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PHD SCHOOL OF BIOLOGICAL SCIENCES

PHD DISSERTATION THESIS

Toxicity of nanoparticulate and bulk ZnO in nematodes with different life strategies; *Xiphinema vuittenezi* and *Panagrellus redivivus*

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1. Introduction

The nanotechnology industry is a rapidly developing segment of the world market and its importance is growing in many areas. This term refers to the use of materials in the nano (10^{-9}) size range, i.e. between 1-100 nm in at least one dimension. The main purpose of nanotechnology is not to produce completely new materials or formulations, but to increase the efficiency of formulations by taking advantage of the beneficial properties of size. There are many examples of this benefit, among others in computer technology, medicine, and the production of cosmetics. Preparations made with this technology have already reached consumers, and their production will increase rapidly in the next decade. As a result, their release into the environment is unavoidable, but their impact on the environment and living beings is still largely unknown.

Nanotechnology is one of the fastest-growing areas, therefore environmental toxicology research also attempts to focus on it. Many of the characteristic properties of nanomaterials are based on their high surface area/volume ratio, which is the reason why most of the atoms are located close to the surface. The increased surface area can be used, for example, in environmental protection and wastewater treatment to remove arsenic from drinking water more efficiently.

Nanoparticles can also be formed naturally, such as during forest fires and volcanic eruptions. They can be generated by human activity, but also as an undesirable by-product, for example during the operation of internal combustion (diesel) engines, or during welding and grilling.

Zinc oxide (ZnO) nanoparticles are used in the manufacture of ceramics, cement, glass and many other products. Due to their effective UV light absorption capacity, ZnO nanoparticles are also used in sunscreens and other cosmetic products. The increased release of ZnO nanoparticles into the environment may pose an increased risk.

Engineered nanoparticles have the potential to be used in a wide range of products due to their specific properties caused by the high surface area per volume ratio and the fact that most of the atoms are located close to the surface. While the number of nanotechnology-powered products is increasing, their bioavailability to organisms and possible toxicity are usually unknown. ZnO nanoparticles are used in various technological applications as a consequence of their specific properties. Due to their high catalytic activity, ZnO nanoparticles are used in ceramics, cement, glass, paint and in many consumer products. Because of their effective UV light-absorbing capacity, ZnO nanoparticles are also applied in sunscreens and in other related cosmetic products.

The increased release of nano-ZnO into the environment may cause intensified risk. That is why improving the understanding of the fate and toxicity of nano-ZnO in the environment is an urgent task. Consequently, ecotoxicological studies were performed in water as well as soil environment. Several studies have investigated the effect of ZnO nanoparticles and their bulk counterpart to aquatic or terrestrial invertebrates, including nematodes.

Data about the ecotoxicological effects of nanoparticles and other xenobiotics on nematodes have been derived almost exclusively from tests on only one species, the rhabditid *Caenorhabditis elegans.* However, former studies assume that K-strategist nematodes, which are included in other taxonomic groups, are more sensitive to xenobiotics than the members of Rhabditida.

The plant-feeder nematode, *Xiphinema vuittenezi* (Penetrantia:Dorylaimida) is considered as an economically important dagger nematode species to harm fruit trees, grape and nut production, particularly in Central and Eastern Europe. It is characterised by a wide host range and is adapted to a variety of soil textures. Former studies showed that it can be used well as a test organism in laboratory toxicity and heavy metal uptake studies. Although no laboratory stock

cultures are available, freshly sampled individuals can be stored under laboratory conditions for some weeks for toxicity testing later on. The heavy metal uptake patterns and effects on the concentration of minor and trace elements by *X. vuittenezi* have been intensely studied using several analytical methods by our research group using total reflection X-ray fluorescence (TXRF) spectrometry, the concentration of minor and trace element was investigated. The free-living bacterivor nematode species *Panagrellus redivivus* (Secernentia: Rhabditida) is a model species in many fields of biology and it is used as a food source in aquacultures. This r-strategist nematode can be easily cultured in laboratories. Therefore, it is widely used as a test organism for aquatic and terrestrial toxicity studies with different toxicity endpoints. These r-strategist nematodes are less sensitive to the xenobiotics due to their higher reproduction rate and their ability to survive adverse conditions in a so-called dauer-larvae. There is not much data published about the toxicity of ZnO for other life-strategy groups, for example, *X. vuittenezi*, but several studies have investigated the toxic effects of nano-ZnO particles on *Caenorhabditis elegans.* These studies presented considerably different toxicity data, presumably due to the differences in the test media and the test methods.

