Evaluation of the toxicity of superfine materials to change the physiological functions of aquatic organisms of different trophic levels

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Abstract. We assessed ecological and biological effects caused by the physical and chemical properties of nanomaterials on the basis of the laboratory researches into water test-organisms of different trophic levels. We studied the physiological functions of water organisms on adding into the environment superfine materials of various chemical nature and structural characteristics: metallic nanoparticles of nikel (nNi), argentum (nAg), platinum (nPt), aurum (nAu), binary NPs (powder of titanium dioxide - $nTiO_2$, aluminum oxide - nAl_2O_3, zink oxide - nZnO, silicon nitride - nSi_3N_4, silicon carbide (nSiC) and carbon nanotubes (BT-50, MCD-material). We observed the dependence of developing the complex of unfavourable biological effects in water plants and entomostracans' organisms on the physical and chemical properties of superfine materials. We determined the values of NOEC, $L(E)C_{20}$ and $L(E)C_{50}$ for aquatic organisms of various regular groups. We found out the most vulnerable elements of the communities' trophic structure and the possibility of a breakdown in the water ecosystem food pyramid.

1. Introduction

In the last two decades nanotechnology development has resulted in a rapid increase in the diversity of engineered nanoparticles (NPs) and nanomaterials (NMs), many of which differ from natural NPs radically in their physical and chemical characteristics including element composition, higher dispersion, inner structure and complicated spatial organization.

Unlike natural NPs engineered nanoparticles that are used increasingly in various life activity spheres may be potentially hazardous for the environment. Despite a growing number of publications on ecotoxicology and ecological effects of a direct or indirect NPs' impact on a living organism, including water organisms, the issue of NPs bio toxicity still remains urgent and is connected with studying both the mechanism of their toxic effect development and their circulation in nature. In connection with this the necessity of complex research is being discussed; such research will make it possible to assess not only biological activity of various NMs, ways of distribution and building NPs into food chains, but also long-term effects of their influence on the ecosystem as a whole.

In the last decade researchers have paid a lot of attention to the studies on NPs ecotoxicity. However, there are still no guidelines for a quantitative assessment of the effects of superfine materials (SFM), and the issue of applying the methodologies at hand to a new class of substances remains open. In connection with this studying SFM influence on the functions of bio-objects, in particular the physiological functions of water organisms, is of greater interest rather than studying SFM biocidal

effects. The most sensitive and appropriate indicators of assessing SFM toxicity are, undoubtedly, the methods of bio-testing that imply creating test-system models (laboratory and field) of NPs' impact on the specially chosen test organisms and the assessment of eco toxicity based on changes in the informative test-parameters. The behaviour, condition and activity of various systematic groups allow stating the toxicity of the environment regardless of the fact what are the substances and what is their combination that cause it.

The literature data at hand concerning the influence of NPs of metals and their oxides on the growth and development of water organisms are limited, controversial and difficult to be compared by dose, NPs size and the species of water plants and animals [1, 2, 3, 4, 5, 6, 7, 8]. The issues of the physiological functions and behavior of organisms in the water ecosystems contaminated with SFM need investigating.

The paper is aimed at revealing the peculiarities of various SFM influence on the physiological functions of water organisms at different trophic levels through experimental modelling.

