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The factors affecting ecotoxicity of engineered nanomaterials

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Abstract

The presented dissertation is focused on the issue of testing of engineered nanomaterials ecotoxicity. Specifically, on to development of a testing method that eliminates factors affecting the rightness and reproducibility of laboratory test results. The results presented in this thesis show that the proposed modifications of the methods for testing the ecotoxicity of nanomaterials have enabled effective monitoring of agglomeration behavior in the exposure media. The method proposed for the aquatic toxicity test has allowed testing of the ecotoxicity of nanoparticles and their agglomerates of precisely defined size. The agar-based exposure medium used for terrestrial toxicity tests allows a homogeneous dispersion of test particles, suppression of dynamic processes during the experiment, and provides the ability to more easily analyze the physico-chemical state of the particles directly in the exposure medium. This method, based on the use of agar exposure medium, could be used as a quick and simple tool in the first tier of environmental risk assessment of nanomaterials or in studies dealing with the effect of physico-chemical conditions on nanoparticles toxicity.

Abstrakt

Předložená disertační práce je zaměřena na problematiku testování ekotoxicity průmyslově vyráběných nanomateriálů. Konkrétně na vývoj metod testování ekotoxicity, které eliminují faktory ovlivňující správnost a reprodukovatelnost výsledků laboratorních testů. Výsledky prezentované v předložené práci ukazují, že námi navržené modifikace metod testování ekotoxicity nanomateriálu umožnily monitorovat aglomerační chování částic v expozičních médiích. Metoda navržená pro test akvatické toxicity umožnila testování ekotoxicity nanočástic a jejich aglomerátů o přesně definované velikosti. Expoziční médium na bázi agaru, které bylo v práci využito pro testy terestrické toxicity, umožňuje homogenní disperzi testovaných částic, potlačení dynamických procesů během experimentu a poskytuje možnost snadněji analyzovat fyzikálně-chemický stav částic přímo v expozičním médiu. Metoda založená na využití agarového expozičního média by mohla nalézt využití jako rychlý

a jednoduchý nástroj v prvním stupni hodnocení environmentálních rizik nanomateriálů nebo pro studie zabývající se vlivem fyzikálně-chemických podmínek na toxicitu nanočástic.

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Introduction

Nanomaterials are defined as materials having at least one dimension in the range of 1–100 nm. Nanomaterials present in the environment come from both natural (forest fires, volcanic activity, rock erosion) and anthropogenic sources. Due to their unique properties, engineered nanomaterials (ENMs) have found application in many areas, for example in medicine, drinking water treatment, optics and electronics. Number of consumer goods also contain ENMs, e.g. cosmetics and health products[1]. This wide application potential of ENMs indicates a high probability of direct or indirect exposure of humans and the environment. Soils and sediments, in contrast to the atmosphere or the aquatic environment, can serve as long-term reservoirs of ENMs[2;3]. The different properties of nanomaterials from other types of matter (soluble substances, larger particles) raise concerns about their effects on living organisms. Nanomaterials interact differently with the biotic and abiotic components of the environment and these specific interactions must be taken into account when assessing the environmental risks of nanomaterials. However, many tools, protocols and guidelines for the determination and evaluation of physicochemical properties, fate, exposure and effect used for conventional chemicals are not suitable for testing of nanomaterials ecotoxicity. These tools therefore need to be modified[2].

Aims of the Thesis

The major factors which complicates the assessment of environmental risks of nanomaterials are changes in physicochemical properties of nanoparticles after their release into the environment and difficult characterization of particles in complex matrices. In laboratory tests where reproducibility is required, transformation processes such as agglomeration, aggregation, sedimentation, dissolution etc. complicate the interpretation of the results. Due to frequently occurring methodological artifacts, there is a high degree of uncertainty about the true nature of physicochemical properties and the effective doses of test materials (specifically micro and nanoparticles). Because of the above, the main aim of this thesis was to design and develop methods for testing the toxicity of nanomaterials, which eliminate or reduce the influence of mentioned factors. The main objective of the work was fulfilled within the framework of partial objectives:

1. Focus on important factors affecting the reproducibility of nanomaterial ecotoxicity tests and optimize the method for toxicity testing in aquatic environment
2. For terrestrial toxicity testing, optimize the preparation and characterization of the exposure medium that suppresses dynamic processes and increases reproducibility of results
3. Test the toxicity of nanomaterials in exposure medium with increased environmental relevance – modified by addition of model soil components (kaolin, humic acids, peat) in order to study the effect of interactions with soil components on the behavior of nanoparticles in the exposure medium

Practical part

1. Toxicity testing in aquatic environment

Fulfillment of the first partial objective of the dissertation thesis consisted in studying important factors influencing reproducibility of nanomaterial ecotoxicity tests, especially in the study of NPs agglomeration behavior. The acquired knowledge should then be used to modify the method of testing NPs toxicity in the aquatic environment in order to suppress the influence of agglomeration on the test results.