1.1 Objectives

The aim of my doctoral research was to investigate the toxic effects of bulk and nano-ZnO on free-living nematodes with different life strategies. In my doctoral research, I aimed to investigate the sensitivity of two free-living nematodes to the toxic effects of zinc. One of the test species was *Panagrellus redivivus*, a formerly widely used ecotoxicological test organism similar to *C. elegans*, while the other was a plant-feeder, K-strategist species, *Xiphinema vuittenezi*.

My experiments focused on three main points:
(i) to study the toxic effects of a commercially available nano-zinc oxide compound in comparison with its bulk counterpart. After the characterization of the particles, we found that the particle size for nano-ZnO was not within the nano size range, either.
ii.) to investigate the toxic effects of targeted nanoparticulated and bulk ZnO.
iii) to support my results, the element content of the nematodes was investigated by microanalytical methods

2. Material and methods

2.1 Test organisms

One of the nematode species used in this research was the K-strategist, plant-feeder *Xiphinema vuittenezi* (Luc, Lima, Weischer and Flegg, 1964). The adult female can be up to 3.8 mm in length, making it a very large species among free-living nematodes. The species breeds mostly parthenogenetically, males being very rare. It is characterised by a wide host range and is adapted to a variety of soil textures. *X. vuittenezi* used in the experiments derived from the same site for all tests. Soil samples were collected from the root system of a cherry tree (Prunus cerasus L., cv. 'Germersdorf') on the outskirts of Budapest, Hungary (N 47° 31' 58.8", E 18° 58' 30"). The soil samples were collected 20-40 cm deep. The nematodes were extracted from the soil using the modified Cobb's sieving method (Brown and Boag 1988). After extraction,

the adult *Xiphinema vuittenezi* females were selected under a microscope with a plastic needle based on such characteristics as the shape, head and tail morphology, vulva position.
The free-living bacterivore nematode species *Panagrellus redivivus* (Secernentia: Rhabditida) is a model species in many fields of biology and it is used as a food source in aquacultures. Stock culture of *Panagrellus redivivus* (Linné 1767) was kept in a cereal-based substrate at 20±1 °C. Adult females of *P. redivivus* were collected under a stereo microscope (Olympus SZH 10, Olympus Optical CO., LTD., Tokyo, Japan) with a plastic needle.

