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Environmental Impact of Zinc Oxide, Titanium Dioxide and Graphene Oxide Nanoparticles – Ecotoxicological Aspects and Applicability in Bioremediation

Thesis Book

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1. Introduction

Nanotechnology has already brought significant advancements to many areas of human society. However, questions regarding the environmental and biological impact of the widespread use of nanoscale materials still need to be answered. Nanoparticles, which are defined as particles with a size range of 1–100 nm[1], have significant potential applications in numerous industries, including electronics, medicine, the energy sector and environmental technology. Their unique physical and chemical properties result in novel applications but also raise the need to thoroughly examine their potential environmental and biological effects.

In contrast to conventional pollutants, nanoparticles may behave differently in the environment through processes such as aggregation, reactivity, or interaction with biological systems. Accordingly, the toxicological profiles of these new materials often differ significantly from those of conventional pollutants. Consequently, research methods and environmental impact assessments must adapt to these new challenges.

2. Scientific background

Nowadays, nanotechnology is receiving increasing attention due to its extremely diverse range of applications. As the use of nanoparticles increases, so does their release into the environment, making it critically important to understand the interactions between living organisms and nanoscale materials, and the consequences of these interactions. Although there are already guidelines for dealing with the special properties of nanomaterials, there is no uniform testing methodology for assessing their impact. Furthermore, revisions to scientific and regulatory guidelines have demonstrated that more detailed requirements and examinations are needed for nanomaterials.

2.1. Application and Fate of Nanoparticles

Nanoparticles have unique characteristics such as small size, composition, surface structure, solubility, shape, and ability to aggregate. These properties can be modified, which makes them preferable to larger-sized bulk phase materials on several fields[2].

The use of nanoparticles has increased significantly in science, medical technology and pharmaceuticals, electronics, telecommunications, and other industrial areas. Especially, metal oxide nanoparticles have gained prominence in agriculture, household products, and energy production. Titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles are the most commonly used nanomaterials in cosmetic products, such as sunscreens, body lotions, soaps, and mouthwashes[3]. Additionally, TiO_2 can also be found in dietary supplements, sauces, plant-based cream substitutes, and sweets[4].

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[1] Martínez, G., Merinero, M., Pérez-Aranda, M., Pérez-Soriano, E.M., Ortiz, T., Begines, B., Alcudia, A. (2021) Environmental impact of nanoparticles' application as an emerging technology: A review. Materials 14. https://doi.org/10.3390/ma14010166

[2] Hughes, S., Asmatulu, E. (2021) Nanotoxicity and nanoecotoxicity: Introduction, principles, and concepts. In: Kumar, V., Guleria P., Ranjan, S., Dasgupta, N., Lichtfouse, E. (eds). Nanotoxicology and nanoecotoxicology Vol. 1. Environmental chemistry for a sustainable world Springer International Publishing, Cham, pp 1–19 https://doi.org/10.1007/978-3-030-63241-0_1

[3] Adawi, H.I., Newbold, M.A., Reed, J.M., Vance, M.E., Feitshans, I.L., Bickford, L.R., Lewinski, N.A. (2018) NanoImpact Nano-enabled personal care products: Current developments in consumer safety. *NanoImpact* 11:170–179. https://doi.org/10.1016/j.impact.2018.08.002

[4] Ropers, M-H., Terrisse, H., Mercier-Bonin, M., Humbert, B. (2017) Titanium dioxide as food additive. In: Janus M (ed). Application of Titanium dioxide. *IntechOpen*, Rijeka. https://doi.org/10.5772/intechopen.68883

Due to the significant increase in the industrial and household use of nanoparticles, they inevitably appear in the environment, raising concerns about their potential risks due to their impact on ecosystems. Nanoparticles can accumulate in the environment, for example in water, sediments, soil, sewage sludge, and air[5]. Moreover, nanoparticles have the capacity to undergo a variety of biotic and abiotic transformation processes. These processes are of pivotal significance in determining their bioavailability and toxicity [6]. As a result, they can induce toxic oxidative stress through the generation of reactive oxygen species (ROS), which can pose potential threats to both environmental and human health. This risk arises, for example, through uptake by plants or entry into organisms in soil and aquatic ecosystems, as nanoparticles can bioaccumulate and ultimately enter the food chain[7].

2.2. Nanoecotoxicology and its regulation

The aim of nanoecotoxicology is to identify and predict the impact of nanoscale materials on ecosystems. To this end, it considers the potential pathways by which nanomaterials enter the environment, as well as the functional changes they cause in organisms, from the cellular level to complex communities. Currently, information on the toxicity of nanoparticles to environmentally relevant species and complex systems is limited. Furthermore, determining their environmental concentrations is challenging, which limits the ability to conduct relevant quantitative risk assessments for nanoparticles. Given the growing need to assess the ecotoxicity of nanomaterials, there is a necessity to further develop standard ecotoxicological test methods. While standard ecotoxicological test methods for assessing the toxicity of other substances can be applied to nanomaterials, adaptations and modifications are required.

Based on studies examining the effects of nanomaterials on various organisms, the impacts of nanomaterials can manifest as behavioral, morphological, cellular, molecular, or genetic effects[8]. Even for nanoparticles considered safe, screening tests are necessary, while comprehensive studies based on the precautionary principle require long-term *in vivo* testing. Relevant testing of new nanomaterials that are constantly emerging is time-consuming, which could be addressed by using less strict but still risk-averse approaches[9].

The lack of comprehensive research on the risks of nanomaterials makes it difficult to assess and prevent their potential harmful effects on the environment. This underscores the need for the development of methodological approaches to research on the environmental risks of nanomaterials, as well as advancements in technological development. Since toxicological and other testing processes are often time-consuming, proposals have been made to develop and apply the so-called precautionary approach to nanotechnology and nanomaterials, thus avoiding the emergence of environmental risks. At the same time, a multi-level risk assessment strategy would be needed, similar to the EU's REACH legislation on chemical regulation.