Because of the above, this part of the work focuses on developing and testing an approach where the maximum size of the agglomerates (to which the test aquatic organism is exposed during the test) is controlled by a concentration-dependent change of exposure medium during the test. In this work was used a mathematical model describing the dependence of agglomerate size on time in liquid medium of given properties (particle concentration, ionic strength of medium). Using this model, the medium exchange frequency was designed so that the experimental organism was always exposed only to agglomerates whose size did not exceed the selected limit value (200 and 400 nm). This approach has been validated in a modified test of silver nanoparticles toxicity to common carp (*Cyprinus carpio*) embryos. Silver nanoparticles are one of the most widely used nanomaterials and can be easily prepared under laboratory conditions. The common carp was chosen because it represents freshwater fish of high commercial importance in the fishing industry in Europe and Asia.

Experiment I lasted 144 h, and the design included repeated periods of exposure to Ag-NP colloids for 6 h on days 0, 1, 2, 3, 4, and 5 followed by 18 h (overnight) periods when the carp embryos were transferred into fresh media without nanoparticles. In experiment II, the embryos were exposed on days 0, 1, 2, 3, 4, and 5, but the colloid media were frequently exchanged during the 6-h exposure. Frequent media exchanges were performed with the aim of checking for the formation of agglomerates. Further experiments were conducted using a modified protocol with variable exchange media periods. Experiment II that lasted same time like in experiment I with the different 6-h exposure periods was divided by freshly prepared media exchange. Length of every part of each period depends on selected size of agglomerates at specific media concentration. The periods were calculated as follows. First, the DLS was used for evaluation of Ag-NP agglomeration in the particular colloid during the time period sufficient to achieve the steady state. The obtained experimental data were then used for determination of the rate constant k in Eq. (1), which describes the growth of agglomerates (more precisely the increase in DH) over time[4].

$$D_H^t = D_H^{\inf} + \left(D_H^0 - D_H^{\inf}\right) \times e^{-kt} \quad \textbf{(1)}$$

In this equation, D_H^0 represents the hydrodynamic diameter of particles in the tested colloid immediately after its preparation D_H^{inf} is the hydrodynamic diameter in the steady state, and t is time. The rate constant k strongly depends on the initial Ag-NPs concentration and thus had to be determined for every concentration level separately. D_H^t represents the average hydrodynamic diameter in time t.

In our study with the continuous 6-h exposures (experiment I), mortalities were observed in all tested concentration of Ag-NPs (mortalities 60–90 %). However, there were no statistical differences among concentrations (Mann–Whitney test, $p > 0.05$), and no apparent dose-response relationship (Fig. 1a). The following experiment II (Fig. 1b, c) provided variable results. The variant where the frequent exchanges of media controlled for a maximum 200 nm agglomerates (Fig. 1b) provided a similar pattern to experiment I with no differences between the doses and a slightly lower toxicity (mortalities 30–50 %). In contrast, the experiment checking for 400 nm agglomerates resulted in a dose-response (statistically different effects among the three tested concentrations, Mann–Whitney $p < 0.05$, Fig. 1c). The highest colloid concentration tested (50 μM) led to 90–100 % mortalities (first observed after 120 h of the experiment). In other words, more pronounced Ag-NPs toxicity was caused by higher concentrations of larger agglomerates in the water column. The continuous presence of the large 400 nm agglomerates (assured by frequent exchanges of media, Fig. 1c) was the most toxic for common carp embryos, while the same concentrations of Ag-NPs colloid without media exchanges led to fast sedimentation of agglomerates and lower toxicity (compare, e.g., effects of 25 and 50 μM Ag-NPs in Fig. 1a, c).