2.2 Toxicity studies involving commercially available zinc oxide

Commercially available ZnO particles (Sigma-Aldrich) were investigated. Based on the measurements of Dr. Zoltán Sávoly, it was found that ZnO is a modification of the two size ranges. Examination of nanoparticle size by dynamic light scattering showed that under nanoscale conditions, the particles typically had a hydrodynamic diameter between 150 nm and 400 nm. This measurement could not be performed with the large particle zinc oxide because the particle size did not allow for it. $ZnSO_4$ (Merck Ltd., Budapest, Hungary) was used to study the effects of zinc ions.
Before starting the tests, nano- and bulk-ZnO suspension of both particles in Milli-Q water and a solution using $ZnSO_4$ were prepared. The suspensions and the solution were sonicated for 30 minutes (Elmasonic S40 device, Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany, 37 kHz, 560 W). Toxicity tests using *Xiphinema vuittenezi* were conducted in 24-well microtiter plates (IWAKI & CO., LTD., Tokyo, Japan). Each well used in the test contained 2 ml treating agent, the control was Milli-Q water. The concentrations were as follows for nano- and bulk-ZnO suspensions: 5, 25, and 50 mg Zn/l. Four replicates per concentration were used.
At the beginning of the test, ten adult females of *Xiphinema vuittenezi* were randomly selected and transferred into each well with a pipette. The animals did not receive food during the 168 hours of exposure time. The experiments were repeated four times for all the treatments. The temperature was kept at 20 ± 1 °C using a thermostat (TS606-CZ/4-WAR; WTW. Weilheim, Germany). At the end of the treatments, mortality was determined by establishing the number of dead nematodes for the given treatment. After an exposure time of 24, 96, 168 hours, the dead nematodes were counted under a stereo microscope. Immobility was considered as the sign of mortality; death was determined as a lack of response to gentle probing with a plastic needle.
The preparation of the suspensions, the preparation of $ZnSO_4$ and toxicity tests with commercially available zinc oxide on *P. redivivus* were the same as described above. In this experiment, lower concentrations were chosen, namely for $ZnSO_4$: 1.625; 3.125; 6.25; 12.5; 25 mg/l, while for bulk- and nano-ZnO suspension: 0.625; 1.25; 3.125; 6.25; 12.5; 25 mg Zn/l. Each well used in the test contained 400 µl treating agent, the control was Milli-Q water. Five replicates per concentration were used. Five adult females were randomly selected and transferred into each well. The animals did not receive food during the exposure time of 24 hours. The microtiter plates were kept in a thermostat during the experiment as mentioned above. Death was clearly established in the case of *P. redivivus*, without any stimulation. The dead individuals assumed a very characteristic straight, elongated shape. The number of dead individuals was recorded.

2.3. Toxicity studies with targeted zinc oxide particles

The targeted ZnO particles were prepared by hydrothermal synthesis at the Department of Applied and Environmental Chemistry, University of Szeged.

The measured particle size (±SD) was 25.08±9.92 nm and 220.92±124.25 nm for nano-ZnO and bulk ZnO particles, respectively. The ZnO nanoparticles and the bulk ZnO were dispersed in Milli-Q water by sonication (Elmasonic S40 device, Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany, 37 kHz, 560 W) for 30 minutes. The toxicity tests were conducted in 24-well polystyrene microtiter plates (IWAKI & CO., LTD., Tokyo, Japan).
Each well used in the tests contained 3 ml treating agent. The concentrations for each test were 0.8; 1.75; 2.5; 5; 10; 25; and 50 mg Zn/l for ZnO nanoparticles and bulk ZnO. The control wells contained only Milli-Q water. Three replicates per concentration were used. At the beginning of the test, ten adult females of *Xiphinema vuittenezi* or *Panagrellus redivivus* were randomly selected and transferred into each well with a pipette. The experiment was conducted on both species without food addition. The plates were incubated at 20±1 °C in a temperature-controlled incubator (TS606-CZ/4-WAR) under dark conditions. After an exposure time of 24 hours, the dead nematodes were counted under a stereo microscope based on the method described in „2.2 Toxicity studies with commercially available zinc oxide".

2.4 Investigation of element uptake of targeted zinc oxide particles

The preparation of nano-ZnO and bulk ZnO suspensions was based on the method described in „2.3 Toxicity studies with targeted zinc oxide particles". The selection of three Zn concentrations for the treatment of nematodes was based on previous results of toxicity tests with targeted zinc oxide particles prepared by the University of Szeged. Low concentration was selected below LC_{50} value, middle concentration around the LC_{50} value and high concentration above the LC_{50} value. Therefore 1, 5 and 25 mg Zn/L concentrations were used for the nano-ZnO and bulk ZnO suspensions. Milli-Q water was used as a control. Five randomly selected adult *X. vuittenezi* or *P. redivivus* females were transferred into each well. The time of exposure was 24 hours, the treatments were performed in 24-well polystyrene microtiter plates as described above. TXRF method was used to determine the concentration of the micro- and trace elements in the nematodes. Before sample preparation, the nematodes were rinsed with Milli-Q water for 1 minute to remove any traces of treating suspension from the body surface. Calcium (Ca), copper (Cu), potassium (K), iron (Fe), zinc (Zn), phosphorus (P) and sulphur (S) concentrations were measured in the nematodes.
After the incubation period of 24 hours, the animals were removed from the treatment medium using a plastic needle. In order to remove any externally adhered ZnO particles from the cuticle, the animals were rinsed for one minute in clean Milli-Q water. According to our previous studies, this step proved to be suitable for the removal of zinc contamination from the body surface of animals. This process was repeated with both treated and control animals.
Then a drop of Milli-Q water was placed on a glass slide, and then the washed nematodes were individually placed in it for taking photographs of their whole body length and greatest body diameter to be measured at vulva level. These pictures served as the basis of calculating the animals' body weights Because nematode masses vary in the µg size range, a volumetric estimation of animal weights was performed, which can be described by the following formula: (Andrássy, 1956):

