



# Determination of the ecotoxicity of silver nanoparticles, chitosan, titanium dioxide and thymol with *Vibrio fischeri*, *Chlorella* sp. and *Lemna minor*

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# Acknowledgment

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# Main goal

Assessment of ecotoxicological impact of silver nanoparticles, chitosan, titanium dioxide and thymol by conducting ecotoxicity tests with the marine bacterium *Vibrio fischeri*, microalgae *Chlorella* sp. and duckweed *Lemna minor*.

# Introduction

Plastic pollution is a growing issue due to the persistence of synthetic polymers in the environment.



- Coatings using inkjet-printed **silver nanoparticles (AgNPs)** on food-grade polymer packaging
- ❖ Inhibition of microbial growth, upgraded food safety and shelf life, without altering food's sensory qualities



## Chitosan

- ❖ Biodegradable polymer which offers an eco-friendlier alternative to conventional polymers
- ❖ Breaks down quicker and reduces long-term harm



## Titanium dioxide (TiO<sub>2</sub>) and thymol

- ❖ Additives used to enhance properties of biopolymers, which often lack the mechanical strength and durability of conventional plastics
- ❖ TiO<sub>2</sub> improves UV stability and strength, while thymol provides antimicrobial protection



→ widely found in a wide variety of products → their release into the environment raises ecological concerns



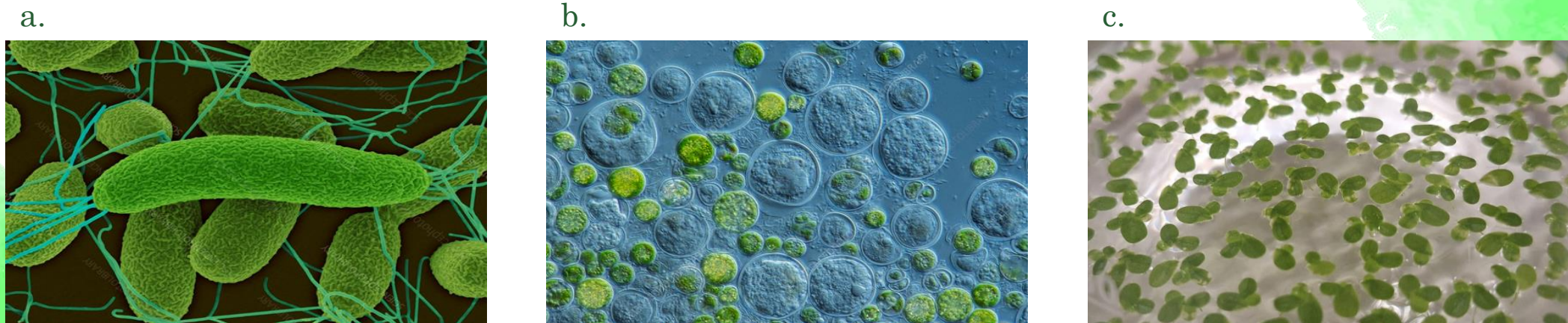
# Introduction

- ❖ **Ecotoxicology** – an interdisciplinary science that studies the effects of the presence of natural or artificially created toxic hazardous substances on all living organisms, i.e. microorganisms, plants, animals, humans and all other components of the ecosystem
- ❖ **Ecotoxicity tests** – are used to assess the effect of a pollutant on the survival, growth, reproduction and behavior of the organisms being tested
- ❖ **Toxicity classification:**
  - Highly toxic substances:  $EC_{50} \leq 1 \text{ mg/L}$
  - Moderately toxic substances:  $1 \text{ mg/L} < EC_{50} \leq 10 \text{ mg/L}$
  - Low toxic substances:  $10 \text{ mg/L} < EC_{50} \leq 100 \text{ mg/L}$

$EC_{20}$   
 $EC_{50}$

# Ecotoxicity tests and test microorganisms

- ❖ Microorganisms used to conduct ecotoxicity tests: bacterium *Vibrio fischeri*, microalgae *Chlorella* sp. and duckweed *Lemna minor*.



**Figure 1.** Presentation of the marine bacterium *Vibrio fischeri* (a.) and microalgae *Chlorella* sp. (b.), as well as duckweed *Lemna minor* (c.).



# Ecotoxicity test with *Vibrio fischeri*

- ❖ Aerobic toxicity determination method - in accordance with *HRN/EN ISO 11348-1:2000 en, Water quality – Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri, Method using freshly prepared bacteria*
  - assessment of reduction of physiological activity of pure culture
- ❖ Bioluminescence – oxidation of the luciferin molecule (Flavin-mononucleotide)