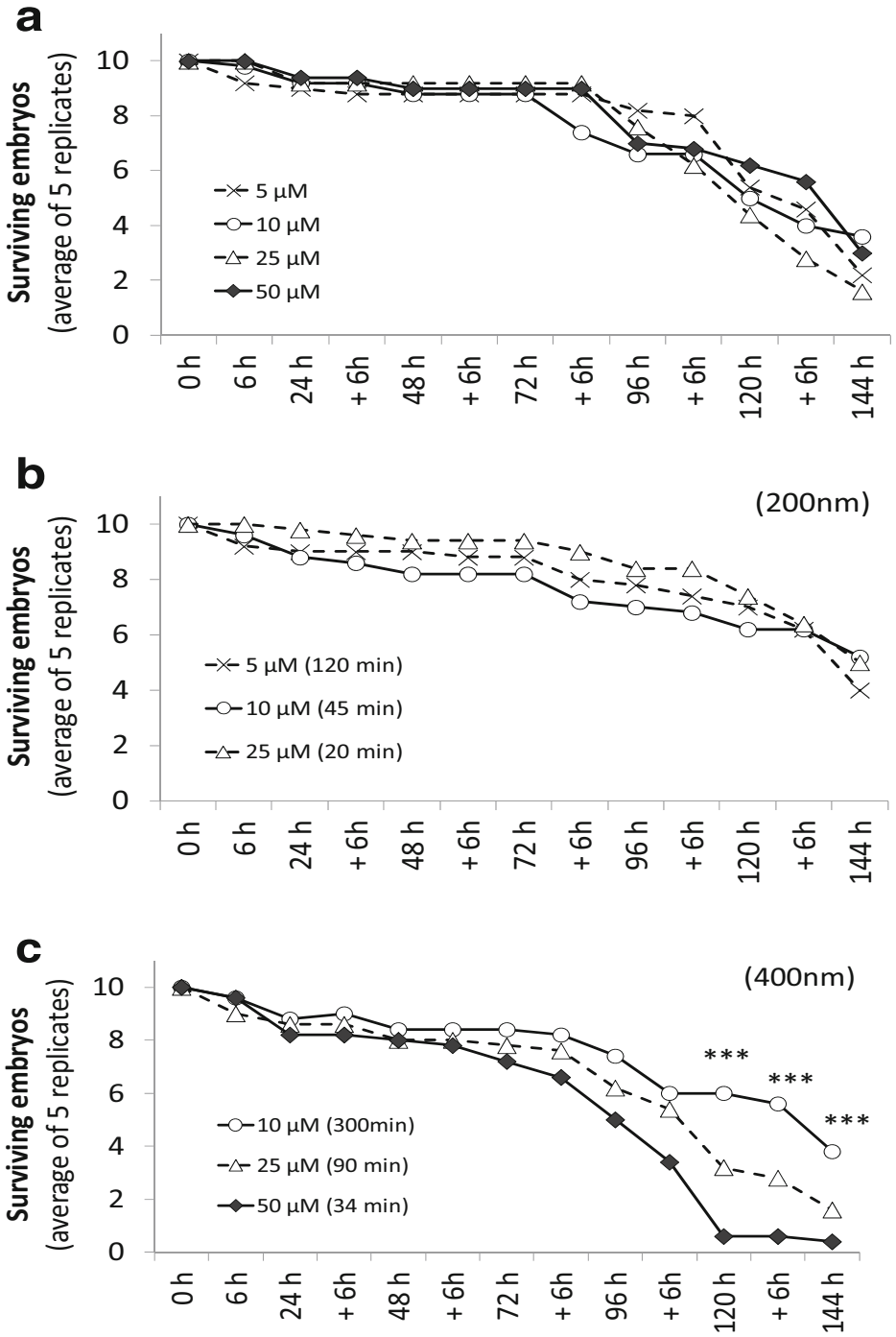

Figure 1 Toxicity testing of silver nanoparticles (Ag-NPs): average surviving embryos of five replicate beakers (error bars not shown for clarity). a Semistatic exposures to four concentrations for 6 h followed by 18 h in fresh media without Ag-NPs, b exposure controlling for maximum 200 nm agglomerates, and c exposure controlling for maximum 400 nm agglomerates. The values in parentheses in b and c indicate the periods of frequent media exchanges for the given concentrations; ***Statistically significant differences among all three concentrations 10 vs 25 vs 50 µM (Mann–Whitney test, $p < 0.05$)

The size-specific effects of particles were also confirmed by the observations of individual embryos in the study with apparent sorption of larger Ag agglomerates into the exposed embryos (Fig. 2). This could dramatically increase the actual Ag concentrations locally at the biological surface and lead to mechanical disruption and blocking of biological functions. The latter phenomenon has also been suggested in other studies[5;6].

Figure 2 Example of the influence of Ag-NPs on the development of carp embryos after 144 h of the experiments. a Control embryo, b embryo with apparent spine curvature and pericardial edema (exposure to media with Ag-NPs concentration 5 μM) in the experiment controlling for 200 nm agglomerates), and c embryo with a chorion covered by Ag-NPs agglomerates (exposure to 25 μM Ag-NPs in the experiment controlling for 400 nm agglomerates)

2. Terrestrial toxicity test in agar medium

The second partial goal of this thesis was to develop a method for testing ecotoxicity of NPs on soil organisms. Fulfillment of the objective consisted mainly in optimizing the preparation and characterization of the exposure medium. The properties of the exposure medium were intended to suppress the dynamic processes, that occur after the addition of NPs during the whole experiment, which affect the reproducibility of the tests. The proposed procedure should also allow for more homogeneous dispersion of particles in the medium and easier characterization of the dispersed particles.

In the terrestrial environment, agglomeration of nanoparticles is a frequent phenomenon too. Nonhomogeneous dispersion of the test material in the exposure medium in combination with the escape behavior typical for some soil organisms further leads to nonmonotonic dose-response curves. Also, characterization of nanoparticles dispersed in the soil matrix is a very difficult task. Because of the above, this part of the thesis deals with the verification of the usability of agar as an exposure medium in acute terrestrial toxicity tests. Because the toxic concentrations (lethal) of

NPs for terrestrial organisms are generally higher than for aquatic organisms, we were unable to prepare Ag-NPs colloids at toxicologically significant concentration levels. Therefore, zinc oxide nanoparticles (ZnO-NPs) with wide range of applications representing another significant environmental contaminant were selected. Ecologically relevant soil organism *Enchytraeus crypticus* was chosen as the test organism. The main advantages of this organism are fast generation time, high natality and easy cultivation on agar.