2. Materials and methods

We studied the functional properties of different NPs: argentum (nAg, with particle size Δ_{50}=2-5 nm and specific surface S_{sp} =60-80 m^2/g), aurum (nAu, Δ_{50}=2-5 nm, S_{sp} = 40 m^2/g), nikel (nNi, Δ_{50}=3-5 nm, S_{sp} = 80-100 m^2/g), platinum (nPt, Δ_{50}=3-5 nm, S_{sp}= 36 m^2/g), zink oxide (nZnO, Δ_{50}=12 nm, S_{sp} = 47 m^2/g), aluminium oxide (α-form) (α-nAl_2O_3, Δ_{50}=7nm and 70 nm, S_{sp} =112-122 m^2/g and 20-25 m^2/g), aluminium oxide (elecrtocorundum) (nAl_2O_3-corundum, Δ_{50}=70 nm, S_{sp} = 45 m^2/g), titanium dioxide (nTiO_2, Δ_{50}=5 nm, 50 nm and 90 nm, S_{sp} = 119 m^2/g, 35 m^2/g and 6 m^2/g), silicon carbide (nSiC, Δ_{50}=95 nm, S_{sp} = 15,7 m^2/g), silicon nitride (nSi_3N_4, Δ_{50}=80 nm, S_{sp}= 11,5 m^2/g) and carbon nanotubes (BT-50 diameter of 4 nm, «Baytubes®»), and MCD-material, diameter of 4 nm, «Sigma Gr.», RF). We used the tested substances in macro form as a control. Nanoparticles with sizes less than 15 nm were prepared by a procedure [9], nTiO_2, Δ_{50}=50 nm obtained by electric explosion («SibTermoChim» Ltd, RF), larger NPs made with the help of the pneumo circuital method («Mipor» Ltd, RF).

SFM influence on the physiological functions was assessed by the change in the test-responces characteristic of water organisms: the chemotactic response of ciliates *Paramecium caudatum* [10], the growth of unicellular algae *Chlorella vulgaris Beijer* [11], the indicators of the trophic activity of entomostracans *Daphnia magna Straus* [10]. We studied SFM effect on the mortality of *Daphnia magna Straus* [12].

The characteristics of the tested SFM were verified by TEM («Phillips CM-12», France), by method of dynamic light scattering with the help of the disperse particles' sizes analyser «Zetasizer Nano ZS» (USA), by method of BET («TriStar 3000», USA) to measure S_{sp}, and the method of X-ray structure analysis of the phase composition and structural characteristics (X-ray diffractometer «Shimadzu XRD-6000», Japan). The disperse systems (DS) SFM were made in accordance with our methodology [13] including the stages of ultrasonic dispersing (30 W/l) during 5 minutes. The toxicity of DS SFM samples were studied in the concentration C=1.0 mg/l corresponding their possible stable concentration in natural environment, which was grounded experimentally in our previous works [13]. The toxicity of the analysed DS SFM was assessed in accordance with the criteria of the certified methods [10, 11, 12].

3. Results and discussion

One of the most important factors that determine organisms' test-responses is the concentration (C) of the substance added to the test-system for analysis, which should correlate with the value of the concentration possible in the natural environment. The previous researches showed that in creating DS SFM test-system the increase in the concentration compared to the initial one (C>5 mg/l) results in the increase in the aggregation speed (V_{ag}) of NPs and the sufficient decrease in their final C [13, 14]. «Self-purification» of the hydrosphere from SFM due to forming conglomerates accompanied by

sedimentation and elimination from water environment solves the problem of assessing effects of large C of NPs (except for short-term effects of emergency discharges).

We stated that in the in-situ conditions SFM concentration in a created DS becomes stable within the interval of several milligrams per a litre in two days after the addition of SFM to water environment. We indicated the following regulations:

– the increase in the initial C results in the increase in V_{ag} of NPs in DS SFM;

– the most rapid growth of V_{ag} of NPs occurs with C >5 mg/l, the ratio "C – Stability of DS SFM " is optimal within the range 1-5 mg/l;

– V_{ag} of NPs grows in the row «Distilled water», «medium of Lozino-Lozinsky» - growth medium (GM) of ciliates, «Drinking water» - GM of fish and daphnids, 0.9 % NaCl and 3.0 % NaCl – GM of luminescent bacteria [13].

Our researches showed that test-organisms' response on SFM in C=1.0 mg/l is ambiguous. The nature of changes in the physiological functions and behaviour depends on the physical and chemical properties of SFM, test-organism type and the test-functions under study. The addition of SFM into GM in most cases did not cause the death of test-organisms but caused serious failures of their physiological functions and behaviour: the negative chemotaxis of ciliates *Paramecium caudatum*, changing mortality/survival in *Daphnia magna Straus,* the failure of the trophic activity of entomostracans *Daphnia magna Straus* and the change in the growth speed of the cellular weight of *Chlorella vulgaris Beijer*. We stated that some SFM kinds have the inhibiting effect on the growth speed the alga *Chlorella vulgaris Beijer*, partially or fully inhibiting its growth (figure 1).

