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ECOTOXICOLOGICAL ASSESSMENT OF EMERGING AQUATIC MICROPOLLUTANTS USING PHYSIOLOGICAL ENDPOINTS

Thesis book

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2017

1. INTRODUCTION

During the last few decades emerging micropollutants have attracted great concern. Due to the low removal efficiency of the conventional wastewater treatment processes and the continuous production and use, these micropollutants are present both in natural as well as treated waters. For the above mentioned problems, the establishment of refined and more sensitive environmental toxicological methods could provide solutions in the field of detection and for early warning of the secondary adverse effects of micropollutants on the ecosystem and human health in environmentally relevant concentrations.

For example, an industrial additive may act as hormone analogue, may impair the hormonal system and the immune system or may sensitize based on different mode of actions, this way poses risk on human health and the ecosystem. These phenomena are challenges to environmental toxicologists, because the presence of hundreds and thousands of these pollutants needs not only the screening of selected pollutants by chemical methods, but investigation of their toxic effect. Therefore, it is necessary to collect the most common adverse effects, to analyze the mode of action, then to find the optimal and quantifiable test endpoint in connection with the adverse effect.
Environmental toxicology investigates the toxic effect on organisms of different chemical substances. The characterization of ecotoxicity is a complex task, because the adverse effect depends on several parameters such as concentration, exposure time, the form of the chemical substance, coexisting pollutants in the matrix and the interaction with them, environmental effects and interactions with the environment. For the above mentioned problems, the establishment of refined and more sensitive environmental toxicological methods could provide solutions in the field of detection and early warning of the secondary adverse effects of micropollutants on the ecosystem and human health in environmentally relevant concentrations.

2. BACKGROUND

The exponentially growing contamination of our natural environment and the aquatic ecosystems by chemicals from industrial and agricultural processes and domestic waste streams is one of the most serious problem of humanity is facing nowadays.

In the last few years the ecotoxicological assessment of emerging micropollutants received great concern. Currently extensive researches are being carried out for the investigation of the effects of pollutants in extremely low concentrations in surface waters, groundwater and treated wastewaters and for the efficiency assessment of wastewater treatment processes.

The continuous exposure of the aquatic ecosystem to emerging micropollutants, the permanent presence of these micropollutants in the aquatic environment in low concentrations (ng/L–µg/L) and the low removal efficiency of the conventionally applied wastewater treatment processes suggest that their subtle effects are probably being underestimated, therefore the appropriate risk assessment is a challenge considering the fundamental concept and methodology of environmental toxicology.

There is an urgent need for a more developed, refined test battery in favor of the more efficient environmental management of these environmental micropollutants. Thus new, innovative methods are being developed in the field of environmental- and human toxicology.

The aim is the development of new, more sensitive ecotoxicological endpoints. These new or modified methods can provide more information compared to conventional endpoints (lethality, growth rate) by applying physiological endpoints or detecting different biomarkers (e.g. the presence of enzyme biomarkers show strong connections with the physiological functions of organisms, thus can be more sensitive and may give a sensitive response earlier to the effects of contaminants).

The detection of stress response, heat production, phagocytic activity or the DNA content of the macronucleus of the *Tetrahymena pyriformis* test organism can be sensitive sublethal endpoints. In case of *Daphnia* species - differently from the conventionally applied lethality and immobilization - the popping activity, feeding activity and heartbeat rate are considered to be innovative endpoints.

In case of the *Lemna minor* (duckweed) test organism the conventionally applied test endpoints are the frond number and wet biomass. The total chlorophyll content and the root length can be considered newer test endpoints compared to the previous ones.

The investigation of endocrine disruptive compounds has been of a great interest in the near past. Though the ecotoxicity of these xenoestrogens and other chemicals mimicking hormonal effects is highly investigated, currently there are no perfectly developed criteria or test battery for the characterization of the endocrine disrupting features of these pollutants.