The *E. crypticus* were exposed in the acute toxicity test for 96 hours, in the dark, at a temperature of (20 ± 2) ° C. 10 enchytraeids were transferred to each test vessel. Each test concentration and control group were prepared in triplicate. Toxicity testing was performed in ventilated plastic Petri dishes filled with 2% agar. The end-point of the test was the mortality of the test organisms compared to the control group.

Influence of different spiking techniques on the ecotoxicological effects of ZnO-NPs was evaluated based on physico-chemical characterization of ZnO-NPs in exposure medium. The two techniques employed in dispersion of ZnO-NPs in the medium were dry method (cryogenic grinding of ZnO-NPs with the agar powder) and wet method (adding the aqueous dispersion of stabilized ZnO-NPs to the hot liquid agar). The relationship between the agglomerate size and the toxicity of ZnO-NPs was studied and the toxicity of nanoparticles was compared to the toxicity of Zn^{2+} ions originating from water soluble salt of zinc. The dose-response curve was obtained by fitting of experimental data using the Boltzmann model:

$$y = y_{min} + (y_{max} - y_{min})/(1 + \exp((P_1 - x)/P_2)) \quad (2)$$

where y is the mortality (%), x is ln of the Zn concentration in agar (mg of Zn per kg of agar), P_1 is the inflection point corresponding to LC_{50}, and P_2 is the slope.

For the soluble zinc salt test, a 96h LC_{50} (with corresponding 95% confidence interval) of 37.2 (35.5-38.8) mg Zn kg^{-1} agar was calculated. Because of the possible contribution of chlorides to zinc toxicity, a test with NaCl was also performed. No mortality was observed in any of the tested concentrations. As seen in Fig. 3 depicting the results of the experiments, the toxicity of zinc cation was higher than the toxicity of ZnO-NPs. The dispersion homogeneity played an important role in the toxic effect of ZnO-NPs. Scanning electron microscopy (SEM) revealed poorly dispersion of ZnO-NPs spiked into agar exposure medium via dry method. On the other hand, in the case of wet spiking, nanoparticles were homogenously dispersed and the size of found agglomerates did not exceed 1 μm. This observation was reflected in nonmonotonic dose-response relationship in agars spiked via dry method, while in the case of wet spiking there was relationship between mortality and concentration of zinc in agar. In

the experiment where nanoparticles were introduced via dry method (Fig. 3 ZnO-NPs-Cryo) observed mortality was in the range 29-34 % and in the experiment where nanoparticles were introduced via wet method (Fig. 3 ZnO-NPs-Colloid) observed mortality was in the range 0-67 %.

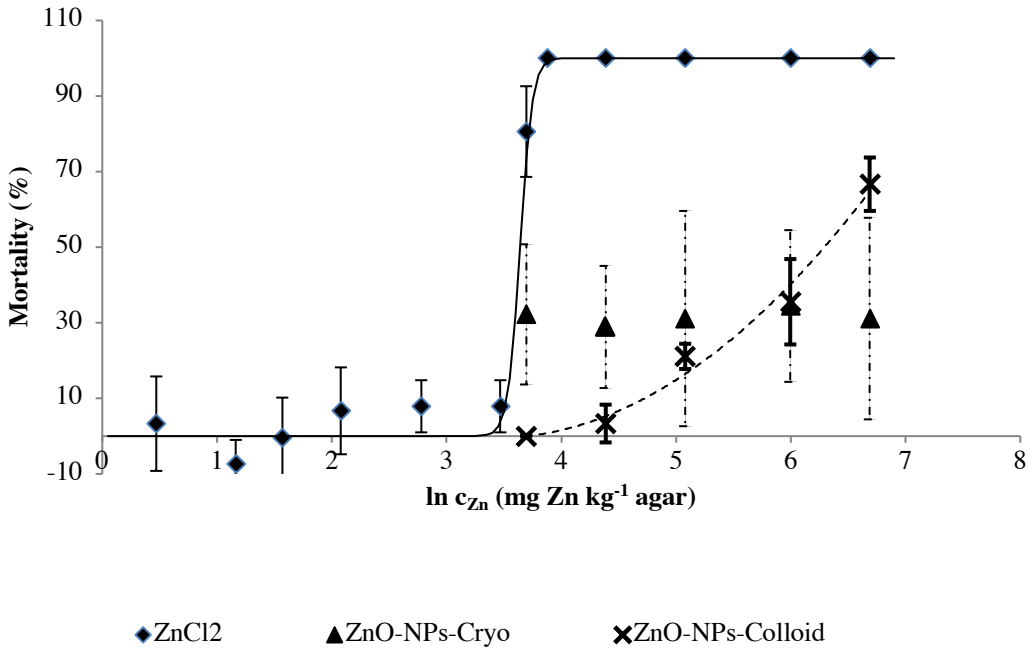

Figure 3 Mortality of *E. crypticus* exposed 96 h to $ZnCl_2$ and ZnO-NPs introduced into the exposure media via dry spiking procedure (ZnO-NPs-Kryo) and wet spiking procedure (ZnO-NPs-Colloid)