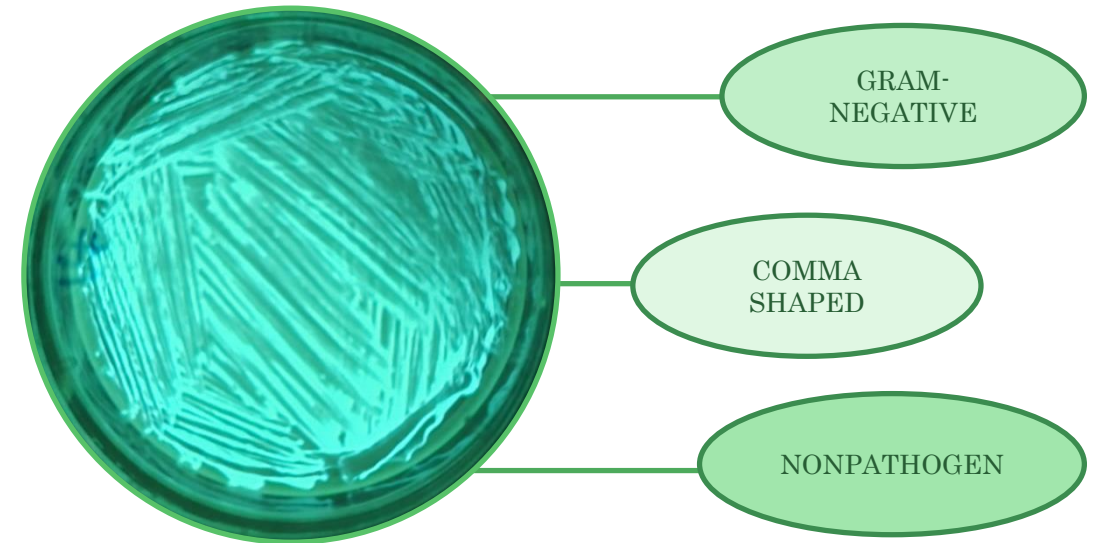


Figure 2. Luminometer.



# Ecotoxicity test with *Chlorella* sp.

- ❖ Principle: exposure of exponentially growing *Chlorella* sp. culture to different concentrations of tested compounds - in accordance with *OECD guidelines for the testing of chemicals - Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011)*
- ❖ Inhibition of microalgae growth is observed over 72 hours



Figure 3. Densely grown suspension of microalgae *Chlorella* sp.

# Ecotoxicity test with *Lemna minor*

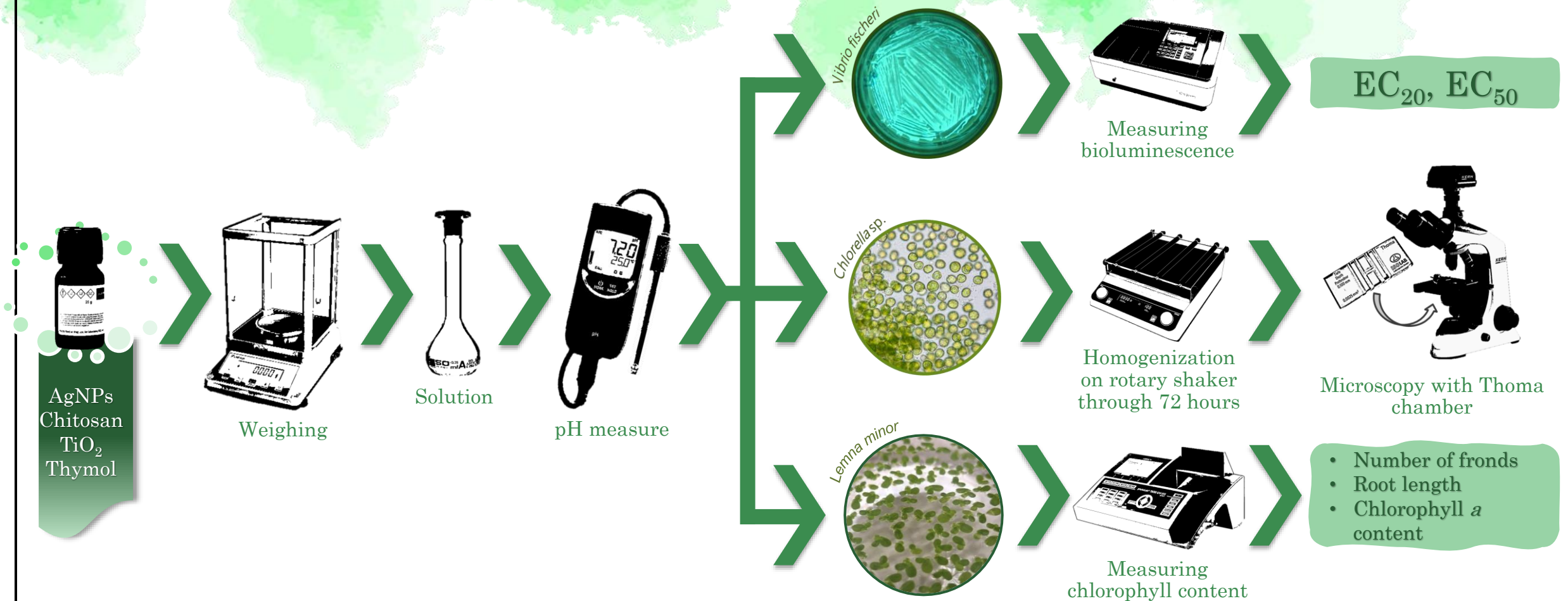
- ❖ Exposure of *Lemna minor* culture to the test substances for 7 days was conducted in accordance with *OECD guidelines for the testing of chemicals - Lemna sp. Growth Inhibition Test (2006)*
- ❖ The following were determined:
  - number of fronds
  - root length
  - chlorophyll *a* content



Figure 4. Duckweed *Lemna minor*.