$$W = (L*D2) / (1,6*106)$$

W: fresh weight (µg)
L: length of the nematode (µm)
D: greatest body diameter (µm)

After this process, the nematodes were moved to a quartz carrier plate using a plastic needle.

One animal was placed on a quartz carrier plate, preferably in the centre. The quartz carrier plate received a unique identification number. Animals were disrupted with 5 µl cc. suprapur HNO_3 solution containing 5 ng gallium internal standard. Subsequently, the quartz carrier plate containing the nematodes were heated at 90 °C for 10 minutes on a glass Petri dish. The element content of the nematodes thus prepared was determined by Dr. Zoltán Sávoly at the TU-Wien Atominstitut in Vienna with an ATOMIKA 8030CTXRF spectrometer (ATOMIKA Instruments GmbH, Germany). The main parameters of the measurements were: Mo Kα excitation (50 kV, 47 mA); Zr20 filter; 500 s measurement time.

3. Results

3.2 Results of the toxicity studies involving commercially available zinc oxide

A concentration-dependent mortality of *Xiphinema vuittenezi* nematode species was observed following an exposure of 24 hours. Nano-ZnO and $ZnSO_4$ exposures had a similar effect on mortality. In contrast, bulk-ZnO was less toxic, with mortality being only 20% at the highest concentration used (50 mg Zn/l) after 24 hours of exposure. The effect of bulk-ZnO on mortality was investigated up to 96 and 168 hours of exposure, respectively. After 96 hours, even the lowest tested concentration (5 mg/l Zn) resulted in 83% mortality. After 168 hours, the mortality was 100% for all tested concentrations.
In the control, which was Milli-Q water, the mortality rate was only 20% after both 96 and 168 hours.
In the case of the other tested species, *Panagrellus redivivus*, in the nano-ZnO test, the first concentration where significant ($p < 0.05$) increase in mortality was found compared to the control was 1.25 mg Zn/l. For bulk-ZnO, 0.625 mg Zn/l was the first effective concentration, while for $ZnSO_4$, this value was 6.25 mg Zn/l. The LC_{50} value for nano-ZnO was 5.48 mg/l. The LC_{50} values for bulk-ZnO and $ZnSO_4$ were calculated as 1.45 mg/L and 7.24 mg/L Zn, respectively.

3.2. Toxicity of targeted ZnO particles (prepared in Szeged)

A concentration-dependent mortality of both nematode species was observed following a 24-h exposure to nano-ZnO and bulk ZnO. Mortality in the control groups was below 10% in all conducted tests. This confirms that both nematode species can be used for this type of test under the conditions applied in this study.
Particle size had a significant effect on the toxicity of ZnO to *X. vuittenezi* (two-way ANOVA, $F = 247.95$, $p<0.001$). Nano-ZnO significantly increased mortality from the concentration of 1.75 mg/L onwards ($p<0.05$). Even at a concentration of 5 mg/L, nano-ZnO was found to be 100% toxic to *X. vuittenezi* (Fig 4. (A)). The 24-h LC_{50} value was calculated as 1.63 mg Zn/L. In contrast, bulk ZnO particles had a considerably lower toxic effect on *X. vuittenezi*, no significant ($p<0.05$) effect was observed up to the concentration of 25 mg Zn/L. The LC_{50} value was determined as 57.77 mg Zn/L for the bulk ZnO.
Particle size also had a significant effect on the toxicity to *P. redivivus* (two-way ANOVA, $F = 12.50$, $p<0.001$). Both materials caused a significant increase in the mortality from a concentration of 1.75 mg/L, and the LC_{50} values for nano-ZnO and bulk ZnO were calculated as 3.34 mg/L and 2.38 mg/L Zn, respectively.