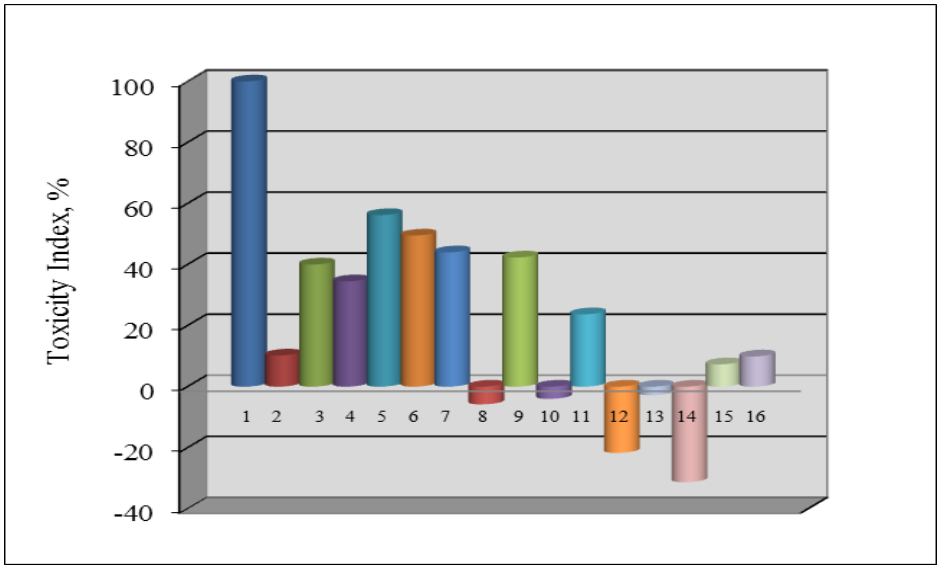

Figure 1. The toxicity index of DS SFM and macro form of the substances with various chemical composition and dispersion rate determined in accordance with the change in the weight growth of unicellular algae *Chlorella vulgaris Beijer* (C=1.0 mg/l): 1 - nAg, Δ_{50} = 2-5 nm; 2 - nPt, Δ_{50} = 3-5 nm; 3 - nAu, Δ_{50} = 2-5 nm; *4 - nNi, Δ_{50} = 3-5 nm; 5 - nZnO, Δ_{50} = 12 nm; 6 - α-nAl_2O_3, Δ_{50} = 7 nm; 7 - α-nAl_2O_3,* Δ_{50} = 70 nm; 8 - nAl_2O_3- corundum, Δ_{50} = 70 nm; 9 - nTiO_2, Δ_{50} = 5 nm; 10 - nTiO_2, Δ_{50} = 50 nm; 11 - nTiO_2, Δ_{50} = 90 nm; 12 - TiO_2, Δ_{50} = 10 mkm; 13 - nSi_3N_4, Δ_{50} = 80 nm; 14 - nSiC, Δ_{50} = 95 nm; 15 - BT-50; 16 - MCD- material

For example, nAg inhibits algae growth completely (100 %), while nAu, nNi, nZnO and α-nAl_2O_3, – only partially: nAu - by 40.0 %, nNi - by 34.5 %, nZnO - by 56.2 %, α-Al_2O_3 - by 49.2 %

(Δ_{50}=7 nm) and 43.9 % (Δ_{50}=70 nm), nTiO_2 (Δ_{50}=5 nm) – by 42.3 % and nTiO_2 (Δ_{50}=90 nm) – by 23.7 %. Some SFM kinds such as nSiC, (Δ_{50}=95 nm) stimulate the unicellular algae growth (by 31.3 %). We found the group of SFM that hardly ever change the chlorella growth speed, for example NPs nPt, nAl_2O_3-corundum (Δ_{50}=70 nm), nTiO_2 (Δ_{50}=50 nm), Si_3N_4 (Δ_{50}=80 nm) and carbon nanotubes (BT-50 and MCD-material).