[5] Mishra, S., Sundaram, B. (2023) Fate , transport , and toxicity of nanoparticles : An emerging pollutant on biotic factors. *Process Saf Environ Prot* 174:595–607. https://doi.org/10.1016/j.psep.2023.04.037

[6] Rajput, V., Minkina. T., Mazarji, M., Shende, S., Sushkova, S., Mandzhieva, S., Burachevskaya, M., Chaplygin, V., Singh, A., Jatav, H. (2020) Accumulation of nanoparticles in the soil-plant systems and their effects on human health. *Ann Agric Sci* 65:137–143. https://doi.org/10.1016/j.aoas.2020.08.001

[7] Jumle, K., Lakhawat, S.S., Ajmera, H., Thakuria, B., Sharma, V., Jain, V., Kumar, V., Singh, S., Kumar, A., Malik, N., Kothari, S.L., Kumar, S., Sharma, P.K. (2025) Chapter 9 - Nanoparticles bioaccumulation an emerging threat to ecosystem. In: Joshi, S., Dua, P., Sarma, H., Velmurugan, P. (eds) Advances in biotechnology and bioengineering. One- and two-dimensional nanomaterials. Academic Press, pp 223–235. https://doi.org/10.1016/B978-0-443-23703-4.00009-1

[8] Boros, B.V., Ostafe, V. (2020) Evaluation of ecotoxicology assessment methods of nanomaterials and their effects. *Nanomaterials* 10(4):610. https://doi.org/10.3390/nano10040610

[9] Choi, J.Y., Ramachandran, G., Kandlikar, M. (2009) The impact of toxicity testing costs on nanomaterial regulation. *Environ Sci Technol* 43:3030–3034. https://doi.org/10.1021/es802388s

The International Organization for Standardization (ISO) is developing nanotechnology-related standards that complement regulatory testing methods[10].

From a regulatory perspective, it is also a problem that some legislation in the EU uses its own definition for nanomaterials, such as the Regulation on Cosmetic Products and the Regulation on Novel Foods, while others do not have a nanomaterial definition at all, such as the Regulation on Plastics in Contact with Food.

2.2.1. Modulation of Microbial Communication by Nanoparticles

Quorum sensing (QS) is a cell-to-cell communication process that depends on cell density and involves signal transduction. Bacteria are single-celled organisms, however they can perform complex tasks as communities that they would not be able to accomplish individually. This collective behavior relies on intercellular communication mediated by signal molecules.

The production of signal molecules and their release into the extracellular space is continuous, but at low cell concentrations, the likelihood of a signal produced by one cell binding to a receptor on another cell is minimal. As the bacterial colony grows, more and more cells release higher amounts of signals into the environment, and when the concentration of secreted signal molecules (and their binding to receptors) reaches a critical threshold, the transcription and the rate of expression of various genes change[11]. As a result, bacteria begin to function in a coordinated manner, enabling the expression of properties (e.g. bioluminescence, biofilm formation, sporulation, and pigment production) that are crucial for adapting to environmental conditions.

Quorum quenching (QQ) reduces the extent of bacterial communication, for example, by degrading signal molecules or using inhibitors that can interfere with cell-to-cell communication through various mechanisms[12]. These inhibitors can be natural compounds or synthetic derivatives, and nanoparticles can also serve as QQ agents. Over the past decade, nanoparticles have become the focus of research on bacterial communication due to their quorum-quenching and antimicrobial effects. Metal oxide nanoparticles, as potential quorum inhibitors, have gained attention in recent years for reducing virulence and biofilm formation[13], alongside other more extensively studied materials. Comprehensive molecular and genetic research is still needed to understand how these "nanoweapons" inhibit QS systems[14].

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[10] Tschiche, H.R., Bierkandt, F.S., Creutzenberg, O., Fessard, V., Franz, R., Giese, B., Greiner, R., Heinz, K., Haase, A., Hartwig, A., Hund, K., Iden, P., Kromer, C., Loeschner, K., Mutz, D., Rakow, A., Rasmussen, K., Rauscher, H., Richter, H., Schoon, J., Schmid, O., Som, C., Tovar, G.E.M., Westerhoff, P., Wohlleben, W., Luch, A., Laux, P. (2022) Environmental considerations and current status of grouping and regulation of engineered nanomaterials. *Environ Nanotechnology, Monit Manag* 18:100707. https://doi.org/10.1016/j.enmm.2022.100707

[11] Papenfort, K., Bassler, B.L. (2016) Quorum sensing signal-response systems in Gram-negative bacteria. *Nat Rev Microbiol* 14:576–588. https://doi.org/10.1038/nrmicro.2016.89

[12] Zhou, L., Zhang, Y., Ge, Y., Zhu, X., Pan, J. (2020) Regulatory mechanisms and promising applications of quorum sensing-inhibiting agents in control of bacterial biofilm formation. *Front Microbiol* 11:1–11. https://doi.org/10.3389/fmicb.2020.589640

[13] Hayat, S., Muzammil, S., Shabana, Aslam B., Siddique, M.H., Saqalein, M., Nisar, M.A. (2019) Quorum quenching: role of nanoparticles as signal jammers in Gram-negative bacteria. *Future Microbiol* 14:61–72. https://doi.org/10.2217/fmb-2018-0257

[14] Salkar, K., Charya, L. (2023) Chapter 11 - Application of nanoparticles as quorum quenching agent against bacterial human pathogens: a prospective therapeutic nanoweapon. In: Morajkar, P., Naik, M. (eds) Advances in nano and biochemistry. Progress in Biochemistry and Biotechnology. Academic Press, pp 261–284. https://doi.org/10.1016/B978-0-323-95253-8.00011-5

2.3. Nano-bioremediation

Various technologies can be applied to reduce the risks of contaminated soils, among which bioremediation techniques based on biodegradation play an important role in the case of organic pollutants. The primary aim of these techniques is to intensify the biodegradation process. The efficiency of biodegradation in the contaminated area can be enhanced by adding microorganisms and/or enzymes, for example in the form of inoculants, a technique called bioaugmentation[15].

Enzyme-based bioremediation can be an effective method for the environmentally friendly removal of recalcitrant xenobiotics (anthropogenic pollutants) from contaminated media.