# Schematic diagram



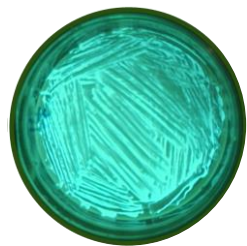
# Used concentrations

Table 1. Concentrations of AgNPs, chitosan, TiO<sub>2</sub> and thymol used in ecotoxicity tests with *Vibrio fischeri*, *Chlorella* sp. and *Lemna minor*.

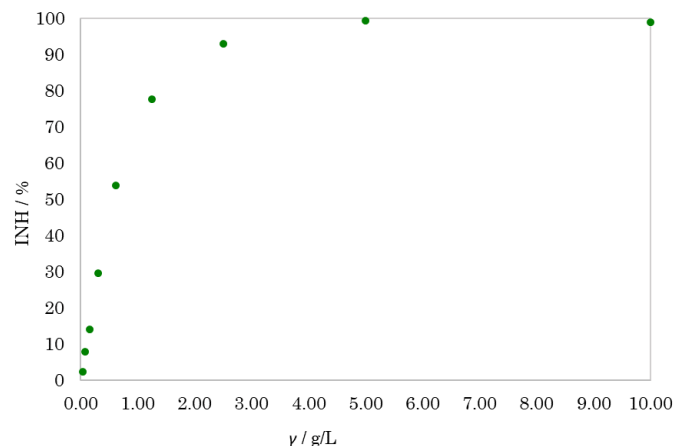
	<i>Vibrio fischeri</i>	<i>Chlorella</i> sp.	<i>Lemna minor</i>
AgNPs	10 g/L	1, 3, 5, 7, 10 g/L	1, 3, 5, 7, 10 g/L
Chitosan	1.0 g/L	0.1, 0.3, 0.5, 0.7, 1.0 g/L	0.1, 0.3, 0.5, 0.7, 1.0 g/L
TiO <sub>2</sub>	1.0 g/L	0.1, 0.3, 0.5, 0.7, 1.0 g/L	0.1, 0.3, 0.5, 0.7, 1.0 g/L
Thymol	1.0 g/L	10, 30, 50, 70, 100 g/L	10, 30, 50, 70, 100 g/L



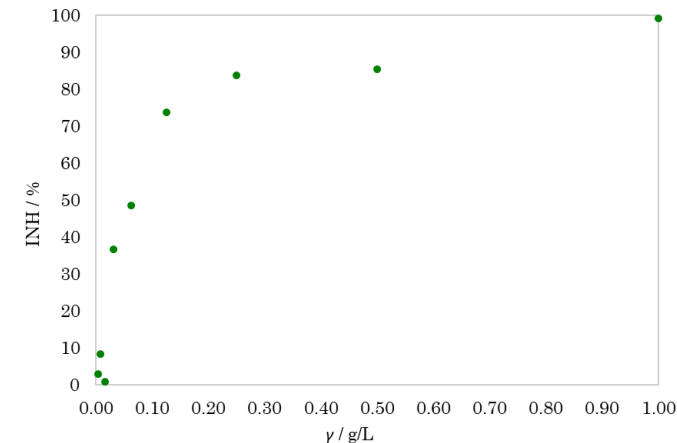
# RESULTS



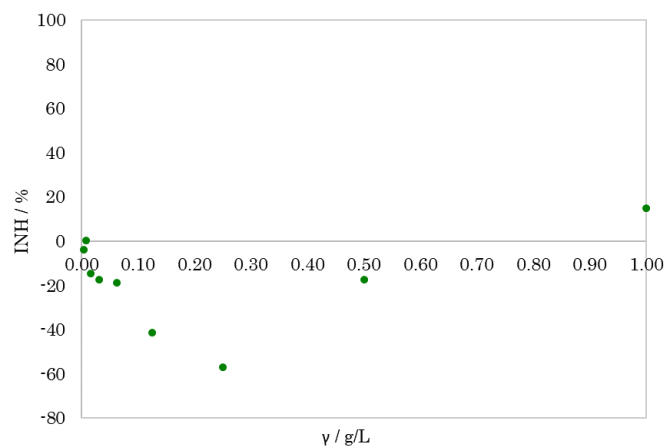
## Results – *Vibrio fischeri*



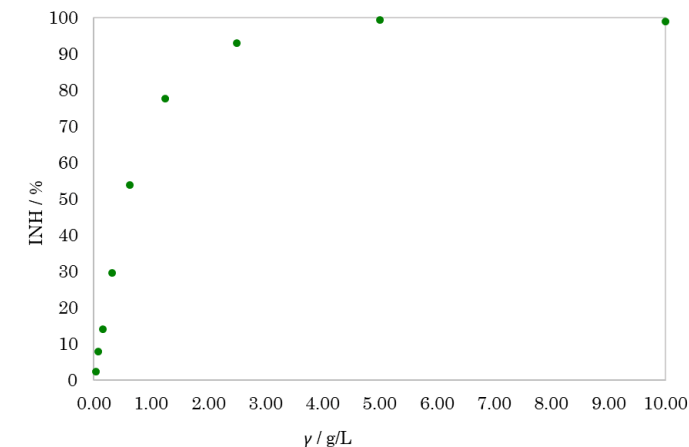
**Figure 5.** Changes in bioluminescence inhibition during exposure to AgNPs at a concentration of 10.0 g/L.