The substances in macro form (Δ_{50}>100 nm, C=1.0 mg/l) do not have a sufficient influence on the chlorella growth speed, i.e. they are biologically inert in relation to unicellular algae.

The addition of SFM into GM of ciliates *Paramecium caudatum* (C=1.0 mg/l) causes changes in the chemotactic response in 7 out of12 testes DS SFM. Only 4 types of DS SFM has a sufficient effect on the chemotaxis of ciliates: nNi (Δ_{50}=3-5 nm) and nAg (Δ_{50}=2-5 nm) completely (100 %) inhibited ciliates' release into the environment with the above-mentioned NPs, nZnO (Δ_{50}=12 nm) – by 85.1 %, nTiO_2 (Δ_{50}=5 nm) - by 51.3 % (figure 2). A less sufficient inhibition of the chemotactic response was observed after the addition of NPs nAl_2O_3 (Δ_{50}=70 nm), nTiO_2 (Δ_{50}=50 nm) and MCD-material into GM. The indicated NPs inhibit ciliates' release by 28.0 %, 18.0 % and 27.5 % correspondingly. The macro form of the substances under study does not cause any changes in the chemotactic response of *Paramecium caudatum.*

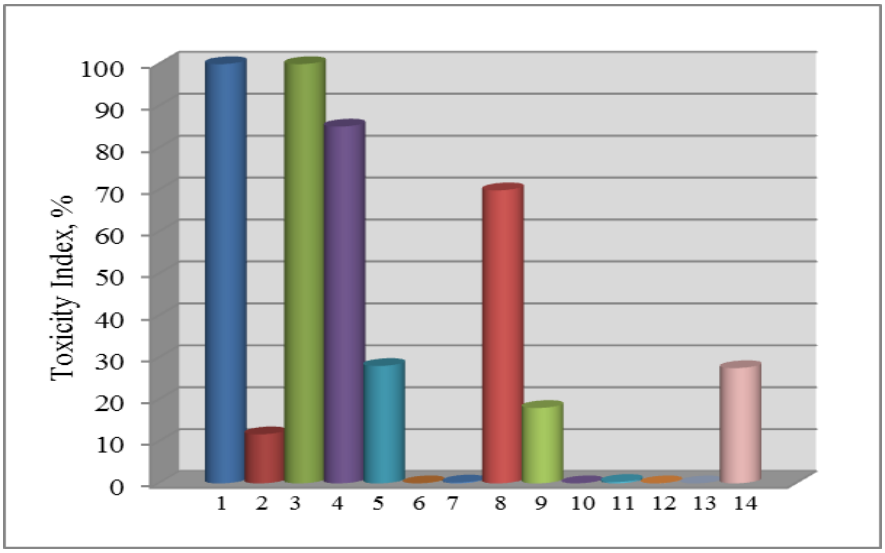

Figure 2. The toxicity index of nanomaterials and macro form of the substances with various chemical composition and dispersion rate determined in accordance with the change in the chemotactic response of ciliates *Paramecium caudatum* (C=1.0 mg/l): 1 - nAg, Δ_{50}=2-5 nm; 2 - nPt, Δ_{50}=3-5 nm; 3 - nNi, Δ_{50}=3-5 nm; 4 - nZnO, Δ_{50}=12 nm; 5 - α-Al_2O_3, Δ_{50}=70 nm; 6 - α-Al_2O_3, Δ_{50}=4 mkm; 7 - nAl_2O_3- corundum, Δ_{50}=70 nm; 8 - nTiO_2, Δ_{50}=5 nm; 9 - nTiO_2, Δ_{50}=50 nm; 10 - TiO_2, Δ_{50}=10 mkm; 11 - nSi_3N_4, Δ_{50}=80 nm; 12 - nSiC, Δ_{50}=95 nm; 13 - BT-50; 14 - MCD-material

The addition of SFM into the composition of GM of entomostracans results in the mortality of *Daphnia magna Straus* only in 2 out of 14 tested DS (figure 3): in DS nAg (Δ_{50}=2-5 nm) and nZnO (Δ_{50}=12 nm) (100 % and 40 % correspondingly).