The number and diversity of products and technologies applying engineered nanomaterials including nano titanium dioxide ($nTiO_2$) have increased dramatically in the past two decades. However, the potential benefits of their use to society require reconsideration regarding their potential adverse effects on the environment and human health. Since risk assessment is based on toxicity and exposure knowledge, the risk assessment of engineered nanoparticles (NPs) demands a considerable amount of reliable well-defined data about the characteristics of the NPs and the environment, as well as the biological effect of NPs. Small morphological changes can significantly alter the physical and chemical properties of NPs, so it is essential to investigate the toxic properties of each modification separately. Due to the heterogeneity of intrinsic properties of NPs (e.g. particle size, surface chemistry, shape, surface area and hydrophilic nature) and the physico-chemical characteristics of the exposure conditions (e.g. pH, ionic strength, mono- and divalent ions and organic matter, concentration) it is important in all cases to document the characteristics of the used materials and testing parameters, because the chemical and physico-chemical properties of a nanomaterial essentially dictate the molecular-level interactions. Consequently, the ecotoxicological data should always be presented in conjunction with sufficiently detailed physico-chemical data. There are plenty of other problems to be further explored. Significant contradictions can be found between the results of different authors which are mostly due to the special properties of NPs and can be attributed to differences in the NP behavior in the test media. Therefore the investigation of the dynamic nature of NP agglomeration behavior is also of paramount interest to current studies of environmental nanoscience and nano(eco)toxicology due to the varying bioavailability and toxicity caused by agglomeration processes of NPs.

The current field of interest and challenges of ecotoxicology includes the testing of complex environmental samples, treated and untreated wastewaters, when the effect of the complex and the multicomponent samples is not equal to the aggregated effects of the

individual substances. Environmental pollutants are typically complex mixtures of chemically and toxicologically different chemical forms and species, showing variable bioavailability and interactions with the biotic and abiotic compartments of the environment. Direct toxicity assessment (DTA) can characterize the aggregated effects of unknown contaminants in environmental samples. DTA may ensure high environmental relevance and represents all possible interactions between contaminants, ecosystem members and the matrix. The results aggregate the effects of all hazardous agents present in the sample.

Although the regulatory and technical background has been created, DTA could not break through and become widespread in risk based environmental management yet. Currently, biological and ecotoxicological methods are still secondary to the chemical methods; mainly because professionals and bureaucrats have got accustomed to the chemical approach. It results in a kind of mechanical chemical data/information management which means checking the table of screening concentrations for comparison and trusting exclusively the chemistry based results.

3. AIMS

I. During my PhD work, in line with the novel directions and different fields of environmental toxicology, my primary goals were the problem-specific adaptation, modification and the testing of the feasibility of ecotoxicological methods for the determination of emerging micropollutants at extremely low concentrations in order to use them for early indication of environmental stress factors. During our experiments we investigated the effects of various types of micropollutants like pharmaceuticals and personal care products, industrial additives, pesticides and nanomaterials by applying standardized ecotoxicological test methods and refined non-conventional, sublethal physiological endpoints of the following test systems:

- *Daphnia magna* waterflea – immobilization, lethality and heartbeat rate test,
- *Tetrahymena pyriformis* protozoa – proliferation and phagocytic activity test,
- *Lemna minor* duckweed – growth inhibition (frond number and chlorophyll content).

During our research, we sought for the answer whether these applied test methods are sensitive enough to detect the secondary adverse effects of micropollutants in environmentally relevant concentrations or whether they are suitable as an early warning system.

II. In case of the *Daphnia magna* heartbeat rate test my further aim was to investigate the effect of the influential test parameters and find the optimal experimental conditions especially for detecting the secondary adverse effects of micropollutants in environmentally relevant concentrations.

III. During our experiments the investigation of the toxic effect of the nano-titanium-dioxide (one of the most significant representatives of nanomaterials) had great importance. Since many of the nano-ecotoxicological studies do not report the physico-chemical characteristics of the tested nanomaterials and the applied test media, or the effect of aggregation and the exposure time-dependency of the exerted effect, my aim was to study the particle size-dependent and exposure time-dependent effect of nano titanium-dioxide suspensions together with the physico-chemical characteristics of the tested nanomaterials and the applied test media. To the comprehensive ecotoxicological assessment of nano titanium-dioxide suspensions non-conventional ecotoxiclogical endpoints (the *Daphnia magna* heartbeat rate, the *Tetrahymena pyriformis* phagocytic activity and the *Lemna minor* chlorophyll content) and standardized ecotoxicological methods were applied, such as the algal growth inhibition test (*Chlorella vulgaris, Scenedesmus subspicatus* és *Pseudokirchneriella subcapitata*) and the *Aliivibrio fischeri* bioluminescence inhibition test.