**Figure 6.** Changes in bioluminescence inhibition during exposure to chitosan solution at a concentration of 1.0 g/L.

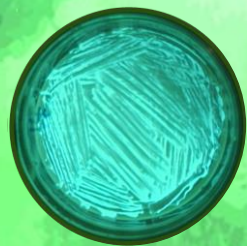


**Figure 7.** Changes in bioluminescence inhibition during exposure to TiO<sub>2</sub> solution at a concentration of 1.0 g/L.



**Figure 8.** Changes in bioluminescence inhibition during exposure to thymol solution at a concentration of 1.0 g/L.





## Results – *Vibrio fischeri*

Table 2. Obtained inhibition values and effective concentrations for AgNPs, chitosan, TiO<sub>2</sub> and thymol by ecotoxicity tests with *Vibrio fischeri*.

Substance	Concentration / g/L	INH / %	EC <sub>20</sub> / g/L	EC <sub>50</sub> / g/L
AgNPs	10	98.89	0.210	0.450
Chitosan	1.0	99.19	0.016	0.049
TiO <sub>2</sub>	1.0	14.82	/*	/*
Thymol	1.0	100	0.016	0.035

/\* - values could not be estimated



# Results – *Chlorella* sp.

Table 3. Initial conditions in ecotoxicity tests with *Chlorella* sp.

Substance	OG <sub>0</sub> / -	CFU <sub>0</sub> / cell/mL	pH <sub>0</sub> / -	ν <sub>0</sub> (O <sub>2</sub> ) / mg/L	T <sub>0</sub> / °C
AgNPs	0.02	8.0*10 <sup>5</sup>	8.071	8.26	23.0
Chitosan	0.04	4.3*10 <sup>5</sup>	8.000	8.07	24.0
TiO <sub>2</sub>	0.07	9.6*10 <sup>5</sup>	7.870	7.33	25.1
Thymol	0.06	7.5*10 <sup>5</sup>	7.800	8.20	23.8

Table 4. Observed final conditions in ecotoxicity tests with *Chlorella* sp.

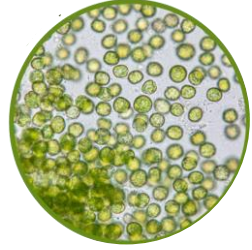
Substance	pH / -	ν(O <sub>2</sub> ) / mg/L
AgNPs	7.670	7.76
Chitosan	7.745	8.10
TiO <sub>2</sub>	7.742	7.87
Thymol	7.510	7.86