Recently, new techniques using nanomaterials have emerged, offering promising opportunities to reduce environmental risks posed by various pollutants, thus cleaning contaminated areas and controlling pollution. The combined application of nanomaterials and/or nanotechnology with bioremediation processes is called nano-bioremediation[16]. Numerous techniques are available for nano-bioremediation, including enhancing microbial activity with nanoparticles, thereby increasing the rate of biodegradation, using microbial cells and enzymes immobilized by nanoparticles, facilitating electron transfer mediated by nanoparticles, and integrated nano-biodegradation, which is the combination of different mechanisms of action of nanomaterials with various biodegradation pathways to achieve the complex remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs)[17].

Several nanoparticles (including ZnO and TiO_2) have been reported to improve the efficiency of PAH removal[18]. However, it is important to note that the application of nanoparticles in the environment, such as for soil remediation purposes, can potentially cause adverse effects[19].

3. Aims and Objectives

The main aspects of my research are environmental risk assessment and risk reduction, focusing on various nanomaterials.

In the first phase of my research, I investigated and characterized the environmental impacts of graphene oxide (nGO), zinc oxide and titanium dioxide nanoparticles, using an ecotoxicological approach. Since many studies have already addressed the assessment of toxic effects of metal oxide nanoparticles, for nZnO and $nTiO_2$, I primarily aimed to explore their effects on aquatic ecosystems, particularly on microbial communities, which have been less studied. Similarly, I evaluated the impact of nGO on freshwater microbial communities.

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[15] Gao, D., Zhao, H., Wang, L., Li, Y., Tang, T., Bai, Y., Liang, H. (2022) Current and emerging trends in bioaugmentation of organic contaminated soils: A review. *J Environ Manage* 320:115799. https://doi.org/10.1016/j.jenvman.2022.115799

[16] Chauhan, P., Imam, A., Kanaujia, P.K., Suman, S.K. (2023) Nano-bioremediation: an eco-friendly and effective step towards petroleum hydrocarbon removal from environment. *Environ Res* 231:116224. https://doi.org/10.1016/j.envres.2023.116224

[17] Gupta, N., Koley, A., Banerjee, S., Ghosh, A., Hoque, R.R., Balachandran, S. (2024) Nanomaterial-mediated strategies for enhancing bioremediation of polycyclic aromatic hydrocarbons: A systematic review. *Hybrid Adv* 7:100315. https://doi.org/10.1016/j.hybadv.2024.100315

[18] Eldos, H.I., Zouari, N., Saeed, S., Al-Ghouti, M.A. (2022) Recent advances in the treatment of PAHs in the environment: Application of nanomaterial-based technologies. *Arab J Chem* 15:103918. https://doi.org/10.1016/j.arabjc.2022.103918

[19] Ge, Y., Schimel, J.P., Holden, P.A. (2011) Evidence for negative effects of TiO_2 and ZnO nanoparticles on soil bacterial communities. *Environ Sci Technol* 45:1659–1664. https://doi.org/10.1021/es103040t

Furthermore, since the availability of comprehensive studies on the toxicity of nGO using single-species test systems is limited, I also aimed to determine the effective concentrations and environmental risks of the tested GO nanomaterials based on concentration-response relationships of test organisms from different trophic levels.

To assess the impact of nanoparticles on aquatic ecosystems, my further goal was to propose an easily applicable methodological approach that can predict significant changes in microbial diversity and activity caused by nanoparticles even at low concentrations. In this context, I evaluated the applicability of the Biolog EcoPlate™ test system as a relatively inexpensive and rapid technique.

Furthermore, by assessing the sensitivity of various endpoints derived from data obtained through Biolog EcoPlate™ measurements, my aim was to determine endpoint(s) that effectively reflect the effects induced by nanoparticles.

The investigation of nanoparticles' effects on bacterial communication is a relatively new research area with several contradictory results. Related to this, my aim was to compare the effects of two metal oxide nanoparticles (nZnO and $nTiO_2$) on the viability of *Pseudomonas aeruginosa* and on its QS-regulated processes controlled by various signal molecules (biofilm formation, pyoverdine production). In this context, I examined the concentration- and time-dependent effects of the studied nanoparticles, as well as their cytotoxic effects.

The second phase of my doctoral research is related to the development of an effective and environmentally friendly biotechnology for risk reduction. My goal was to design and implement the parameters of an enzyme-based bioremediation process for PAH-contaminated soils, which can prove the efficiency of the technology and the applied enzymes.

I investigated the biodegradation efficiency of novel PAH degrading enzymes (gentisate dioxygenase and catalase peroxidase) designed by bioinformatics and evaluated the applicability of the Biolog EcoPlate™ for determining microbial activity and functional diversity in PAH-contaminated soils. My further aim was to evaluate the effect of nZnO on PAH-degrading enzymes, thereby on the efficiency of bioremediation, and to assess the impact of nZnO on the activity and diversity of the indigenous microflora in the soil.

4. Experimental Methodology

In my experiments, I used nano zinc oxide obtained from Sigma-Aldrich Inc. (with an average particle size of less than 40 nm), AERODISP® VP Disp. W2730X nano titanium dioxide purchased from Evonik Resource Efficiency GmbH (90% anatase and 10% rutile; average particle size below 16 nm), and AF 96/97 and PM 995 graphene oxide nanoparticles (with average crystallite sizes of 8.24 nm and 6.74 nm, respectively) manufactured at the Department of Physical Chemistry and Materials Science at the Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics.

4.1. Assessment of The Impact of Nanoparticles on Organisms

I studied the effects of nanoparticles on organisms using several types of test systems with different sensitivities, including single-species and multi-species (microbial communities) ecotoxicological systems with sublethal and lethal testing endpoints.

In assessing the environmental risk of nGO, I applied the following test systems and endpoints:

- *Aliivibrio fischeri* (bacterium)
  - Measurement of bioluminescence intensity
  - Measurement of the activity of enzymes involved in respiratory chain
- *Escherichia coli* (bacterium)
  - Reproduction
  - Measurement of the activity of enzymes involved in respiratory chain
  - Measurement of the reactive oxygen species production intensity
  - Genotoxicity assessment using SOS ChromoTest™
- *Tetrahymena pyriformis* (protozoan)
  - Reproduction
  - Measurement of the activity of enzymes involved in respiratory chain
- *Panagrellus redivivus* (nematode)
  - Reproduction
- *Sinapis alba* (White mustard) and *Triticum aestivum* (Common wheat)
  - Germination capacity
  - Measurement of root- and shoot length.