The assessment of the pollutants uptake by the organism is also important part in ecotoxicological studies. Following a previous study, it was necessary to develop a method for analyzing the elements in samples of *E. crypticus* biomass and samples of agar exposure medium. The quantitative analysis of the elements contained in enchytraeid tissues, which are often used in terrestrial toxicity tests, is complicated by the low weight of these organisms. The most common expression of internal pollutant contents is a concentration per fresh or dry biomass weight or content per number of organisms. Taking into account a very small weight less than 1 mg per worm and water content of more than 80%, there are obvious problems with accuracy both in weighing and determination of dry weight (DW) and results related to it[7;8;9].

Because of the above, a simple, reliable and routinely applicable procedure for analysis of samples from the ecotoxicological experiments was developed. A fast and fairly simple sample preparation procedure was based on dissolution of *E. crypticus* in 25% tetramethyamoniumhydroxide and dissolution of agar in 65% HNO_3. The samples were then analyzed using the inductively coupled plasma optical emission spectrometry (ICP-OES) method. The fresh, wet mass of the *E. crypticus* organism was determined. The content of analytes was then referred to wet biomass. This allowed the simplification of developed method.

The mean wet weight value 0.64 mg per *E. crypticus* individual was obtained from repeated measurement of 100 *E. crypticus* individuals. Considering target elements, this procedure is usable in connection with other detection analytical techniques. The ICP-OES method is sufficiently sensitive and suitable for zinc determination in the *E. crypticus* and agar test gel. The LOD 0.90 mg kg^{-1} was estimated for 10 mg of the *E. crypticus* biomass dissolved in a final volume of 10 mL. Even in the case of analysis of one worm (weight 0.64 mg, 38 ng background zinc in one body, final volume 10 mL), the zinc signal can be reliably distinguished from the background.

3. Terrestrial toxicity test in modified agar medium

The third part of the thesis aimed to test the toxicity of nanoparticles on *E. crypticus* cultivated in agar exposure medium with increased environmental relevance. Studying the transformation processes of NPs in soils and describing the interaction of NPs with inorganic and organic soil components should be an integral part of ecotoxicity studies. These processes affect the fate of NPs and strongly depend on environmental conditions. The proportion of inorganic and organic constituents significantly affects the bioavailability of NPs in soil and thus toxicity[10]. For the study of the influence of specific soil components on the fate of NPs it is more convenient to use a less complex medium. In view of the above, in terrestrial toxicity test was used our optimized method based on using agar as an exposure medium. The agar was modified with kaolin, humic acids and peat within two studies. These agar modifications should allow us to easily observe how the soil components interact with the tested ZnO-NPs and how these interactions affect the resulting material state and the associated changes in observed toxicity. The toxicity of nanoparticles was compared to the toxicity of Zn^{2+} ions originating from water soluble salt of zinc. For creating of dose-response curves and for calculation of LC_{50} values, the module of nonlinear regression in GraphPad Prism 7 software was used.

The *E. crypticus* were exposed in the acute toxicity test for 96 hours, in the dark, at a temperature of (21 ± 2) ° C. Twenty adult individuals were transferred to each test vessel. Each test concentration and control group were prepared in triplicate. Toxicity testing was performed in ventilated plastic Petri dishes filled with 1.5% agar. The end-point of the test was the percent mortality of the test organisms compared to the control group.

In the first study zinc toxicity was tested in pure agar and in three agar modifications: in the presence of 1% kaolin, 0.1% humic acids derived from weathered brown coal called oxyhumolite and in the presence of both components. As seen from the Fig. 4 and Tab 1. toxicity of zinc originating from $ZnCl_2$ after 96 h of exposure was highest in pure agar (LC_{50} = 13.2 mg kg^{-1}) followed by agar with HA (LC_{50} = 28.8 mg kg^{-1}) and agar with kaolin and HA (LC_{50} = 39.4 mg kg^{-1}) and the lowest toxicity was observed in agar with kaolin (LC_{50} = 75.4 mg kg^{-1}). Toxicity of zinc originating from ZnO-NPs (Fig. 4b) was highest in agar with HA (LC_{50} = 15.8 mg kg^{-1}), lower toxicity was found in pure agar (LC_{50} = 43.5 mg kg^{-1}), followed by agar with kaolin (LC_{50} = 111 mg kg^{-1}) and the lowest toxicity was observed in agar with both components (LC_{50} = 122 mg kg^{-1}). In this case HA even increased the bioavailability and toxicity of zinc. On the contrary, the presence of kaolin reduced the toxicity of zinc originating from ZnO-NPs. In the presence of both soil components, the effect of kaolin was shown to be dominant.