IV. The current field of interest and challenges of ecotoxicology includes the testing of complex environmental samples, treated and untreated wastewaters, when the effect of the complex and multi-component samples is not equal to the aggregated effects of the individual substances. For this problem the so called *Direct Toxicity Assessment (DTA)* approach can be applied, which measures the toxicity of the sample taking into account all possible way of exposure without translating the results into the language of

chemistry. There is a continuous aspiration for the establishment and harmonization of DTA protocols. Although the regulatory and technical background has been created, DTA could not break through and become widespread in risk based environmental management yet and the list of the accepted DTA protocols is very short.
Thus my aim was to prove the feasibility of the *Lemna minor* test system for DTA, however this method currently is not on the list of accepted DTA protocols. My experiments aimed to investigate the feasibility of the total chlorophyll content for the reliable risk assessment of treated and untreated wastewaters.

The above mentioned research fields are connected to each other via the current challenges of environmental toxicology and the applied ecotoxicological test systems as potential solutions to the problems.

4. APPLIED TEST METHODOLOGY

4.1 Ecotoxicological assessment of individual substances

4.1.1 Ecotoxicological assessment of organic micropollutants

The feasibility of different ecotoxicological methods applying sublethal physiological endpoints for the early indication of the toxic effects of aquatic micro-pollutants at environmentally relevant concentrations was investigated.

Tested micropollutants: Na-diclofenac, paracetamol, metamizole-Na, 17β-estradiol, nicotine, triclosan, bisphenol A, dibutyl-phthalate, 3,4-dichloro-phenol, acetochlor, atrazine, diuron, metazachlor, metolachlor in a wide concentration range (0.005 µg/L-100 mg/L).

The applied test battery:

- *Tetrahymena pyriformis* phagocytic activity test (average number of vacuoles in 80 cells)
- *Daphnia magna* heartbeat rate test (determination of the heartbeat rate of 10 organisms under stereomicroscope)
- *Lemna minor* growth inhibition test (total chlorophyll content)

4.1.2 Method development – the investigation of the influential test parameters of the *Daphnia magna* heartbeat rate test

During the method development of the *D. magna* heartbeat rate test to set up the optimal experimental design we studied the following factors at two different levels: the composition of the test medium, the age of the test organism, and the exposure time, at triclosan concentrations 0.2; 2; 20; 200 and 2000 µg/l, which resulted in eight different experimental setups with two parallels.
The optimal experimental setup was chosen based on the following criteria:

- There are as many data point as possible and the imbalance is low.
- Test organisms show an intensive response to increasing pollutant concentrations in the whole tested concentration range.

– The spread of the responses should be low.

4.1.3 The comprehensive ecotoxicological assessment and the particle size-dependent effect of nano titanium-dioxide

For the comprehensive ecotoxicological assessment of nano titanium-dioxide suspensions, beside the ecotoxicological testing, the complex physico-chemical characterization was also carried out in the applied test media.

Test substances: 0,1 M HCl activated Degussa VP P90 $nTiO_2$ suspension, AERODISP® W 740 X and W 2370 X $nTiO_2$ suspensions (Evonik Resource Efficiency Kft.), AFDC200 (Kemira Ltd.).

The zeta potential and the hydrodynamic diameter were determined by the Dynamic Light Scattering method (DLS) with Malvern Zetasizer ZS (Malvern Instruments, UK). In the applied test media the pH and electric conductivity were also determined.

The components of the applied test battery:

- *Aliivibrio fischeri* bioluminescence intensity test with prolonged exposure time (120 min)
- Algal growth inhibition tests (*Chlorella vulgaris, Scenedesmus subspicatus, Pseudokirchneriella subcapitata*)
- *Tetrahymena pyriformis* phagocytic activity test (average number of vacuoles in 80 cells)
- *Daphnia magna* heartbeat rate test (determination of the heartbeat rate of 10 organisms under stereomicroscope)
- *Lemna minor* growth inhibition test (total chlorophyll content)

4.2 Direct toxicity assessment (DTA)

Tested wastewater samples: Samples were taken from a biological wastewater treatment plant containing a tertiary treatment unit. The effect of the treated and untreated wastewater samples from two different sampling times (July and August, 2010) was determined.

The applied test battery:

- *Aliivibrio fischeri* bioluminescence intensity test
- Algal growth inhibition tests (*Chlorella vulgaris, Scenedesmus subspicatus, Pseudokirchneriella subcapitata*)
- *Daphnia magna* immobilization
- *Lemna minor* growth inhibition (frond number and chlorophyll content)

For the detection of micro-pollutants in the treated and untreated wastewater samples a sensitive chemical-analytical method was applied. Analytical characterization was carried out at the Eötvös Loránd University by trimethylsilyl (oxime) ether/ester derivatives, gas

chromatography-(tandem) mass spectrometry (Varian 4000-GC-MS/MS system (Walnut Creek, CA, USA)).