Based on the results of these tests, concentration-response curves have been prepared to determine the effective concentration values causing 20% inhibition (EC_{20}).

Using a conservative approach, taking into account the lowest EC_{20} value, the PNEC (Predicted No Effect Concentration) value of nGOs can be determined, which is necessary for calculating the environmental risk quotient (RQ). The RQ value is calculated as the ratio of the predicted environmental concentration (PEC) to the PNEC. The higher the RQ value, the greater the environmental risk posed by the chemical released into the environment.

I assessed the impact of nanoparticles on microbial communities using laboratory microcosm system with freshwater collected from a real environment (Lake Balaton). In this experiment, I measured the activity of enzymes involved in the respiratory chain as a function of time and concentration. Additionally, using the Biolog EcoPlate™, I determined various indices and endpoints (Substrate Richness (SR), Average Well Color Development (AWCD), Substrate Average Well Color Development (SAWCD), Area Under the Curve (AUC), Shannon Index (H), Shannon Evenness (E), Simpson Index (D), McIntosh Index (U), Gini Index (G)), which serve to characterize microbial activity.

The effects of metal oxide nanoparticles on microbes through their influence on bacterial communication also were studied. In this context, the effect on biofilm formation and pyoverdine siderophore production of *Pseudomonas aeruginosa* DSM 1117 (ATCC 27853) strain was examined. Furthermore, I assessed bacterial growth, enzyme activity, and ROS production in order to distinguish the toxic effects from their impact on bacterial communication.

4.2. Bioremediation of Polycyclic Aromatic Hydrocarbons-Contaminated Soil

I examined the activity and efficiency of enzymes designed using metagenomic approaches (catalase–peroxidase (E39), gentisate 1,2-dioxygenase (E99 and E105)) with the aim of developing a remediation process for PAH-contaminated soils in two successive steps within microcosm systems.
In the first phase, 1 mg of purified enzyme and 20 mmol of calcium peroxide (CaO_2) were added to 200 g of contaminated soil in various combinations.
In the second phase of the research, I also assessed the effect of nZnO on the efficiency of bioremediation of PAH-contaminated soil within technological microcosm systems.

I added nZnO at concentrations of 200 and 1000 mg/kg, as well as 10 mmol of calcium peroxide (CaO_2) and enzymes (E39 and E105) in various combinations to the contaminated soil, to evaluate their impact on remediation efficiency.
The efficiency of the various treatments was evaluated based on the gas chromatography analytical measurement of the concentration of contaminants and the determination of microbial activity. For the latter, I used the previously described Biolog EcoPlate™ tool and characterized the observed effects based on the endpoints calculated from it.

4.3. Statistical Analysis

Depending on the test system, 3-6 replicates were applied in the various measurement methods. These measurement results were used for further evaluation, including the determination of inhibition percentages and effective concentrations compared to the controls without nanoparticles. The data obtained during the experiments were subjected to statistical analysis in order to identify the significant effects. StatSoft® Statistica 13.1 (TIBCO Software, Inc., Palo Alto, CA, USA) software was used for this purpose, with a significance level of $p < 0.05$ applied. To determine significant differences, Fisher's LSD (Least Significance Difference) test was performed during one-way analysis of variance (one-way ANOVA) or repeated measures analysis of variance (RMANOVA). One-way ANOVA was applied in cases where the measured endpoint was measured only once. The prerequisite of the test, data homogeneity, was verified using Cochran's C-test. Repeated measures ANOVA (RMANOVA) was used when the given endpoint was measured at multiple times. The fulfilment of the test precondition was determined by Mauchly's sphericity test. Significant differences are indicated by different letters on the diagrams.

5. Results and Discussion

My doctoral research was built around two different but interconnected aspects of environmental risk management: environmental risk assessment and environmental risk reduction, with a focus on various nanomaterials.

5.1. Characterization of the Environmental Impact of Nanoparticles Using an Ecotoxicological Approach

There were only small differences in the physicochemical properties of the examined GO nanosuspensions, thus in several cases (e.g., in bacterial test systems) only slight differences were detected in the toxicity of the two nGOs. However, significant differences were observed in toxicity and the behavior of the nanoparticles for some test organisms (e.g., *Tetrahymena pyriformis* and *Panagrellus redivivus*). The results of the complex ecotoxicological assessment clearly highlight that the application of test organisms from different trophic levels is recommended for effect evaluation. Based on our results, the level of toxicity increased with the progression of exposure time, indicating that the transformation of nGO may enhance their harmful effects on the tested organisms. According to the concentration-response relationships and the EC_{20} values causing 20% inhibition (Table 1), the sensitivity of the test systems and endpoints was as follows:

Sinapis alba shoot length < *Triticum aestivum* root length < *Triticum aestivum* shoot length < *Sinapis alba* root length < *Panagrellus redivivus* mortality < *Aliivibrio fischeri* enzymatic activity < *Aliivibrio fischeri* bioluminescence intensity < *Escherichia coli* enzymatic activity < *Tetrahymena pyriformis* enzymatic activity < *Tetrahymena pyriformis* reproduction.