$$CFU = \frac{m * n * 16 * 10^4}{K}$$

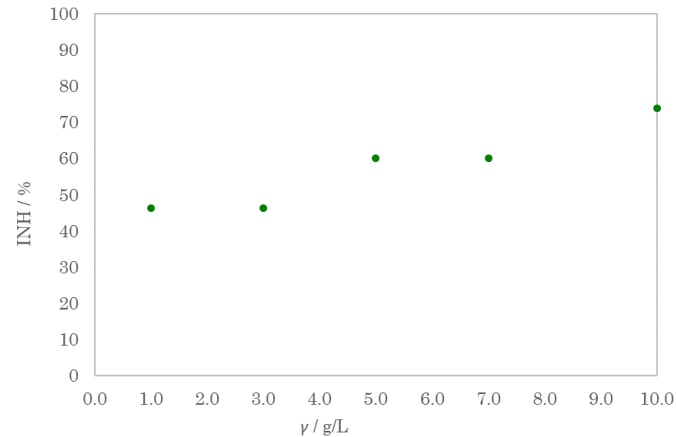


$$INH = \frac{CFU(control) - CFU(sample)}{CFU(control)} \times 100$$

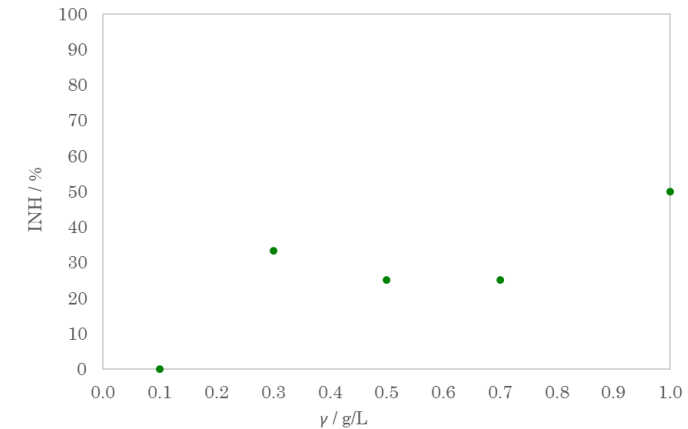




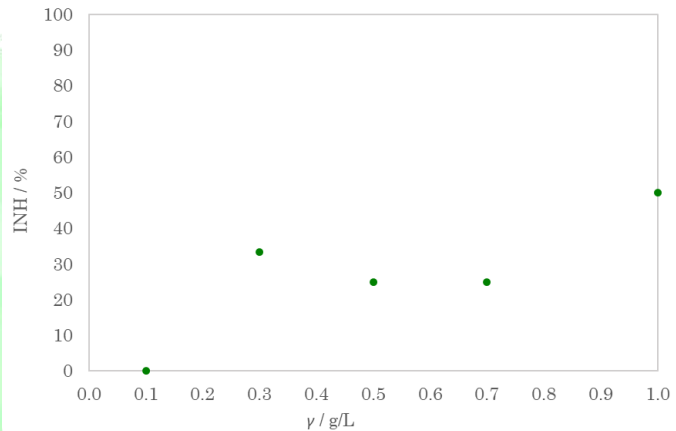
## Results – *Chlorella* sp.



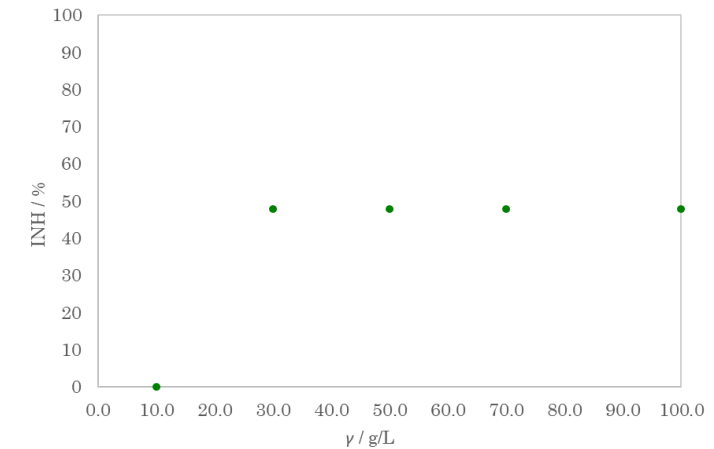
**Figure 9.** Inhibition of microalgal growth during exposure to AgNPs at a concentration of 10.0 g/L after 72 hours.



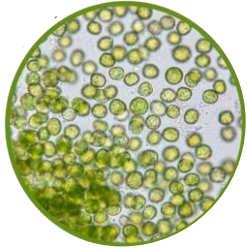
**Figure 10.** Inhibition of microalgal growth during exposure to chitosan solution at a concentration of 1.0 g/L after 72 hours.



**Figure 11.** Inhibition of microalgal growth during exposure to TiO<sub>2</sub> solution at a concentration of 1.0 g/L after 72 hours.



**Figure 12.** Inhibition of microalgal growth during exposure to thymol solution at a concentration of 100.0 g/L after 72 hours.



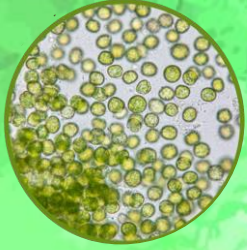
# Results – *Chlorella* sp.

**Table 5.** Obtained inhibition values and effective concentrations for AgNPs, chitosan, TiO<sub>2</sub> and thymol used by ecotoxicity tests with *Chlorella* sp.