5. RESULTS AND DISCUSSION

5.1 *Tetrahymena pyriformis* phagocytic activity test

In the frame of my PhD work one of my aims was to test the feasibility of the modulation of the phagocytic activity as a sensitive ecotoxicological endpoint for the determination of the toxic effect of aquatic micropollutants (diclofenac, 17β-estradiol, 3,4-dichloro-phenol, atrazine, bisphenol A, and dibutyl-phthalate) at ng–µg/L concentrations.

According to our results, the *T. pyriformis* phagocytic activity test is a feasible test method for the early indication of the adverse effects of these micropollutants at environmentally relevant concentrations (0,1–0.005 µg/L). We proved that the *T. pyriformis* phagocytic activity test system with its short exposure time (30 min) was highly sensitive to xenoestrogens at environmentally relevant concentrations (Table 1). The supposed mode of action is the binding of these xenoestrogens to the steroid receptor-like structures of the *T. pyriformis* cell, hence the disturbance of the hormonal homeostasis of the cell.

Table 1: The effect of the tested model substances on the phagocytic activity of *T. pyriformis*

Atrazine		**Bisphenol A**		**Dibutyl-phthalate**		**17β-estradiol**		**3,4-dichloro-phenol**		**Na-diclofenac**	
Conc. [µg/L]	**H [%]**	**Conc. [µg/L]**	**H [%]**	**Conc. [µg/L]**	**H [%]**	**Conc. [µg/L]**	**H [%]**	**Conc. [µg/L]**	**H [%]**	**Conc. [µg/L]**	**H [%]**
0.05*	16	0.1*	58	0.05*	19	0.01*	14	0.005*	23	0.01*	−36
0.5	35	1	88	0.5	22	0.1	51	0.05	26	0.1	−42
5	80	10	−67	5	38	1	64	0.5	59	1	−21
50	86	100	−33	50	48	10	57	5	100	10	−29
500	81	1000	81	500	74	100	79	50	100	100	−30

*Lowest Significant Effect (LSE)
H%: inhibition in % values

Due to the high importance of protozoa in the food web, they are an important member of the aquatic ecosystem. The modulation of their feeding activity may affect the ecological health of aquatic ecosystems.

5.2 *Daphnia magna* heartbeat rate test

One of my goal was to test the feasibility of the *Daphnia magna* heartbeat rate as a sensitive ecotoxicological endpoint for the determination of the toxic effect of a wide array of aquatic micropollutants. Based on our results of the *Daphnia magna* heartbeat rate test it has been proved that 17β-estradiol, diclofenac, triclosan, and metazachlor may exert their toxic effect in environmentally relevant concentrations and affect the heartbeat rate of this planktonic crustacean (Table 2). However, the exact mechanism underlying the toxic effect of these individual substances on the heartbeat rate of *Daphnia magna* are not clear yet, it can be assumed that it happens through the inhibition of the COX-2 enzyme system or oxidative

stress. The decreased or accelerated heartbeat rate may be an early bioindication of the secondary adverse effects of these emerging micropollutants.

Table 2: The comparision of the sensitivity of the *D. magna* heartbeat rate and the immobilization tests

Model substance	LSE [µg/L] – 48 h	
	Heartbeat rate	**Immobilization**
Metazachlor	***0.05***	***> 5000***
Na-diclofenac	***0.1***	***> 10,000***
17β-estradiol	***0.1***	***> 1000***
Triclosan	***0.5***	***50***
Paracetamol	500	>5000
Nicotine	1000	1000

5.3 Analysis of the influential test parameters of the *Daphnia magna* heartbeat rate test

After the sensitivity of the *Daphnia magna* heartbeat rate test system was proved, in order to find the optimal test conditions of the *Daphnia magna* heartbeat rate test system the effect of the exposure time, the age of the test organism and the applied test media were investigated. To the evaluation of the results of the eight different experimental setups, we established a statistical method based on the relative inhibition sum of ranks (SOR) (Table 3). This evaluation method eliminates the error of evaluation due to the unbalanced design caused by the perished test organisms, the segregation of test runs in time and the limits of randomization of the experiments. In contrary, this method takes into account the sensitive response of the measurement endpoint in the whole range of applied test concentrations and the standard deviations. According to the SOR evaluation the optimal test setup assumes 10-day-old test organisms, 48 h exposure time and dechlorinated tap water as test medium.