Table 1 *– EC_{20} values [mg/L] of the applied graphene oxide suspensions in the examined test organisms. The more toxic effects are indicated in red (and its shades), while green (and its shades) show lower ecotoxicity. The lower the EC_{20} value, the more toxic is the nGO.*

Examined endpoint of the test organisms	Incubation time	AF 96/97	PM 995
		EC_{20} [mg/L]	EC_{20} [mg/L]
Escherichia coli enzymatic activity	24 h	3.97	2.57
Aliivibrio fischeri bioluminescence intensity	30 min	5.86	5.06
	120 min	4.01	4.40
Aliivibrio fischeri enzymatic activity	120 min	22.86	16.13
Tetrahymena pyriformis reproduction	24 h	0.008	0.033
	48 h	0.011	0.004
Tetrahymena pyriformis enzymatic activity	24 h	0.038	0.020
	48 h	0.014	0.004
Panagrellus redivivus mortality	24 h	79.74	217.56
	72 h	56.09	11.33
Sinapis alba root length	72 h	76.71	40.73
Sinapis alba shoot length	72 h	380.13	554.01
Triticum aestivum root length	72 h	224.78	392.53
Triticum aestivum shoot length	72 h	223.98	203.49

Using the smallest effective concentration (SC) values and applying 1000 as a safety assessment factor (AF), the $PNEC_{AF\ 96/97}$ value was determined to be 8 ng/L, while the $PNEC_{PM995}$ value to be 4 ng/L, following the methodology recommended by the EU[20]. The potential environmental risk (RQ) can be quantified, which is the quotient of the PEC and PNEC values. Literature data were used as the basis for determining the PEC value. Applying the worst-case scenario approach, the highest possible concentration value (1.82 ng/L)[21] was used for the calculations. Consequently, the RQ value was calculated to be 0.23 for nGO AF 96/97 type, and 0.46 for nGO PM 995. Since both values are less than one, it can be stated that the examined nGO types (AF 96/97 and PM 995) do not pose an environmental risk even when applying a conservative environmental risk assessment approach, i.e., the *worst-case scenario*.

During my research, it was established that GO nanoparticles affect the metabolic activity and diversity of freshwater microbial communities; however, the effect is significantly influenced by the type and concentration of the applied nGO. My investigations revealed that nGO stimulates microbial activity even at low concentrations, as 0.16 mg/L had a significant effect on the examined endpoints. Based on my results, nGO enhanced microbial activity and functional diversity, which effect was concentration-dependent. When low concentrations (0.16 – 0.8 mg/L) were applied, negative effects were detected on the AWCD and Shannon index for PM 995, and the Gini index also indicated adverse effects for both types of nGO.

[20] European Commission (2003) Technical Guidance Document on risk assessment in support of Commission Directive 93/67/EEC on risk assessment for new notified substances, Commission Regulation (EC) No 1488/94 on risk assessment for existing substances, Directive 98/8/EC of the European. https://op.europa.eu/en/publication-detail/-/publication/9aebb292-39c5-4b9c-b4cb-97fb02d9bea2/language-en

[21] Zhao, J., Lin, M., Wang, Z., Cao, X., Xing, B. (2021) Engineered nanomaterials in the environment: Are they safe? *Crit Rev Environ Sci Technol* 51:1443–1478. https://doi.org/10.1080/10643389.2020.1764279

However, the substrate richness and McIntosh index values demonstrated beneficial effects of these GO nanoparticles even at low concentrations. Based on the SAWCD values, no significant differences were observed in the utilization rates of the specific substrate groups by the treatments.

During the impact assessment of TiO_2 and ZnO nanoparticles, I demonstrated that the tested nanoparticles could exert a significant adverse effect on microbial activity, depending on their concentration and exposure time. nZnO had a more pronounced effect compared to $nTiO_2$ for all examined endpoints. The results of the Biolog EcoPlate™ used in the studies confirmed that the effect of metal oxide nanoparticles on microbial communities is significantly dependent on the type of particle. For most of the examined endpoints, a significant decrease was observed at all applied concentrations, in some cases even at a dose of 0.8 mg/L. However, in most cases, the lowest concentration applied (0.8 mg/L) did not cause a significant difference compared to the control.

The McIntosh index (U) was found to be the most sensitive diversity index for evaluating the effect of nZnO on microbial communities. The U value decreased with increasing nZnO concentration and with longer exposure time (Figure 1). The application of 20 mg/L nZnO resulted in a 74–76% reduction in the McIntosh index compared to the control, while a dose of 100 mg/L nZnO led to a 90–92% decrease. Statistical analysis (RMANOVA) revealed a clear, significant effect (p=0.000) for the treatment, contact time, and their interaction.

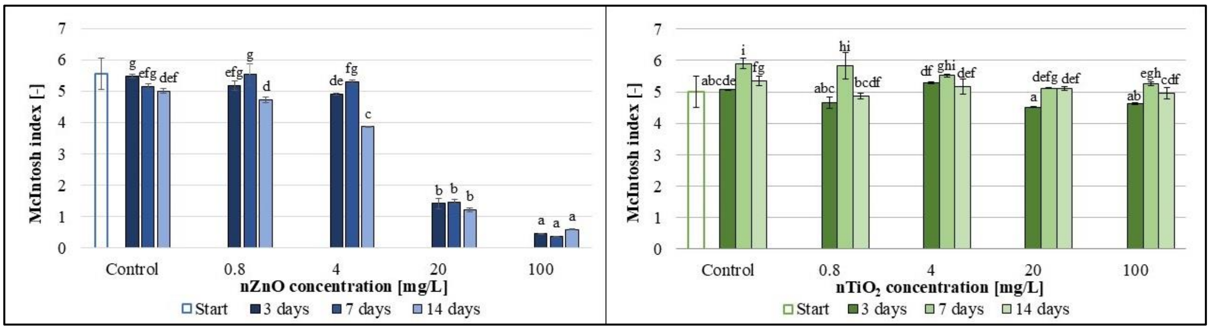


Figure 1. *The effect of ZnO and TiO_2 nanoparticles on freshwater McIntosh index value (U). Letters on the columns indicate significant differences (level of significance: $p < 0.05$).*

According to literature data, the estimated environmental concentration of nZnO in surface waters is 0.38 µg/L, while that of $nTiO_2$ is 2.17 µg/L. Thus, according to current information, these concentrations suggest that nZnO and $nTiO_2$ do not have a significant risk on microbial communities in surface waters at environmentally relevant concentrations.

Examining the endpoints with Biolog EcoPlate™ revealed that the sensitivity of each endpoint differs in response to nZnO and $nTiO_2$ treatments and exposure time. This suggests that Biolog EcoPlate™ is a suitable method for evaluating the impact of nanoparticles on microbial communities.

5.1.1. Effect of Metal Oxide Nanoparticles on Microbial Communication

The production of pyoverdine and the formation of biofilms by *P. aeruginosa* are processes that are regulated by QS and were significantly influenced by the tested nanoparticles. (Figure 2).