SEM analysis of particles in exposure media revealed that in agar containing 1 % of kaolin, nanoparticles were better dispersed than in the case of pure agar. Nanoparticles were coated with kaolin during agar preparation, which in all probability prevented the formation of larger ZnO agglomerates. The behavior of ZnO was completely different in the presence of 0.1 % HA. Larger agglomerates of ZnO (> 1 μm) were observed in these samples, however, in a smaller amount in the same area than in pure agar. EDX analysis showed that the Zn/C intensity ratio in the flat surface was higher than in the case of pure agar, indicating that a larger amount of zinc was dispersed in the agar containing HA.

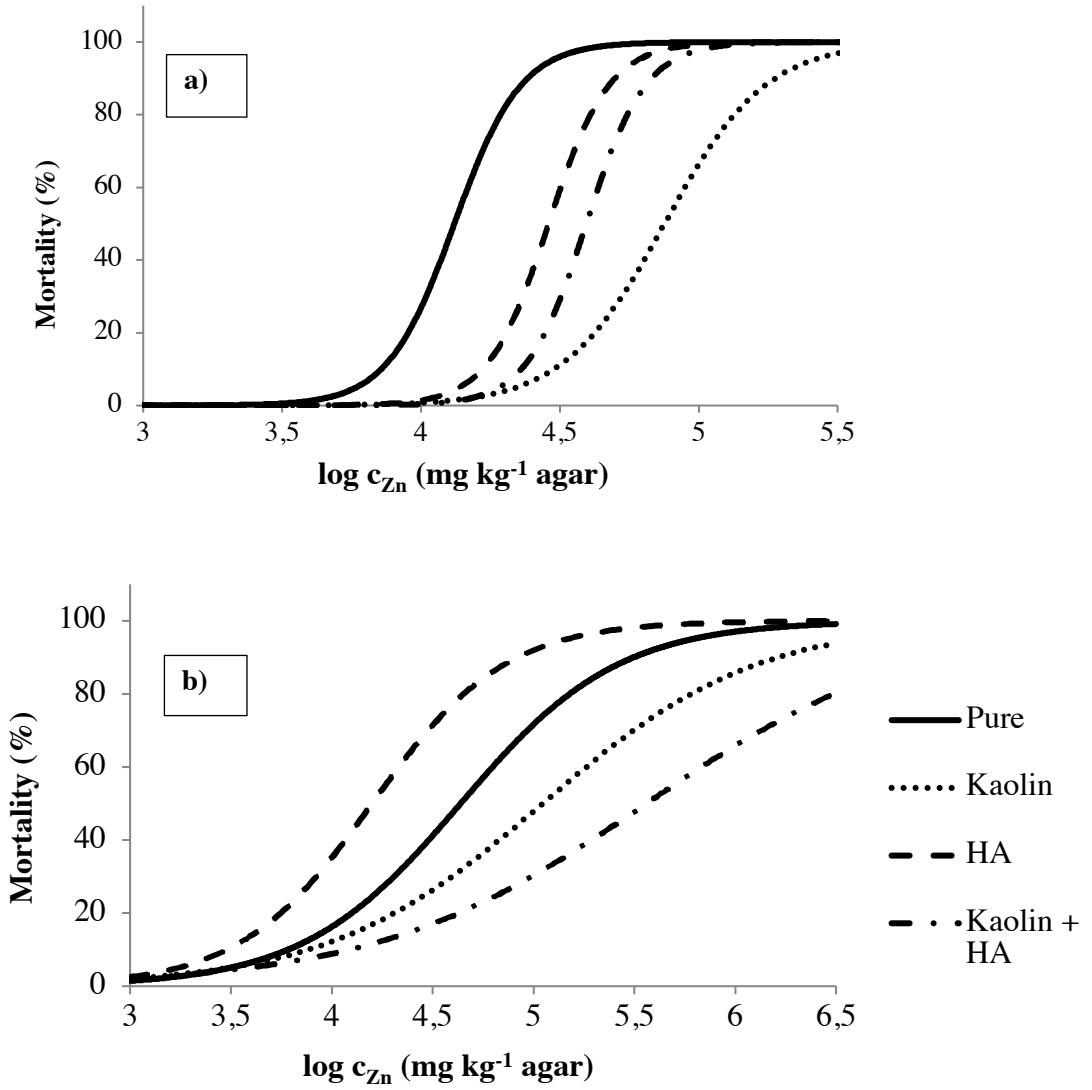

Figure 4 Dose-response curves describing the acute toxicity of zinc originating from $ZnCl_2$ (a) and ZnO-NPs (b) for *E. crypticus* in four different agar-based exposure media

Table 1 LC_{50} values for both form of zinc in four different agar-based exposure media

Exposure media	LC_{50} (mg kg^{-1})	
	$ZnCl_2$	ZnO-NPs
Pure agar	13.2 (12.1–14.3)	43.5 (23.8–60.1)
Agar with kaolin	75.4 (65.8–86.5)	111 (73.6–157)
Agar with HA	28.8 (27.7–30.0)	15.8 (3.3–36.9)
Agar with kaolin and HA	39.4 (36.7–42.2)	122 (80.1–171)

In the second study zinc toxicity was tested in pure agar and in the agar exposure medium modified by two sources of humic substances, namely sodium humate and peat. The effect of HA on the behavior and acute toxicity of ZnO-NPs for *E. crypticus* was studied. Experimental conditions were the same as for previous described study.