3.3 Results of element uptake of targeted zinc oxide particles

The zinc content and other element content of the animals were investigated by TXRF method.

Summary of results for the element content of untreated individuals from both species are shown in Table 1. The results for the element duration of the animals (mean (± standard deviation)) were given. The element content expressed in ng was divided by the body weight of the animal expressed in µg.

1. table Element content of untreated X. vuittenezi *and* P. redivivus

	Ca	Cu	Fe	K	P	S	Zn
X. vuittenezi	0.79 (±0.09)	0.09 (±0.08)	0.14 (±0.06)	1.95 (±0.19)	6.80 (±5.33)	3.23 (±0.79)	0.26 (±0.07)
P. redivivus	2.4 (±0.97)	0.02 (±0.02)	0.29 (±0.27)	3.09 (±0.52)	7.67 (±3.81)	2.47 (±1.28)	0.11 (±0.09)

Zinc content

The second table shows the measured zinc content of the nematodes. A clear dose-response effect was found at both species (multi-way ANOVA, $F = 10.47$, $p<0.001$). The particle size did not cause any effect on the zinc content of the nematodes in either case. The zinc content in the treated *X. vuittenezi* was significantly higher at the concentration of 25 mg Zn/l as compared to the untreated nematodes, both in the case of nano and bulk ZnO treatments. In case of *P. redivivus*, the zinc content in the treated animals was significantly higher from the concentration of 5 mg Zn/l onwards, as compared to the untreated nematodes both in the case of nano and bulk ZnO treatments.
P. redivivus took up significantly higher zinc than *X. vuittenezi* (multi-way ANOVA, $F = 5.31$, $p = 0.025$).

2. table Zinc content of the nematodes

Test material	Concentration	***X. vuittenezi***	***P. redivivus***
Control	0 mg/l	0.26 (±0.073)	0.11 (±0.09)
Nano-ZnO	1 mg/l	0.83 (±0.120)	0.44 (±0.264)
	5 mg/l	0.70 (±0.091)	1.62 (±0.237)**
	25 mg/l	2.15 (±0.727)**	1.66 (±0.523)**
Bulk-ZnO	1 mg/l	0.63 (±0.344)	0.83 (±0.385)
	5 mg/l	0.70 (±0.223)	1.48 (±0.575)*
	25 mg/l	1.17 (±0.437)*	1.33 (±0.602)*

Potassium content

A significant difference was observed between the two species in the potassium content (multi-way ANOVA, $p<0.001$; $F = 16.5$). The potassium content between the control groups of the two species was different. The control group of *X. vuittenezi* contained significantly less potassium (1.94 ± 0.18 ng/µg) than the control group of *P. redivivus* (3.09 ± 0.52 ng/µg). Regarding the treated *X. vuittenezi* specimens, there were no statistically significant differences compared to the control animals, except in the 1 mg Zn/L nano-ZnO treatment. In case of the treated *P. redivivus* nematodes, the potassium content was significantly lower at each concentration compared to the control animals. The particle size did not have a significant effect on the potassium content of the animals.

Calcium content

There were significant differences in the calcium content between the two species, not only in the treated animals but also in the controls. The calcium content of the control P. redivivus was measured as 2.4 3± 0.97 ng/µg and the calcium content of the control *X. vuittenezi* was 0.79 ± 0.09 ng/µg. The nano-ZnO and bulk ZnO treatments did not have any effect on the calcium content of *X. vuittenezi*, however, the calcium levels of *P. redivivus* in the treated samples were significantly lower compared to the untreated nematodes and showed an apparently decreasing trend in the nano-ZnO treatments. Furthermore, the particle size did not have a significant overall effect on the calcium content of the animals (ANOVA, $F = 1.26$; $p = 0.27$).

