifestation.

In contrast to $nTiO_2$ the introduction of nAl_2O_3 into GM did not cause the expected changes in the growth of *Chlorella vulgaris Beijer* depending on the particle size. The exposure of *Chlorella vulgaris Beijer* to DS nAl_2O_3 Δ_{50}=7 nm and Δ_{50}=70 nm within the range C=0.000001 mg/l – 0.1 mg/l caused the inhibition of alga cells' growth вызывала ингибирование роста клеток водоросли within tolerance (figure 5). A pronounced toxic effect was observed only with C=1.0 mg/l: *Chlorella vulgaris Beijer* mass growth decreased compared to the control values by 49.1±5.9 % for DS nAl_2O_3 Δ_{50}=7 nm and 44.0±3.9 % for DS nAl_2O_3 Δ_{50}=70 nm. The macro form Al_2O_3 Δ_{50}=4000 nm did not have a toxic effect on *Chlorella vulgaris Beijer,* the toxicity index changing in range of control values.

The researches in the test-organism of *Daphnia magna Straus* in contrast to biosensor «Ekolyum», *Chlorella vulgaris Beijer* and *Paramecium caudatum* did not reveal toxic effects of $nTiO_2$ and nAl_2O_3. The exposure of *Daphnia magna Straus* in DS with particles TiO_2 (Δ_{50}=5 nm, Δ_{50}=50 nm and Δ_{50}=350 nm) and into DS with particles Al_2O_3 (Δ_{50}=7 nm, Δ_{50}=70 nm and Δ_{50}=4000 nm) did not cause the mortality of more than 10 % of the entomostracans for 48 hours, which indicated the absence of the toxic effect for all the tested concentrations DS TiO_2 and Al_2O_3 (figure 1, 2, 3).

On the basis of the data of the eco toxicological analysis obtained with the help of test-systems with the basic set of test-organisms we did the integral assessment of the toxicity of TiO_2 and Al_2O_3 with various dimension characteristics (table 1).

According to GHS, while making the conclusion on the ecotoxicity of the tested samples of NPs we took into account the principle of assessment by the lowest index available $L(E)C_{50}$ [11].

Table 1. Values of N-dimensional $L(E)C_{50}$ TiO_2 and Al_2O_3

$L(E)C_{50}$, (mg/l) / Test-organism	TiO_2				nAl_2O_3		
	NPs			macro form	NPs		macro form
	5 nm	50 nm	90 nm	350 nm	7 nm	70 nm	4000 nm
«Ekolyum»	>100	>100	-	47.6	>100	>100	>100
Paramecium caudatum	0.0256	>100	>100	>100	-	1.22	-
Chlorella vulgaris Beijer	1.153	>100	7.7	>100	30.8	15.15	-
Daphnia magna Straus	>100	>100	>100	>100	>100	>100	>100
Conclusion	Acute toxic class 1	No acute toxicity	Acute toxic class 2	Acute toxic class 3	Acute toxic class 3	Acute toxic class 2	No acute toxicity

Consequently, the research results showed that the toxicity of DS $nTiO_2$ with dimension Δ_{50}=5 nm were sufficiently higher than the toxicity of DS $nTiO_2$ with particle size Δ_{50}=50 nm, Δ_{50}=90 nm. A different dependence is observed for nAl_2O_3. Per totality of the test-parameters under study nAl_2O_3 Δ_{50}=70 nm are most toxic. nAl_2O_3 with dimension Δ_{50}=7 nm are less toxic. The comparison of the toxicity of $nTiO_2$ and nAl_2O_3 with close dimensional characteristics indicated that the toxicity of $nTiO_2$ (Δ_{50}=5 nm) is much higher than the toxicity of nAl_2O_3 Δ_{50}=7 nm. At the same time, nAl_2O_3 (Δ_{50}=70 nm) have a more pronounced toxic effect compared to $nTiO_2$ (Δ_{50}=50 nm) correspond to $nTiO_2$ (Δ_{50}=90 nm) by toxicity category. The macro form TiO_2 per totality of the test-responses under study belongs to the category «Acute toxicity 3», and the macro form Al_2O_3 is «non-hazardous substance».

4. Discussion

The absence of standardized toxicological methods of assessing the hazard NPs can cause to living objects of nature together with little information in published papers about the size, crystal structure, morphology of NPs, methods of their synthesis, experiment conditions, methods of preparation of DS NPs makes it difficult to compare the obtained results with the literature data.

In the literature available the data on the influence of $nTiO_2$ and nAl_2O_3 on the test-parameters of aquatic organisms are few. Most researches in the recent years have been devoted to the genotoxic and cytotoxic effect of $nTiO_2$ and nAl_2O_3 as well as the cancerogenic effect of $nTiO_2$ [1, 2, 3, 12, 13, 14].