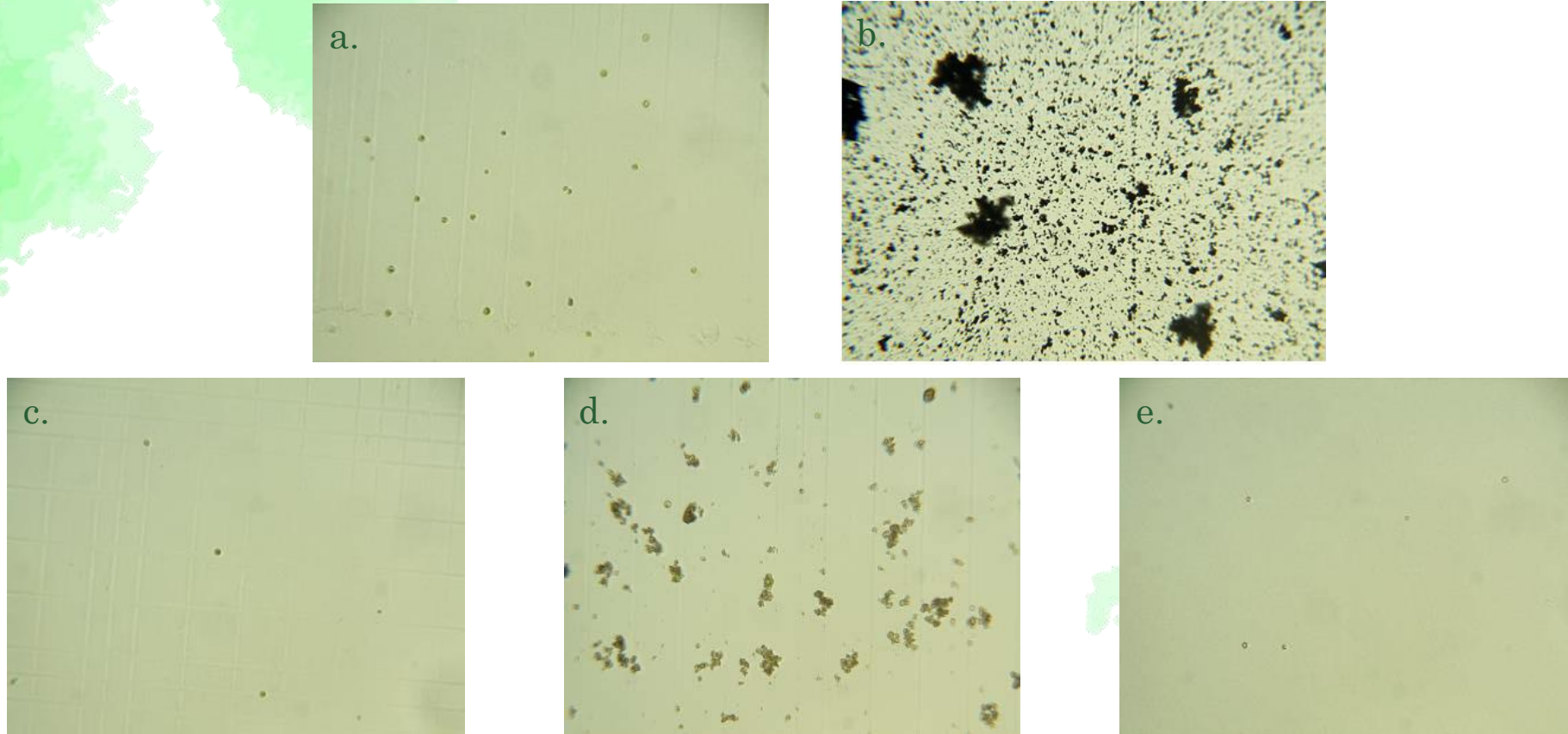
Substance	Concentration / g/L	INH / %	EC <sub>20</sub> / g/L	EC <sub>50</sub> / g/L
AgNPs	1.0	46.25	/*	1.518
	3.0	46.25		
	5.0	60.00		
	7.0	60.00		
	10	73.75		
Chitosan	0.1	0	/*	0.410
	0.3	33.33		
	0.5	25.00		
	0.7	25.00		
	1.0	50.00		
TiO <sub>2</sub>	0.1	6.67	0.169	0.357
	0.3	60.00		
	0.5	60.00		
	0.7	73.33		
	1.0	73.33		
Thymol	10	0	/*	8.845
	30	48.00		
	50	48.00		
	70	48.00		
	100	48.00		

/\* - values could not be estimated





## Results – *Chlorella* sp.



**Figure 13.** Microphotographs of the microalgae *Chlorella* sp. in 72 hours in a blank test (a.) and at concentration of 10 g/L of AgNPs (b.), and at concentrations of 1 g/L of chitosan (c.), TiO<sub>2</sub> (d.) and thymol (e.).

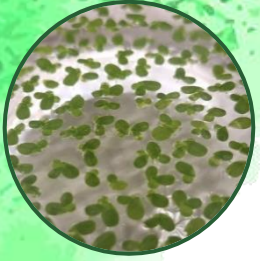


## Results – *Lemna minor*

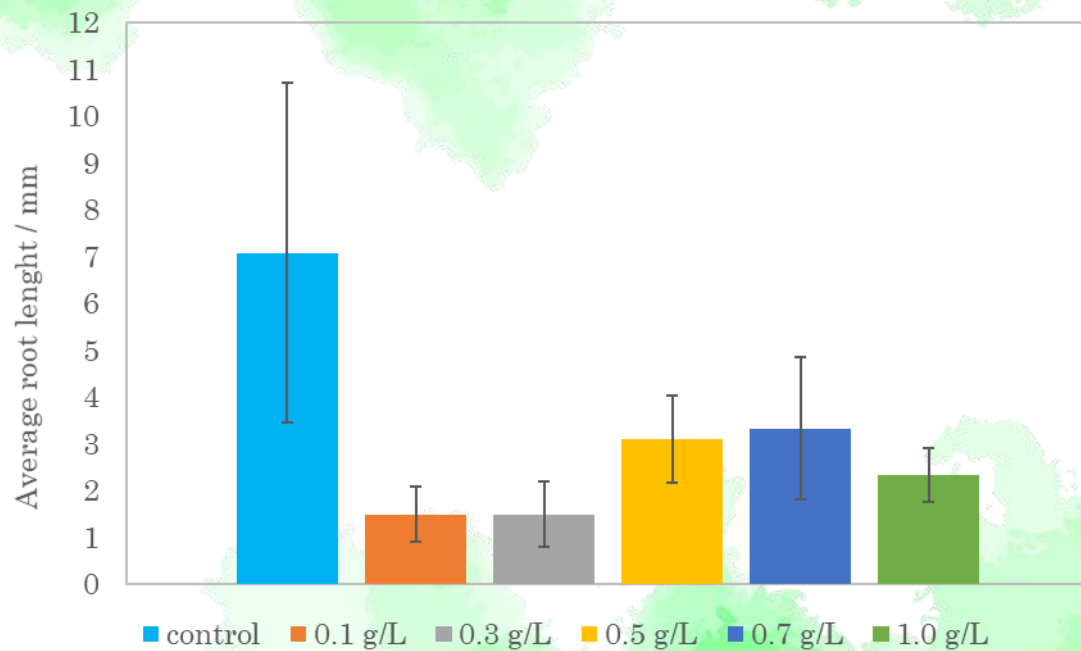
**Table 6.** Inhibition of duckweed growth during exposure to different concentrations of AgNPs, chitosan, TiO<sub>2</sub> and thymol.