A concentration-dependent positive effect of nZnO on pyoverdine production was observed. Treatments with concentrations of 1.95–125 mg/L had a positive influence on this endpoint, while a slight inhibitory effect was observed at a concentration of 0.98 mg/L nZnO after 24 hours. The highest increase in pyoverdine production was detected after 24 hours of exposure (Figure 2A).

The effect of $nTiO_2$ on pyoverdine production was found to be different compared to nZnO, as all applied doses of $nTiO_2$ significantly inhibited siderophore formation, although the rate of this inhibition was similar across all treatments (Figure 2A).

My investigations revealed that both the concentration of ZnO nanoparticles and the length of exposure time significantly influence the effect on *P. aeruginosa* biofilm formation, as a significant decrease in biofilm formation was observed after 6 hours of incubation time, while the rate of the inhibitory effect reduced after 24 and 48 hours (Figure 2B).

By contrast, the effect of $nTiO_2$ on biofilm formation was determined to be similar to that observed for pyoverdine production, as significant inhibitory effects were evident in all treatments, though the extent of inhibition decreased slightly with increasing $nTiO_2$ concentration and contact time (Figure 2B).

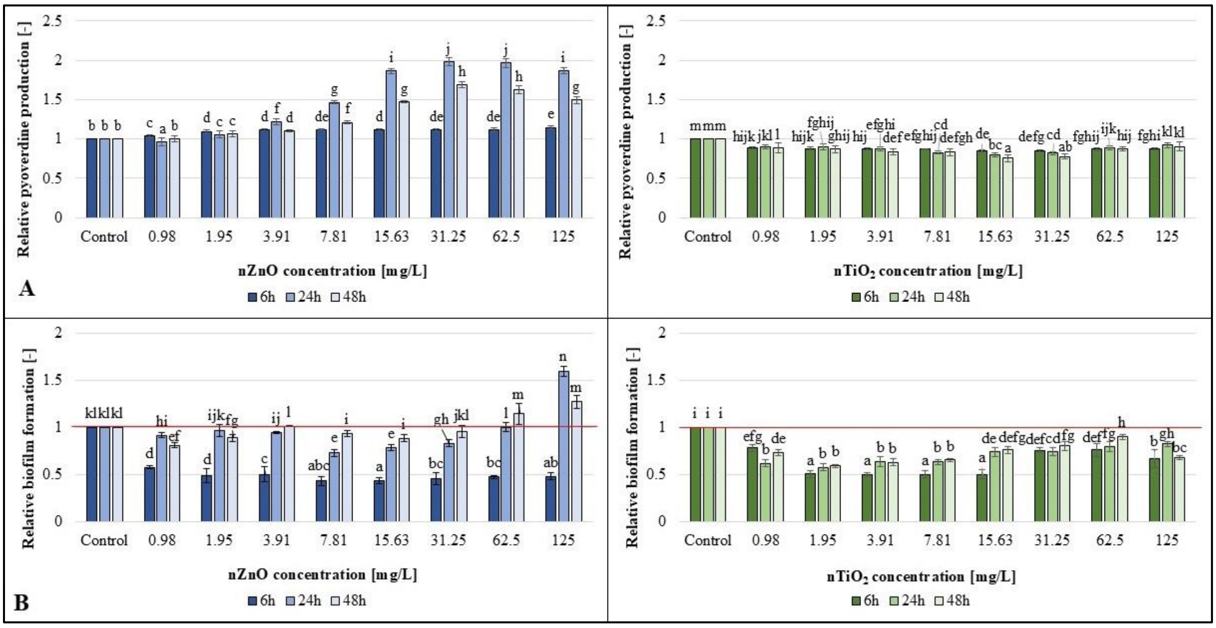


Figure 2. *The effect of ZnO and TiO_2 nanoparticles on the pyoverdine production (panel A) and biofilm formation (panel B) of Pseudomonas aeruginosa.*
Letters on the columns indicate significant differences (level of significance: $p < 0.05$).

An important finding from my results is that a given type of nanoparticle can differentially influence various bacterial communication-regulated processes, such as biofilm formation and siderophore production.

Furthermore, the effect of nZnO on biofilm formation is found to be dependent on the applied concentration and exposure time, with both stimulatory and inhibitory effects observed depending on these factors. In contrast, $nTiO_2$ consistently inhibited both biofilm formation and pyoverdine production under all experimental settings.

5.2. Enzyme-based Technology Development for Bioremediation of Soils Contaminated with Polycyclic Aromatic Hydrocarbons

During the development of a new enzyme-based bioremediation method for PAH-contaminated soils, significant PAH removal was detected even without the addition of the newly created enzymes, which indicates the PAH-degrading capability and activity of the natural microflora present in the soil (Figure 3A). The addition of the new catalase-peroxidase and gentisate 1,2-dioxygenase enzymes (E39, E99, and E105) significantly increased the intensity of naphthalene and phenanthrene degradation; however, the removal of anthracene and pyrene was not enhanced by the addition of enzymes.