Copper, iron, phosphorus and sulphur content

There were no significant differences between the treated and control nematodes. The copper content was significantly different between the two species ($p<0.001$, $F = 46.2$) but was not affected by the treatments.
There was no significant effect of ZnO treatment on the iron content in either the nano or bulk form. The iron content of the untreated *X. vuittenezi* individuals was 0.14 (± 0.06) ng / µg, while that of the untreated *P. redivivus* individuals was 0.29 (± 0.27) ng / µg.
In contrast, neither species nor particle size had any effect on this parameter. Similar phosphorus content was measurable for the two species.

3.4 New scientific results

- Based on the test results on *Xiphinema vuittenezi* in polyester microtiter plate in Milli-Q aqueous medium, this plant-feeder nematode species proved significantly more sensitive to the toxic effects of nano-ZnO than to its bulk counterpart.

- In an ecotoxicological study with *Xiphinema vuittenezi* and *Panagrellus redivivus* nematode species on a polyester microtiter plate in Milli-Q aqueous medium, the plant-feeder *Xiphinema vuittenezi* and the bacterivore *Panagrellus redivivus* showed comparable sensitivity at 25 nm.

- Regarding the elemental uptake and elemental content of the nematode species, I had the following result under the experimental conditions: there was no difference between *Xiphinema vuittenezi* and untreated individuals of *Panagrellus redivivus* in terms of zinc content, iron content, phosphorus content, sulphur content and copper content. However, untreated individuals of *Xiphinema vuittenezi* and *Panagrellus redivivus* differed in potassium and calcium content. Both elements occurred in significantly higher amounts in individuals of *Panagrellus redivivus.*

- I found that nano-and bulk-ZnO exposure may change the element content of nematodes. Zinc-oxide treatment reduced the calcium content and potassium content of the treated *Panagrellus redivivus* individuals, respectively.

4. Conclusions and recommendations

Based on mortality studies involving commercially available zinc oxide, nano-ZnO was found to be much more toxic than its bulk counterpart. However, the rate of mortality was nearly the same as the effect of $ZnSO_4$ solution on mortality. Although this commercial nano-ZnO cannot be classified as a nanomaterial based on its actual particle size, it had different effects on nematodes than its bulk counterpart. Based on studies with $ZnSO_4$ solution, dissolved Zn^{2+} ions are responsible for the toxicity of the nano-ZnO material.

The ZnO particles were prepared at the University of Szeged (nano- (~25 nm) and bulk-ZnO (~221 nm)) had a similar dose-response effect on mortality of *P. redivivus*. This is consistent with the result of a study wherein *C. elegans* was used as a test. The toxic effect of nano-ZnO was stronger in plant-feeder *X. vuittenezi* as compared to bulk ZnO in our study, the effect was shown in LC_{50} values.

Our results showed the difference between the sensitivity of the two species. Although *X. vuittenezi* belongs to the order Dorylaimida, which is in general considered to be a sensitive group (Bongers, 1990).

Element uptake tests were performed with three zinc concentrations. The two species responded to the treatments in a different way. Significantly higher zinc uptake was recorded in *P. redivivus* than in *X. vuittenezi*. The element content of *X. vuittenezi* was less affected by the ZnO treatment. In *X. vuittenezi*, only the highest zinc concentration (25 mg/L) caused a significant increase in the zinc content as compared to the control group. However, in *P. redivivus*, we measured significantly higher zinc amounts as compared to the control, even at a concentration of 5 mg/l. The particle size did not have an effect on the zinc uptake in neither of the two species.

Based on the results, it can be stated that the particle size has a role if the nematodes with different life-strategies reacted differently to nanoparticulate ZnO and bulk counterpart. Furthermore, the plant-feeder *Xiphinema vuittenezi* is significantly more sensitive to nano- than bulk-ZnO. In the case of *P. redivivus*, the particle size did not affect its sensitivity to the toxic effect of ZnO. The sensitivity of the two species is considered to be similar for the 25 nm nano-ZnO treatments performed under the laboratory conditions of this study.