A number of researches have shown that it is impossible to correctly assess the medium quality with the help of the principle, widely used in ecotoxicology, that takes into account the characteristics of 2 test-organisms, especially in the cases when one of the criteria is the mortality of entomostracans *Daphnia magna Straus* [10, 12, 15].

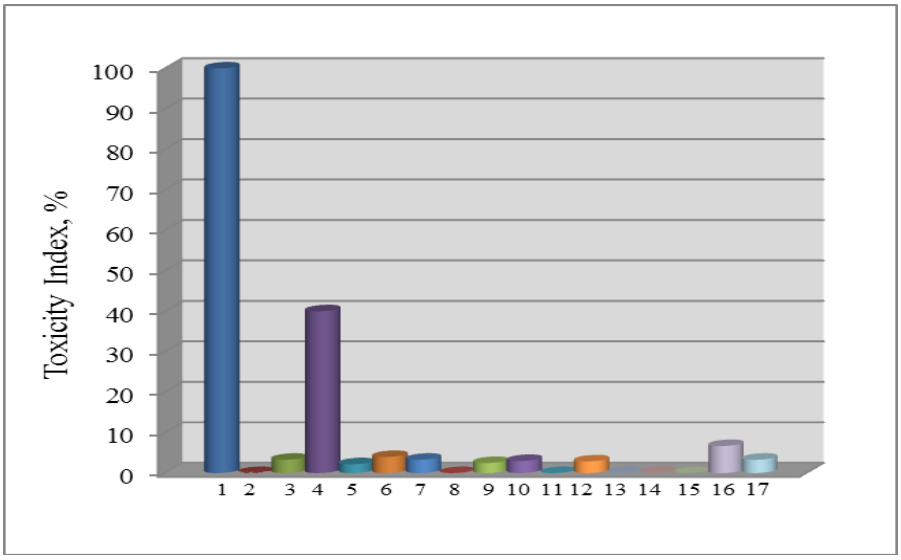

Figure 3. The toxicity index of NMs with various chemical composition and dispersion rate determined in accordance with the indicator of mortality of entomostracans *Daphnia Magna Straus* (C=1.0 mg/l): 1 - nAg, Δ_{50}=2-5 nm; 2 - nPt, Δ_{50}=3-5 nm; 3 - nNi, Δ_{50}=3-5 nm; 4 - nZnO, Δ_{50}=12 nm; 5 - α-Al_2O_3, Δ_{50}=7 nm; 6 - α-nAl_2O_3, Δ_{50}=70 nm; 7 - α-Al_2O_3, Δ_{50}=4 mkm; 8 - nAl_2O_3- corundum, Δ_{50}=70 nm; 9 - nTiO_2, Δ_{50}=5 nm; 10 - nTiO_2, Δ_{50}=50 nm; 11 - nTiO_2, Δ_{50}=90 nm; 12 - nTiO_2, Δ_{50}=350 nm; 13 - TiO_2, Δ_{50}=10 mkm; 14 - nSi_3N_4, Δ_{50}=80 nm; 15 - nSiC, Δ_{50}=95 nm; 16 - BT-50; 17 - MCD-material

It is possible to solve this problem using more sensitive test-functions based on the behavioural and physiological responses of entomostracans. One of such functions is trophic activity (TA) *Daphnia magna Straus*.

The change in the trophic function in comparison with the validation batch is illustrated in the group of NPs of metals and metal oxides for which no biological effects on the daphnids' mortality indicator were registered (figure 4).

We considered studying the trophic activity while adding nAg and nZnO into GM composition to be unreasonable because of the high mortality rate of entomostracans under the influence of DS of the indicated NPs. Moreover, in accordance with our data, the mortality rate of entomostracans grows because of additional impact of NPs after their bio-accumulation and adsorption by the cells of chlorella added as feed-stuff. The possibility of NPs accumulation in unicellular algae and ciliates is indicated by the data obtained by the authors in the experiments with nAg, nZnO, nTiO_2, nAu and quantum dots [14, 16, 17].