Substance	Concentration / g/L	INH / %
AgNPs	1	88.65
	3	100.00
	5	88.65
	7	100.00
	10	100.00
Chitosan	0.1	16.69
	0.3	10.82
	0.5	10.82
	0.7	16.69
	1.0	16.69
TiO <sub>2</sub>	0.1	0
	0.3	0
	0.5	0
	0.7	0
	1.0	0
Thymol	10	100
	30	100
	50	100
	70	100
	100	100

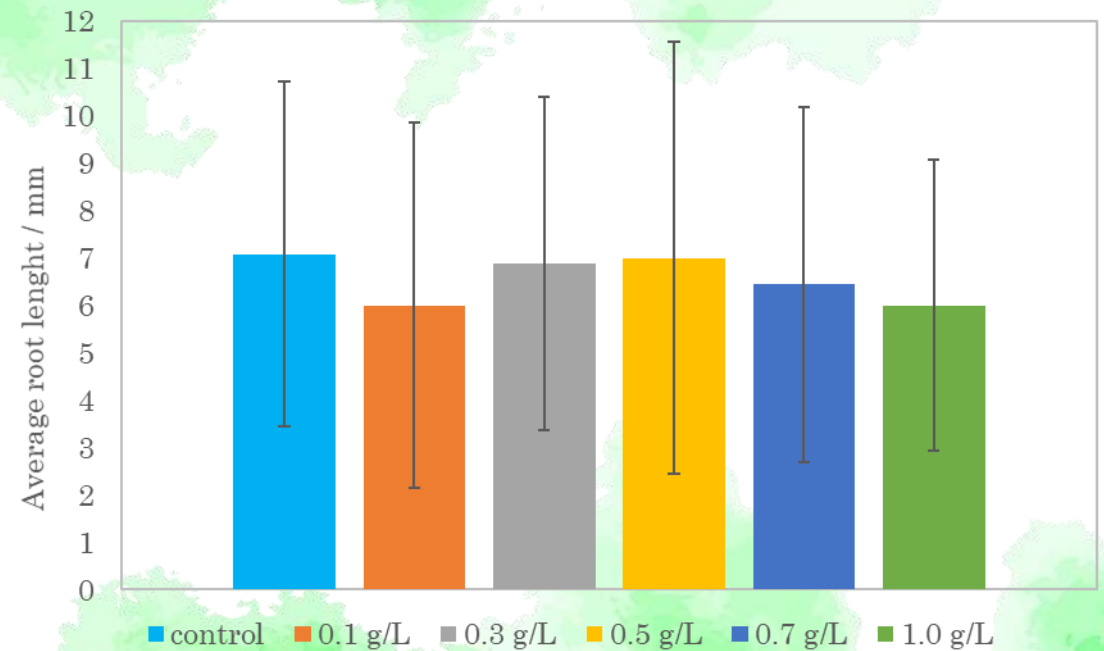




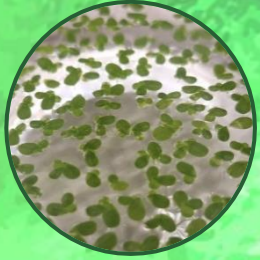
## Results – *Lemna minor*



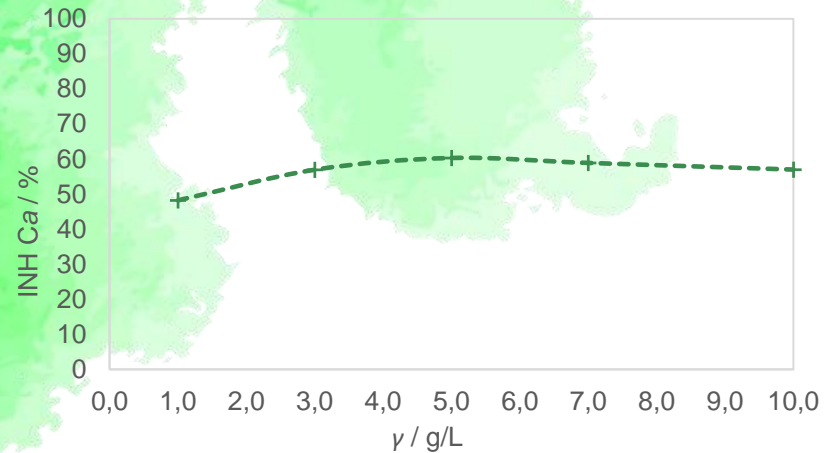
**Figure 14.** Inhibition of root length of *Lemna minor* during exposure to different concentrations of chitosan.