As seen from the Fig. 5a and Tab 2. toxicity of zinc originating from $ZnCl_2$ after 96 h of exposure was highest in pure agar (LC_{50} = 13.2 mg kg^{-1}) followed by agar with SH (LC_{50} = 28.8 mg kg^{-1}), whereas the lowest toxicity was observed for agar with peat (LC_{50} = 161 mg kg^{-1}). Toxicity was slightly decreased by adding SH but, in the presence of peat, the toxicity decreased by one order of magnitude. From such results, it is obvious that the addition of soluble SH did not reduce the bioavailability of zinc as much as the solid peat. Chemical and compositional differences of HA originating from different sources could lead to different interactions HA-NPs.

In case of ZnO-NPs (Fig. 5b), the toxicity was the highest in agar with SH (LC_{50} = 15.8 mg kg^{-1}) followed by pure agar (LC_{50} = 43.5 mg kg^{-1}) and the lowest toxicity was observed in agar with peat (LC_{50} = 304 mg kg^{-1}). As in the case of $ZnCl_2$, different composition of HA and the solid phase of peat reduced toxicity of zinc almost by one order of magnitude compared to pure agar. Interestingly, there was even an increase in the toxicity of nanoparticles in the presence of SH compared to pure agar. SEM analysis showed that more zinc was dispersed in the volume of agar in the presence of SH. Apparently, HA stabilized ZnO-NPs which resulted in the increase of amount of well-dispersed nanoparticles and Zn^{2+} ions. Ability of HA to increase dissolution of zinc (or other metals) was shown in other studies[11;12]. Zinc was very well dispersed throughout the volume of the area in the presence of peat when very small amounts of ZnO-NPs agglomerates (< 1 μm) were found in the sample with 1 000 mg ZnO-NPs kg^{-1} agar.