Our research showed that the trophic activity is inhibited in 5 out of 10 tested NMs (figure 4). The trophic activity of *Daphnia magna Straus* decreases with exposing entomostracans into DS nTiO_2 (Δ_{50}=5 nm) by 20.6 %, nSi_3N_4 (Δ_{50}=80 nm) – by 25.1% and nAl_2O_3-electrocorundum (Δ_{50}=70 nm) - by 30.9 %. The addition of NPs α-nAl_2O_3 (Δ_{50}=7 nm) or nTiO_2 (Δ_{50}=50 nm) into the test-system stimulates the trophic activity by 17.6 % and 10.0 % correspondingly.

Consequently, the entomostracans *Daphnia magna Straus* react on the addition of most SFM into GM by changing the physiological function – the trophic activity. Daphnids are known to be classical test-organisms while studying water medium contamination. It is related to the fact that daphnids being active filterers react quickly on the change in the medium quality by means of changes in their physiological functions such as locomotory, breathing and trophic activity, heart-beating rhythm, fertility, etc., which predict more serious anthropogenic loading [15, 18].

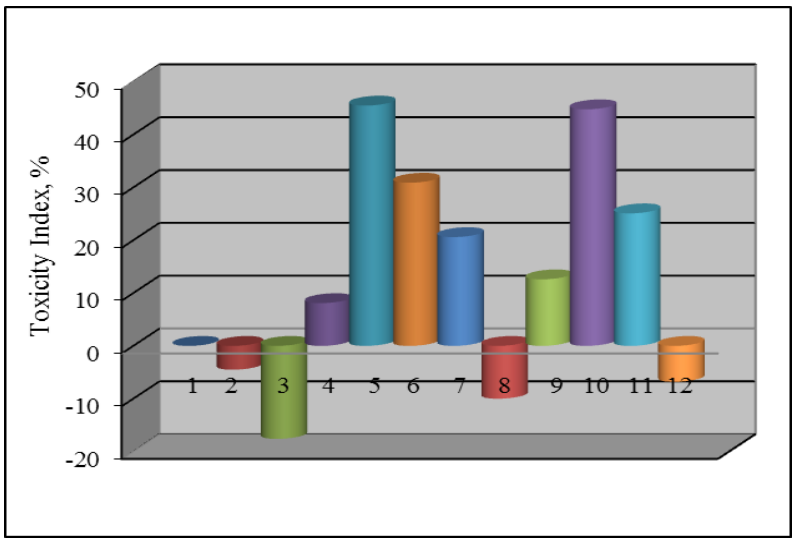

Figure 4. The toxicity index of NMs with various chemical composition and dispersion rate determined in accordance with the indicator of trophic activity of entomostracans *Daphnia magna Straus* (C=1.0 mg/l): 1 - nPt, Δ_{50}=3-5 nm; 2 - nNi, Δ_{50}=3-5 nm; 3 - α-Al_2O_3, Δ_{50}=7 nm; 4 - α-nAl_2O_3, Δ_{50}=70 nm; 5 - α-Al_2O_3, Δ_{50}=4 mkm; 6 - nAl_2O_3-corundum, Δ_{50}=70 nm; 7 - nTiO_2, Δ_{50}=5 nm; 8 - nTiO_2, Δ_{50}=50 nm; 9 - nTiO_2, Δ_{50}=90 nm; 10 - nTiO_2, Δ_{50}=350 nm; 11 - nSi_3N_4, Δ_{50}=80 nm; 12 - nSiC, Δ_{50}=95 nm

However, the researches into medium quality assessment using the physiological functions of entomostracans are represented in literature by few works. The works on studies of the influence of heavy metals on the heart beating of *Daphnia Cucullata* G.O.SARS, 1862 [18] and the trophic activity of *Daphnia magna Straus* [15] are of great interest. We have not found any works studying the bio effects of SFM on the physiological parameters in the literature available.