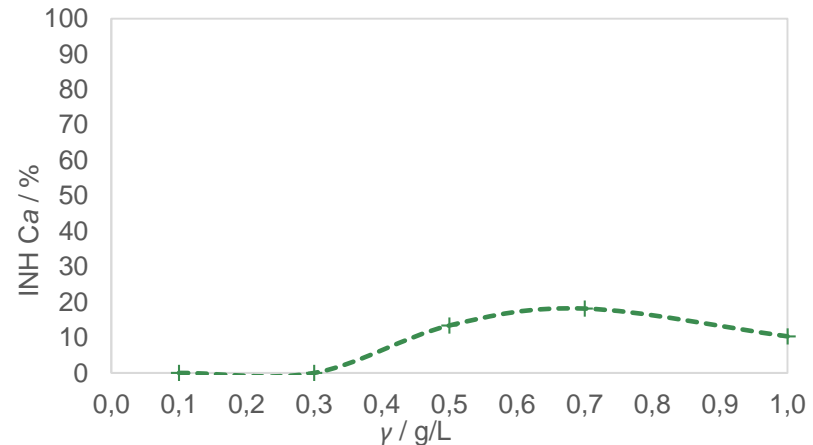
**Figure 15.** Inhibition of root length of *Lemna minor* during exposure to different concentrations of  $\text{TiO}_2$ .



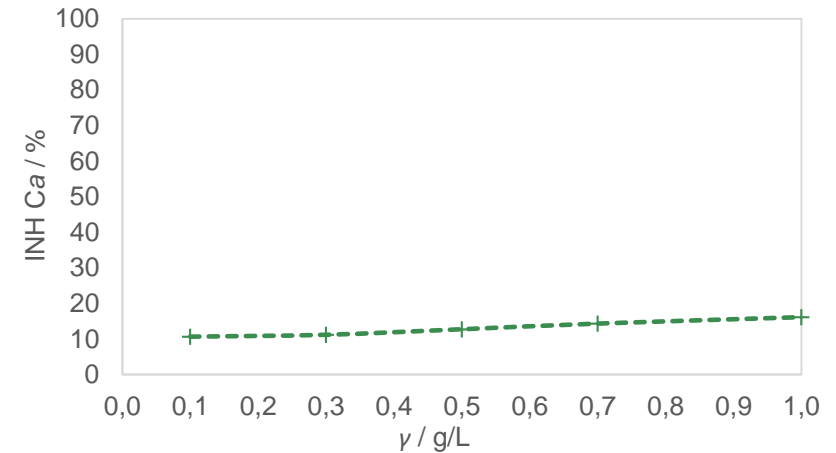
## Results – *Lemna minor*



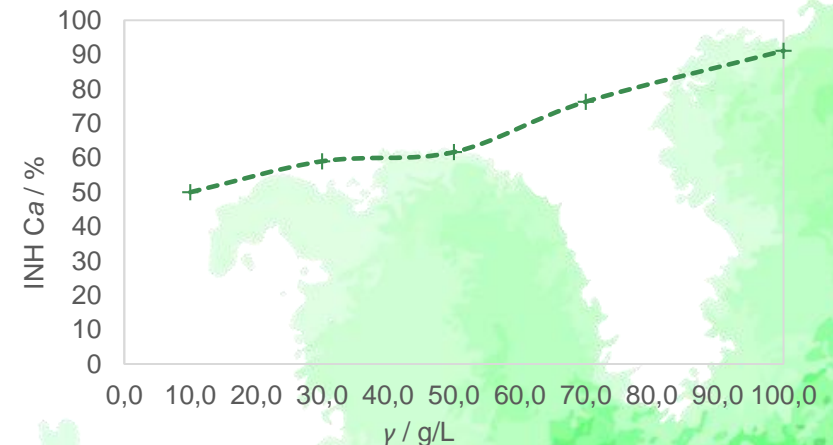
**Figure 16.** Inhibition of chlorophyll *a* production during exposure to different concentrations of AgNPs.



**Figure 18.** Inhibition of chlorophyll *a* production during exposure to different concentrations of TiO<sub>2</sub>.



**Figure 17.** Inhibition of chlorophyll *a* production during exposure to different concentrations of chitosan.



**Figure 19.** Inhibition of chlorophyll *a* production during exposure to different concentrations of thymol.



# Conclusion

❖ AgNPs and thymol exhibited the highest overall toxicity

Substance	<i>Vibrio fischeri</i>	<i>Chlorella</i> sp.
AgNPs	0.450 g/L	1.518 g/L
Chitosan	0.049 g/L	0.410 g/L
TiO <sub>2</sub>	/*	0.363 g/L
Thymol	0.035 g/L	8.845 g/L

/\* values could not be estimated

❖ TiO<sub>2</sub> and AgNPs caused morphological changes in *Chlorella* sp.

❖ Chitosan and TiO<sub>2</sub> showed negligible or no toxic effect on *Lemna minor*, while AgNPs and thymol caused complete inhibition

The results highlight the potential environmental risk posed by widespread use and release of these substances into aquatic ecosystems.

# Literature

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# Thank you for your attention.



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