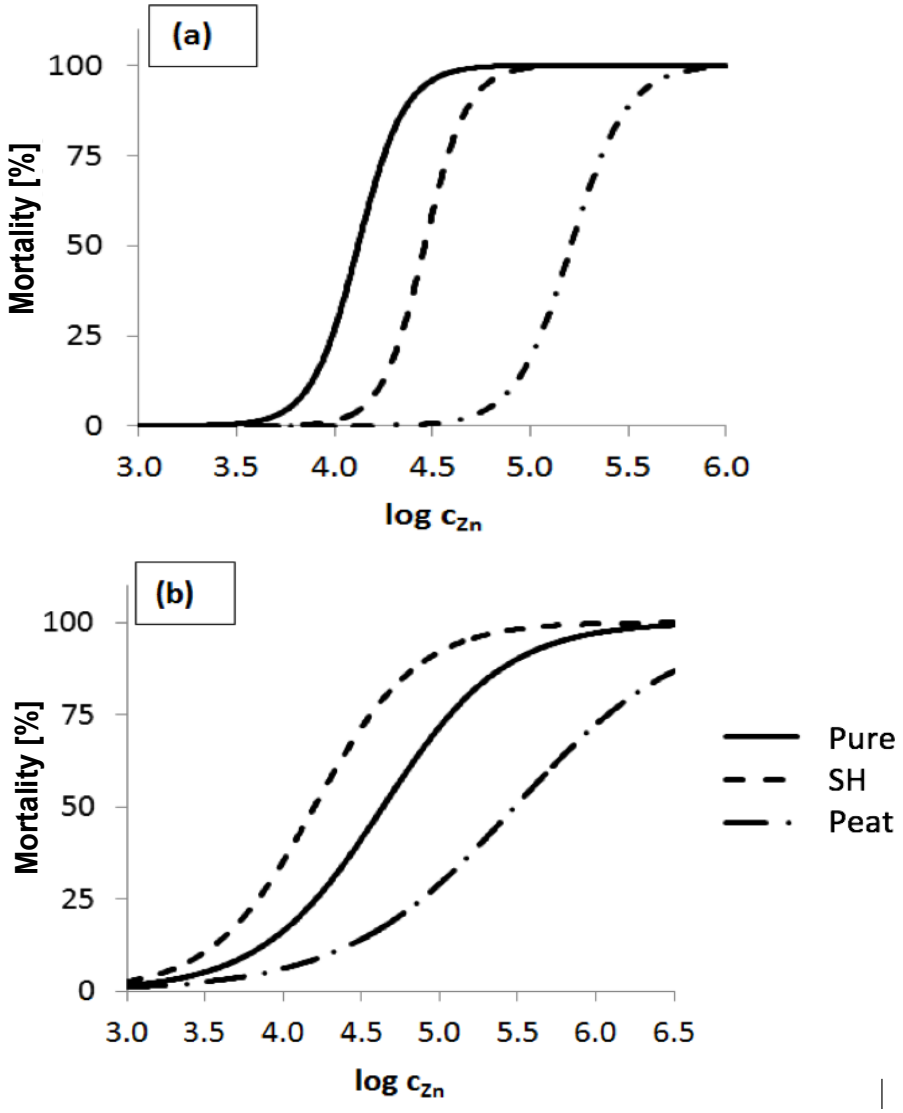

Figure 5 Dose-response curves describing the acute toxicity of zinc originating from $ZnCl_2$ (a) and ZnO-NPs (b) for *E. crypticus* in three different agar-based exposure media. Concentration of Zn expressed in 10^{-3} mg Zn kg^{-1} of agar

Table 2 LC_{50} values for both form of zinc in three different agar-based exposure media

Exposure media	LC_{50} [mg kg^{-1}]	
	$ZnCl_2$	ZnO-NPs
Pure agar	13.2 (12.1–14.3)	43.5 (23.8–60.1)
Agar with SH	28.8 (27.7–30.0)	15.8 (3.3–36.9)
Agar with peat	161 (140–287)	304 (255–365)

Conclusion

The results of our modified methods for ecotoxicity testing highlighted the importance of assessment of physico-chemical properties of nanomaterials during the ecotoxicity tests. The method for testing aquatic toxicity of nanoparticles based on an approach where the maximum size of agglomerates to which the test organism is exposed during the test is controlled by a concentration-dependent variable medium exchange was developed. This method was successfully published in the journal Environmental Science and Pollution Research (IF – 2.914). However, the probability of using our method for the practical evaluation of the ecotoxicity of nanomaterials for aquatic organisms is rather low, because the experiment without flow-through system or automatization of the colloid exchange is very time consuming and very demanding in terms of personnel.

The method for testing terrestrial toxicity based on using agar instead of soil was developed. An integral part of the proposed methodology was an optimized procedure for introducing NPs into the exposure media, procedure for preparation of exposure media samples for NPs secondary characterization and procedure for quantitative analysis of samples from ecotoxicological experiments. This method was successfully published in the journal Chemical Papers (IF – 1.246) and Environmental Science and Pollution Research. Highly artificial character of the proposed agar system, which is connected with reduced degree of its environmental relevance, obviously prevents the use of this method as a substitute of assays performed in a real soil matrix. On the other hand, application of the proposed cheap and simple approach as a tool in a first tier of environmental risk assessment or for studies dealing with an influence of physicochemical conditions on the nanoparticle toxicity could be beneficial.

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List of Students' Published works

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[IF 2018 = 2.914] (CIT = 1)

Contribution of P.M.: (estimated **60 %**) contributed to planning the study, conducted experimental work, evaluated the data and draft the manuscript

Patocka J, Krejcova A, Stojarova K, **Hrda K** a Pouzar M: The ICP-OES method for determination of zinc in *Enchytraeus crypticus* and agarose gel from ecotoxicological tests. *Chem Pap* 2018, 73(1):159-164.

[IF 2018 = 1.246] (CIT = 0)

Contribution of P.M.: (estimated **40 %**) contributed to planning the study, conducted the ecotoxicological experiments, contributed to manuscript writing

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[IF 2018 = 1.246] (CIT = 6)

Contribution of P.M.: (estimated **70 %**) contributed to planning the study, conducted experimental work, evaluated the data and draft the manuscript

Oprsal J, Blaha L, Pouzar M, Knotek P, Vlcek M a **Hrda K**: Assessment of silver nanoparticle toxicity for common carp (*Cyprinus carpio*) fish embryos using a novel method controlling the agglomeration in the aquatic media. *Environ Sci Pollut Res* 2015, 22:19124-19132.

[IF 2018 = 2.914] (CIT = 5)

Contribution of P.M.: (estimated **25 %**) contributed to characterization of NPs colloids, conducted part of experimental work and contributed to the manuscript writing

Publications in other scientific reviewed journals

Novotny L, Kabutey A a **Hrda K**: Tentative assessment of the agglomeration kinetics of waste nanopraticles based on its partial similarity to the electrosorption processes. *Waste Forum* 2018, (2):124-131.

> **Contribution of P.M.**: (estimated **20 %**) conducted the characterization of NPs colloids, contributed to the manuscript writing

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> **Contribution of P.M.**: (estimated **60 %**) contributed to planning the study, conducted part of experimental work, evaluated the data and draft the manuscript

Contributions presented at international scientific conferences

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