Table 3: Sum of Ranks (SOR) and EC_{50} values [µg/L] of different experimental setups

Test medium	Age of test organism	Exposure time	SOR	EC_{50}	LCI[a]	UCI[b]
Csapvíz	***10 day***	***48 h***	***53***	***35.2***	***6.55***	***276***
Tapwater	3 day	24 h	75	303	207	466
Tapwater	10 day	24 h	81	327	200	587
M7	3 day	48 h	86	334	243	480
M7	3 day	24 h	91	329	247	450
M7	10 day	24 h	92	340	259	453
M7	10 day	48 h	96	379	310	469
Tapwater	3 day	48 h	106	369	315	437

[a]LCI: Lower Confidence Interval

[b]UCI: Upper Confidence Interval

[c]EC_{50} values were derived with OriginLab 8.0 software logistic dose-respons function y = A1 + (A2−A1)/(1 + 10^((LOGx0−x)*p)).

5.4 Determination of the toxic effect of nano titanium-dioxide on the aquatic ecosystem in terms of particles size and concentration

We proved that the aggregation phenomenon of nano titanium-dioxide affects toxicity both in ecotoxicological test media as well as in natural waters.

The aggregation of nanoparticles do not necessarily lower the inhibitory effect due to decreased bioavailability, namely aggregated micro-size particles can exert their toxic effect via binding the nutritional components essential for the test organism. We proved that the primary and secondary particle size of nano titanium-dioxide essentially dictate the interaction between the nanoparticles and the test organism, however their toxicity is highly affected by the exposure routes. E.g. in case of *D. magna* the aggregated micro-size particles can exert toxic effect during feeding and may stuck in the alimentary canal of the test organism.

We demonstrated, that the exposure time has a significant effect on the toxicity of nano titanium-dioxide, and we proved that the physico-chemical characterization (pH, electric conductivity, hydrodynamic diameter) of the aggregation phenomenon of nanoparticles in the applied test media is necessary in every case, because this information is essential for the discussion of the exerted toxicity in terms of exposure and bioavailability (Table 4).
We demonstrated that the ecotoxicological test battery (test organisms representing different trophic levels of the ecosystems: the *A. fischeri* bioluminescence inhibition test with prolonged exposure time, the *Tetrahymena pyriformis* phagocytic activity assay, the *Daphnia magna* heartbeat rate test and the *Lemna minor* chlorophyll content test system) used in our study sensitively indicated the effect of nano titanium-dioxide as well as of the toxicity of their aggregated forms.

Table 4: The lowest significant effect concentrations of the tested $nTiO_2$ suspensions

Test organism	Ecotoxicity endpoint	Lowest significant effect [µg/L]			
		W 2730X 16 nm	W 740X 36 nm	P90[M] 89 nm	AFDC200 3264 nm
Aliivibrio fischeri	bioluminescence (120 min)	1	0.1	10,000	10
Tetrahymena pyriformis	phagocytic activity (30 min)	0.1	1000	0.1	0.1
Daphnia magna	heartbeat rate (48 h)	100	10,000	10,000	1
Lemna minor	chlorophyll content (7 day)	1	1	1	0.1

5.5 *Lemna minor* growth inhibition test with the determination of the chlorophyll content.

According to the results of the *L. minor* chlorophyll content assay outstanding sensitivity was experienced in case of pesticides and 3,4-dichloro-phenol in environmentally relevant concentration range (ng/L– µg/L) (Table 5). The decrease of chlorophyll content caused by pesticides can obviously be attributed to their primary mode of action. The structural similarity of 3,4-dichloro-phenol assumes a similar mode of action as in case of pesticides.

Table 5: Lowest Observed Effect Concentration (LOEC) values of the tested chemical substances

Model substance	LOEC [mg/L]
Acetochlor	***0.00005***
Metolachlor	***0.0005***
Diuron	***0.0005***
3,4-dichloro-phenol	***0.0025***
Metazachlor	***0.005***
Metamizole-Na	0.01
Atrazine	0.1
Na-diklofenák	3.125
Biszfenol A	6.25

5.6 Direct toxicity assessment with the *Lemna minor* test system

The practicability of the *Lemna minor* test system was examined through the assessment of the efficiency of a biological wastewater treatment plant and the risk characterization of the treated wastewater samples, by characterizing the aggregated effects of unknown contaminants and representing all possible interactions between contaminants, ecosystem members and the matrix. Although the regulatory and technical background has been created, DTA could not break through and become widespread in risk based environmental management yet.