We observed the decrease in the trophic activity of *Daphnia magna Straus* while exposing entomostracans into GM with the substances in macro form such as NPs α-nAL_2O_3 (4000 nm) - by 45,5 %, nTiO_2 (350 nm) - by 44,8 %. Entomostracans are known to have a filter feeding type. They can filter great amounts of water and swallow the particles whose size is 0.4-40.0 mkm, consisting of algae cells, large bacteria and other organic and inorganic particles. Entomostracans can seize small particles especially the ones that are less than 50 micron in diameter and suspended aggregates NPs (TiO_2 and C_{60}), which have micro- rather than nano-size [19]. Large particles are too difficult to digest, and entomostracans can prevent them from penetrating to a filtration chamber. It is possible that while filtrating GM with added substances in macro form the filtration mechanism of entomostracans becomes cluttered, which results in the decrease in their trophic activity and often in the death of entomostracans.

We determined ecotoxicological parameters of DS SFM: NOEC, (L(E)C_{20} and L(E)C_{50} for each test-organism. Table 1 contains biologically active tested SFM and their concentrations L(E)C_{20}, which result in toxicity effect appearing. We showed that L(E)C_{20} depends on the test-organism type and the physical and chemical properties of SFM.

We revealed 6 types of the tested SFM that have a toxic effect on chlorella growth in smaller concentrations than on the chemotaxis of ciliates and mortality of entomostracans: nAg Δ_{50}=2-5 nm, nAu Δ_{50}=2-5 nm, nZnO Δ_{50}=12 nm, nAl_2O_3 (α-form) Δ_{50}=7 nm, nTiO_2 Δ_{50}=90 nm, nSi_3N_4 Δ_{50}=80 nm and BT-50. We found 3 types of the tested SFM that have toxic impact on the chemotaxis of ciliates in

fewer amounts than on chlorella growth, mortality and trophic activity: nNi Δ_{50}=3-5 nm; α-nAl_2O_3 Δ_{50}=70 nm and nTiO_2 Δ_{50}=5 nm.

Table 1. The value L(E)C_{20} for the biologically active, tested SFM

NPs	*Chlorella vulgaris Beijer*	*Paramecium caudatum*	*Daphnia magna Straus*	
			Dead	Trophic activity
Ag, Δ_{50}=2-5 nm	0.00009	0.00010	0.00003	-
Au Δ_{50}=2-5 nm	0.043	>100	-	-
Ni Δ_{50}=3-5 nm	0.067	0.042	15.21	32.77
ZnO Δ_{50}=12 nm	0.0087	0.044	0.029	-
Al_2O_3 (α-form)Δ_{50}=7 nm	0.0087	>100	>100	0.062
Al_2O_3 (α-form) Δ_{50}=70 nm	3.121	0.056	>100	0.208
Al_2O_3 (corund) Δ_{50}=7 nm	>100	>100	>100	0.041
TiO_2 Δ_{50}=5 nm	0.053	0.000009	>100	0.274
nTiO_2 Δ_{50}=50 nm	>100	>100	>100	0.465
nTiO_2 Δ_{50}=90 nm	0.279	>100	>100	2.965
nSi_3N_4 Δ_{50}=80 nm	>100	>100	>100	0.122
BT-50	1.796	>100	>100	-

We found 3 SFM types that disrupt the trophic activity of entomostracans in lower concentrations, than the growth of algae and the chemotactic response of ciliates: nAl_2O_3 (corund) Δ_{50}=7 nm, nTiO_2 Δ_{50}=50 nm and nSi_3N_4 Δ_{50}=80 nm.

4. Conclusion

Our research showed the vulnerable elements of the ecosystem resistance to water contamination with SFM. We stated that phytoplankton (unicellular algae) and zooplankton (unicellular animals and entomostracans) representatives are susceptible to harmful influence of SFM. For some SFM kinds algae are the most vulnerable element in the chain «SFM-algae-water animals». Water animals (ciliates and entomostracans) are more sensible to other SFM kinds. The toxic effect of SFM is reflected in species composition of communities and the correlation of species populations, which may bring about a change in the structure of trophic connections and the distortion of the food pyramid and the ecosystem as a whole.

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