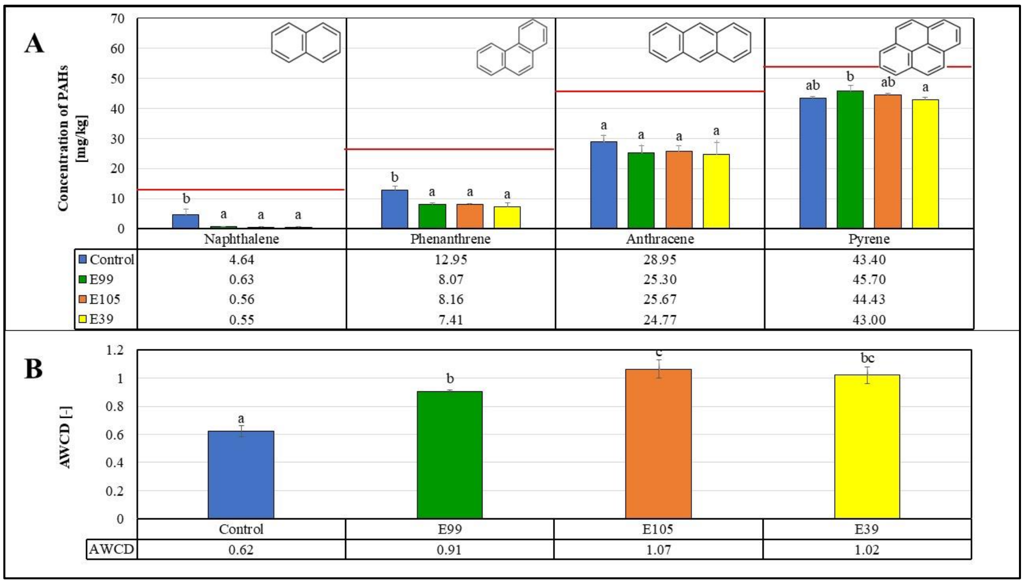


Figure 3. *Concentration of PAH compounds (panel A) and microbiological activity (panel B) in soils treated with new enzymes.*
Letters on the columns indicate significant differences (level of significance: $p < 0.05$).
Red lines show the initial concentrations of the specific PAH compounds.

The effect of the soil's natural microbial activity was also reflected in the results of the Biolog EcoPlate™ test (Figure 3B). The significant increase in AWCD values observed after the addition of enzymes suggests that the enzyme proteins may have a positive impact on the microbes in the soil.

Based on our microcosm experiment, significant degradation activity of the new enzymes was demonstrated for some, but not all of the tested PAH compounds (Figure 3A).

In further experiments, it was investigated whether the addition of enzymes combined with an inorganic oxidizing additive, calcium peroxide (CaO_2) would enhance the beneficial effect on PAH degradation (Figure 4A). By the addition of CaO_2, no reduction in PAH compound concentrations was observed compared to the initial concentration (Figure 4A), which can presumably be attributed to CaO_2 inhibiting the soil's natural microflora, as the metabolic activity - which was also observed in the control soil - was completely eliminated by the addition of CaO_2 (Figure 4B).

However, according to our investigations, the combined application of CaO_2 and the enzymes increased the degradation of the more recalcitrant PAH compounds, such as anthracene and pyrene (Figure 4A). The concentration of anthracene was reduced by approximately 57–70%, while pyrene concentration decreased by approximately 56–66% when enzymes and CaO_2 were used in combination. This result suggests that the applied enzymes can effectively degrade PAH pollutants even in the absence of microbial activity.

The applied enzymes increased the remediation efficiency, while the addition of CaO_2 reduced the removal efficiency for lower molecular weight PAH compounds. It is noteworthy that for naphthalene, the removal efficiency was approximately 95% during enzymatic treatments without CaO_2, while this was reduced to 73–83% when enzymes and CaO_2 were applied in combination. Similar results were obtained for phenanthrene, where degradation efficiency was 68–71% with enzyme addition alone but decreased to 41–53% with the combined use of enzymes and CaO_2.

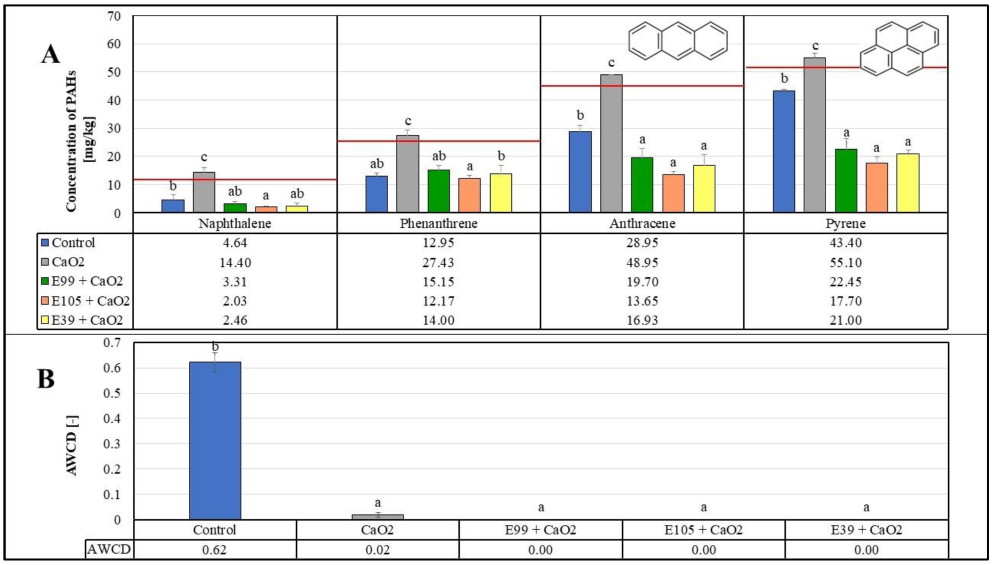


Figure 4. *Concentration of PAH compounds (panel A) and microbiological activity (panel B) in soils treated with CaO_2 and/or new enzymes.*
Letters on the columns indicate significant differences (level of significance: $p < 0.05$).
Red lines show the initial concentrations of the specific PAH compounds.

In summary, it can be stated that the applied new enzymes effectively degraded the contaminants, among which enzyme E105 should be highlighted, as the pyrene removal achieved by it was significantly greater compared to that observed with enzymes E99 and E39. The combined application of CaO_2 and the enzymes resulted in a lower removal rate of naphthalene and phenanthrene compared to the treatments that applied only enzymes, while achieving higher efficiency in the removal of anthracene and pyrene.

CaO_2 alone was not proven to be effective, in the removal of contaminants. While it exerted a considerable adverse effect on the natural microbial activity in the soil, enzymatic degradation - and consequently the elimination of PAH compounds - was significantly enhanced when combined with CaO_2 treatment.

In addition, it is important to mention that our research demonstrated that the Biolog EcoPlate™ is a suitable tool for characterizing the activity of microbial communities during the remediation of PAH-contaminated soils, and is even considered a necessary complementary method to the analytical measurements of the concentrations of contaminants for a broader interpretation of the effectiveness of bioremediation.

Further investigations were conducted with enzymes E105 (gentisate 1,2-dioxygenase) and E39 (catalase-peroxidase), which resulted in the highest microbial activity and contaminant removal, and it was assessed how 200 and 1000 mg/kg nZnO influence the remediation efficiency.