The data we obtained are in accord with the data of Liu et. al. [15], who studied the effect of $nTiO_2$ (Δ_{50}<20 nm and Δ_{50}=30 nm) on the survival rate of *Daphnia magna Straus* and stated that $nTiO_2$ with particle size Δ_{50}<20 nm induced a higher toxicity compared to $nTiO_2$ Δ_{50}=30 nm, which contradicted the data of L. Adams et. al. [16] and J. Verran [17], who showed in their studies that the particle size of $nTiO_2$ does not influence its toxicity. The inconsistency of the data on the dependence of $nTiO_2$ toxicity on the particle size may be caused by the authors' employing different methods of assessing the toxicity of NPs. For example, Liu et. al. are known to have studied the survival rate of *Daphnia*

magna Straus in a long-term experiment. The research in a long-term toxicity of $nTiO_2$ for 21 days displayed the disorder of fertility and the increase in the mortality of entomostracans up to 70 % [18, 19]. The analysis of toxicity of nAl_2O_3 with dimensional characteristics Δ_{50}=40-100 nm on immobilisation and further death of *Ceriodaphnia dubia* displayed a clear temporal dose-dependent trend to reveal a toxic effect taking into account aggregation and bioavailability reduction of NPs [20]. One of the basic mechanisms of the toxic effect of $nTiO_2$ and nAl_2O_3, in the authors' view, is an oxidative stress that arises in the condition of NPs impact on aquatic organisms.

According to the literature, the absence of toxic effect of $nTiO_2$ with particle size Δ_{50}<50 nm was found for luminescent bacteria *Vibrio fischeri* [21], which is in accord with the data we obtained.

V. Aruoja et.al. [22] studied the influence of $nTiO_2$ on the freshwater alga *Pseudokirchneriella subcapitata.* It was stated that the macro form TiO_2 ($L(E)C_{50}$=35.9 mg/l) was less toxic than $nTiO_2$ ($L(E)C_{50}$=5.83 mg/l). I.M. Sadiq et.al. [23] revealed the inhibitory effect of nAl_2O_3 with particle size Δ_{50}<50 nm on the growth of microalgae *Scenedesmus sp.* and *Chlorella sp*. The value of $L(E)C_{50}$ for 72 hours of incubation was 45.4 mg/l for *Chlorella sp*. and 39.4 mg/l for *Scenedesmus sp.* The macro form Al_2O_3 (Δ_{50}<5 mkm) also displayed toxicity, though to less extent ($L(E)C_{50}$= 110.2 mg/l for *Chlorella sp*. and $L(E)C_{50}$=100.4 mg/l for *Scenedesmus sp.*). The researchers revealed selective sensitivity of various algae species to nAl_2O_3. The authors suppose that $nTiO_2$ and nAl_2O_3 form specific aggregates settling on the surface of algae cells, which causes toxic effect.

We were the first to show the influence of $nTiO_2$ and nAl_2O_3 with various size characteristics on the chemotactic response of ciliates *Paramecium caudatum*.

5. Conclusion

Eco toxicological analysis showed that $nTiO_2$ Δ_{50}=5 nm belong to the category « Acute toxicity 1», $nTiO_2$ Δ_{50}=90 nm and nAl_2O_3 Δ_{50}=70 nm - to the category «Acute toxicity 2», macro form TiO_2 Δ_{50}=350 nm and nAl_2O_3 Δ_{50}=7 nm - to the category «Acute toxicity 3». The absence of acute toxicity was found for $nTiO_2$ Δ_{50}=50 nm and the macro form Al_2O_3 Δ_{50}=4000 nm.

In most test-systems with both $nTiO_2$, and nAl_2O_3 having various size characteristics there is no monotonic dependence between the concentration of DS and the level of the test-response change, the general trend being the increase in the test-response with the concentration increased. The degree of hazard of DS containing $nTiO_2$ and nAl_2O_3 is determined by the size of NPs, their chemical nature and concentration in DS.

The toxicity of $nTiO_2$ and nAl_2O_3 is different for various test-organisms. Most sensible to the presence of $nTiO_2$ Δ_{50}=5 nm and nAl_2O_3 Δ_{50}=70 nm in the environment are the ciliates *Paramecium caudatum*, of $nTiO_2$ Δ_{50}=90 nm and nAl_2O_3 Δ_{50}=7 nm – the unicellular algae *Chlorella vulgaris Beijer*, of macro form TiO_2 Δ_{50}=350 nm – bacterial sensor «Ekolyum».

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