In case of the toxicological assessment of treated and untreated wastewaters the *Lemna minor* chlorophyll content test system showed outstanding sensitivity compared to the *Aliivibrio fischeri* bioluminescence intensity test and standardized algal growth inhibition tests (already accepted DTA protocols) however this method currently is not on the list of accepted DTA protocols.

In order to characterize the efficiency of the wastewater treatment plant and the potential environmental risk or the necessary risk reduction rate, the following practical indicators were derived (Table 6):

- ESP (%): effective sample proportion;
- RCR (–): risk characterization ratio or relative risk based on toxicity: $ESP_{sample}/ESP_{reference}$, $ESP_{reference}$ is 100%;
- RRR (–): necessary toxicity attenuation i.e. risk reduction rate to reach the targeted toxicity: $ESP_{sample}/ESP_{target}$, $RRR_{20} = ESP_{sample}/80\%$.

Table 6: Practical indicators of the toxicity of the treated wastewater samples

	[a]ESP (%)		**[b]RCR**		**[c]RRR_{20}**	
Sampling time	1	2	1	2	1	2
Frond number	84	67	1.2	1.5	1.0x	1.2x
Chlorophyll content	53	40	1.9	2.5	1.5x	2.0x

6. NOVEL SCIENTIFIC FINDINGS

1. I proved the feasibility of test organisms of different trophic levels (*Tetrahymena pyriformis* and *Daphnia magna*) – refined with the examination of physiological endpoints – to detect biological effects of micro-pollutants at environmentally relevant concentrations.

    a. I concluded that the *Tetrahymena pyriformis* phagocytic activity test gives quantifiable and significant response to 17β-estradiol, and the tested xenoestrogens – 3,4-dichlorophenol, atrazine, bisphenol A and dibutyl-phthalate – at environmentally relevant concentrations (ng/L–µg/L). This method is a feasible qualitative tool for the early warning of the adverse effects of xenoestrogens. (VIII)

    b. I demonstrated with the *Daphnia magna* heartbeat rate test that 17β-estradiol, diclofenac, triclosan, and metazachlor exert their toxic effect in environmentally relevant concentrations (17β-estradiol: 0.1 µg/L; diclofenac: 0.1 µg/L; triclosan: 0.5 µg/L; metazachlor: 0.05 µg/L) and affect the heartbeat rate of this planktonic crustacean. The heartbeat rate as a sublethal endpoint proved to be more sensitive than the conventional mortality and immobilization endpoints; furthermore its great advantage that we gain plus information about the mode of action of the model substances. (III).

2. In order to find the optimal test conditions of the *Daphnia magna* heartbeat rate test system, I established a statistical method based on the relative inhibition sum of ranks (SOR). This statistical method takes into account the extent of the intensive response to increasing pollutant concentrations in the whole concentration range and the spread of the responses. According to the evaluation of test results the optimal test condition for the heartbeat rate test assumes tap water as test medium, 10-day-old test organisms and 48 h exposure time. (VII)

3. I proved that the nano titanium dioxide can exert toxic effect at environmentally relevant concentrations by carrying out the comprehensive ecotoxicological assessment of the effect of nano titanium-dioxide suspension on several aquatic test organisms using sublethal endpoints. (I, VI)

    a. I demonstrated, that the exposure time has a significant effect on the toxicity of nano titanium-dioxide.

    b. Based on my results I considered to be grounded that the physico-chemical characterization (pH, electric conductivity, zeta potential, hydrodynamic diameter) of the aggregation phenomenon of nanoparticles in the applied test media is necessary in every case, because this information is essential for the discussion of the exerted toxicity in terms of exposure and bioavailability.

    c. I demonstrated that the ecotoxicological test battery (test organisms representing different trophic levels of the aquatic ecosystem (the *A. fischeri* bioluminescence inhibition test with prolonged exposure time, the *Tetrahymena pyriformis* phagocytic activity assay, the *Daphnia magna* heartbeat rate test and the *Lemna minor* chlorophyll content test) used in my study sensitively indicated the effect of nano

titanium-dioxide as well as of the toxicity of its aggregated forms based on different toxicity mechanisms. I suggest the use of this complex test battery when assessing the toxic effect of nano titanium-dioxide.