6. Scientific publications

Scientific articles

Publications related to the topic of the dissertation in peer-reviewed journals:

Hrács K., Sávoly Z., Seres A., Kiss L.V., Papp I.Z., Kukovecz Á., Záray G., Nagy P. (2018) Toxicity and uptake of nanoparticulate and bulk ZnO in nematodes with different life strategies. **Ecotoxicology** 8:1058-1068.
DOI: 10.1007/s10646-018-1959-8; IF=1,94

Kiss L.V., **Hrács K.,** Nagy P.I., Seres A. (2018) Effects of Zinc Oxide Nanoparticles on *Panagrellus redivivus* (Nematoda) and *Folsomia candida* (Collembola) in Various Test Media. **International Journal of Environmental Research**, 12:233-243.
DOI: 10.1007/s41742-018-0086-y; IF=1,488

Sávoly Z., **Hrács K.**, Pemmer B., Streli C., Záray Gy., Nagy P.I. (2016)
Uptake and toxicity of nano-ZnO in the plant-feeding nematode, *Xiphinema vuittenezi*: the role of dissolved zinc and nanoparticle-specific effects
Environmental Science and Pollution Research 23 (10): 9669–9678
DOI: 10.1007/s11356-015-5983-4; IF=2,741

Sávoly Z., Buzanich G., Pepponi G., Streli C., **Hrács K.**, Nagy P.I., Záray G. (2015) The fate of nano-ZnO and its bulk counterpart in the body of microscopic nematodes: An X-ray spectrometric study. **Microchemical Journal** 118:80-87.
DOI: 10.1016/j.microc.2014.08.011; IF=2,893

Kiss L.V., **Hrács K.**, Nagy P. I., Seres A. (2015)
Különböző szemcseméretű cink-oxid hatása talajlakó ugróvillás és fonálféreg tesztszervezetekre. ***Állattani Közlemények*** 100:77-88.
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Sávoly Z., Nagy P., Varga G., Havancsák K., **Hrács K.**, Záray G. (2013)
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Microchemical Journal 110:558-567.
DOI: 10.1016/j.microc.2013.07.007, IF=3,05

Kiss L. V., **Hrács K.**, Nagy P. I., Seres A. (2016)
Nano szemcseméretű fém-oxidok hatásai a talajban élő kiemelt ökológiai jelentőségű mikroorganizmusokra – Szemle.
Agrokémia és Talajtan 65:115-134.
DOI: 10.1556/0088.2016.65.1.8

Informative publications related to the topic of the dissertation:

Sávoly Z., **Hrács K. (2015)**
Talajszennyezésről árulkodó fonálférgek: Röntgensugárzás az ökotoxikológia szolgálatában

Élet és Tudomány LXX: 272-274.

Publications not related to the topic of the dissertation:

Daragó Á., Szabó M., **Hrács K.**, Takács A. P., Nagy P.I. (2013)
In vitro investigations on the biological control of *Xiphinema index* with Trichoderma species.
Helminthologia 50: 132-137.
DOI: 10.2478/s11687-013-0121-7; IF=0,776

Seres A., **Hrács K.,** Gyurcsó G., Sárospataki M., Szakálas J., Nagy P.I. (2016)
Laboratory studies on the effects of a neonicotinoid-containing seed treatment product on non-target soil animals. **Columella: Journal of Agricultural and Environmental Sciences** 3:7-14.
DOI: 10.18380/SZIE.COLUM.2016.3.2.7

Conference publications

Scientific presentation:
Seres A., **Hrács K.** , Kiss L. V. , Posta K., Nagy P. (2015)
Nano- és nagyszemcsés cink-oxid hatásainak vizsgálata talajlakó szervezeteken
TOX'2015 Tudományos Konferencia . Konferencia helye, ideje: Harkány, 2015.10.14 - 2015.10.16. p. 30. 1 p.

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