4. I proved the feasibility of the *Lemna minor* chlorophyll content test system for the *Direct Toxicity Assessment (DTA)* of treated and untreated wastewater samples and for the assessment of the aggregated effect of a multi-component, complex sample and for the sensitive indication of the actual toxicity. (II; IV; V)
    a. I proved that the *Lemna minor* growth inhibition test based on the chlorophyll content determination was a far more sensitive method - for the toxicity assessment of wastewater samples containing the complex mixture of micro-pollutants – than the already accepted DTA tests: the *Pseudokirchneriella subcapitata* growth inhibition test and the *Daphnia magna* immobilization test.
    b. The practical indicators derived from the direct toxicity assessment results – *ESP%: Effective Sample Proportion* and *RCR: Risk Characterization Ratio* – give direct information on the toxicity of the samples.
    c. Based on my results from *RRR: Risk Reduction Rate* we can identify the necessary dilution rate to reach no inhibition or the targeted toxicity.

7. CONCLUSIONS, POTENTIAL APPLICATIONS

In recent years attention was focused on pharmaceuticals, cosmetics, industrial additives, pesticides and nanomaterials, because their appearance in different water matrices, hence the exposure of the aquatic ecosystem is continuous. At the same time in most cases we know very little about the secondary adverse effects of these micropollutants and their metabolites.

The *Tetrahymena pyriformis* phagocytic activity test and the *Daphnia magna* heartbeat rate test may be feasible solutions – with the refined physiological endpoints – for the early indication of the adverse effects of aquatic micropollutants at environmentally relevant concentration.

The *Lemna minor* test system based on the determination of the chlorophyll content may be a feasible solution for the direct toxicity assessment of treated and untreated wastewaters, furthermore the practical parameters – as for example the *RRR: Risk Reduction Rate* – derived from the direct toxicity assessment are suitable for the determination of the necessary dilution rate of the tested wastewater samples.

The above mentioned test system may have significant role both in the early indication of the risk of emerging pollutants as well as in the monitoring systems investigating water quality and in early warning systems. Besides, these results may serve as a basis for the separated treatment of the risky wastewaters, their specific treatment and the creation of inciting laws.

8. PUBLICATIONS

Papers on which the thesis was based:

I. **Fekete-Kertész, I.**, Maros, G., Gruiz, K., Molnár, M., 2016. The effect of TiO_2 nanoparticles on the aquatic ecosystem: a comparative ecotoxicity study with test organisms of different trophic levels. *Periodica Polytechnica Chemical Enginering* 60(4),231–243. IF: 0,46

II. Gruiz, K., **Fekete-Kertész, I.**, Kunglné-Nagy, Zs., Hajdu, Cs., Feigl, V., Vaszita, E., Molnár, M., 2016. Direct toxicity assessment – Methods, evaluation, interpretation. *Science of the Total Environment* 563–564, 803–12. IF: 3,976

III. **Fekete-Kertész, I.**, Kunglné-Nagy, Zs., Molnár, M., 2016. Ecological impact of micropollutants on aquatic life determined by an innovative sublethal endpoint *Daphnia magna* heartbeat rate. *Carpathian Journal of Earth and Environmental Sciences* 11(2), 345–354. IF: 0,73

IV. **Fekete-Kertész, I.**, Kunglné-Nagy, Zs., Gruiz, K., Magyar, Á., Farkas, É., Molnár, M., 2015. Assessing toxicity of organic aquatic micropollutants based on the total chlorophyll content of *Lemna minor* as a sensitive endpoint. *Periodica Polytechnica Chemical Enginering* 59(4), 262–271. IF: 0,46

V. Nagy, Zs.M., Molnár, M., **Fekete-Kertész, I.**, Molnár-Perl, I., Fenyvesi, É., Gruiz, K., 2014. Removal of emerging micropollutants from water using cyclodextrin. *Science of the Total Environment* 485–486, 711–719. IF: 3,163

Papers under review:

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VII. **Fekete-Kertész, I.**, Stirling, T., Ullmann, O., Farkas, É., Kirchkeszner, Cs., Feigl, V., Molnár, M., 2017. How does experimental design modify the result of *Daphnia magna* heartbeat rate test? ─ Analyses of factors affecting the sensitivity of the test system. *Marine and Freshwater Behaviour and Physiology*

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