Based on the monitoring investigation performed with the Biolog EcoPlate™ technique, the values of the McIntosh index (Figure 5) - following a pattern similar to AWCD - were significantly increased by both enzymatic treatments, while the CaO_2 treatment significantly decreased them. Furthermore, the addition of nZnO also led to an increase in the microbial activity indicator.

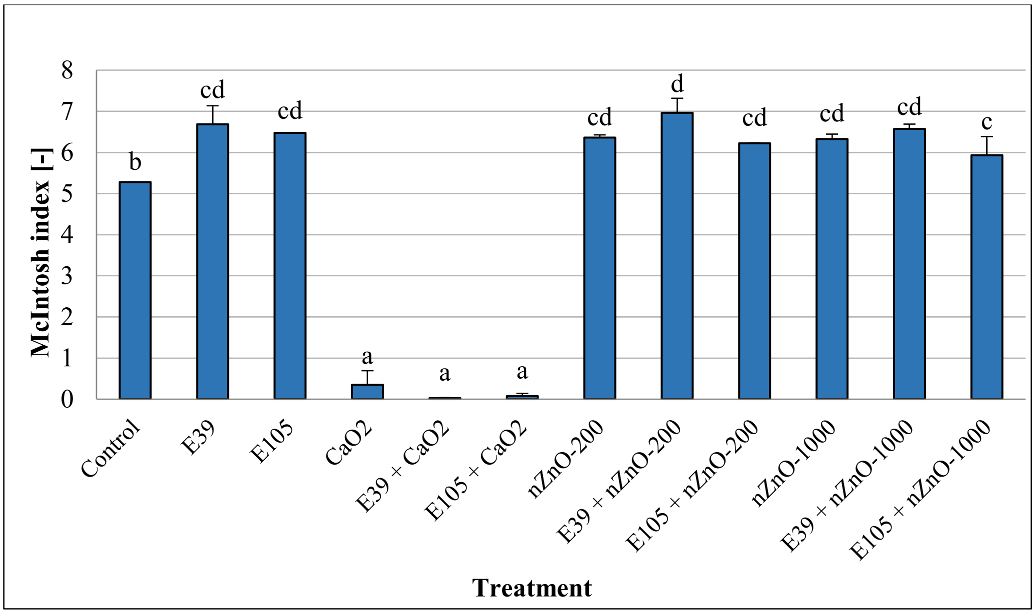
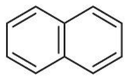
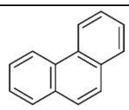
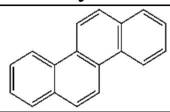
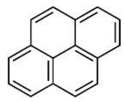


Figure 5. *Effect of the treatments on McIntosh index value (U).*
Letters on the columns indicate significant differences (level of significance: $p < 0.05$).

The removal efficiency relative to the initial concentrations of the various contaminants is presented in Table 2.

Table 2 *– The average pollutant degradation efficiency of the different treatments compared to the initial pollutant concentrations determined by GC MS. In each column, the low efficiency rates are indicated in red (and its shades), while green and its shades show high removal efficiencies.*

Treatments	**Average removal [%] with the standard deviation**				
	Naphthalene	Phenanthrene	Chrysene	Pyrene	Total PAH
					
Control	51.7 ± 5.1	29.5 ± 0.9	59.1 ± 6.4	16.7 ± 6.9	26.6 ± 3.1
E39	59.4 ± 1.9	37.2 ± 2.0	54.6 ± 9.1	10.3 ± 0.5	28.6 ± 1.2
E105	42.5 ± 0.4	29.7 ± 1.7	63.6 ± 0.0	2.1 ± 0.5	19.9 ± 1.0
CaO_2	28.3 ± 18.0	6.9 ± 0.6	60.6 ± 5.3	10.8 ± 8.0	11.2 ± 2.0
E39 + CaO_2	42.9 ± 5.2	20.9 ± 0.3	72.7 ± 0.0	22.6 ± 0.0	22.9 ± 1.7
E105 + CaO_2	47.0 ± 12.4	17.3 ± 2.6	63.6 ± 0.0	19.5 ± 6.3	23.2 ± 3.5
nZnO-200	80.8 ± 1.8	29.1 ± 8.2	63.6 ± 0.0	19.2 ± 3.9	24.5 ± 9.4
E39 + nZnO-200	91.4 ± 0.9	43.7 ± 1.4	69.7 ± 5.3	15.2 ± 0.2	35.5 ± 1.2
E105 + nZnO-200	93.5 ± 0.4	47.0 ± 2.0	69.7 ± 5.3	21.6 ± 3.9	39.2 ± 3.5
nZnO-1000	84.7 ± 0.8	34.3 ± 2.0	60.6 ± 5.3	11.8 ± 5.6	29.1 ± 4.7
E39 + nZnO-1000	92.4 ± 0.2	32.7 ± 2.6	63.6 ± 0.0	11.1 ± 0.2	29.4 ± 3.2
E105 + nZnO-1000	96.4 ± 1.5	38.2 ± 0.6	69.7 ± 5.3	20.7 ± 5.6	37.2 ± 0.1

Based on our results, CaO_2 was found to significantly inhibit the degradation of contaminants compared to the control, indicating its inhibitory effect on microbial activity. In most cases, nZnO stimulated the rate of degradation compared to control, at both 200 and 1000 mg/kg concentrations. Its combined use with enzymes was also had a beneficial effect on the contaminant removal, except for pyrene when combined with the E39 enzyme.

Although the combined application of nZnO and enzymes resulted in the effective degradation of the tested PAH compounds, and the combined use of the E105 enzyme with 1000 mg/kg nZnO yielded in higher naphthalene removal efficiency than with 200 mg/kg nZnO, our results indicate that the highest removal efficiency is achieved by simultaneously applying the E105 enzyme and 200 mg/kg nZnO.

In conclusion, based on the results of our research, the most effective method for achieving the highest degradation rates of recalcitrant PAH contaminants is the combined application of the E105 enzyme and 200 mg/kg nZnO in a rock flour (*Aleurite*) medium.

However, it should be noted that the combination of the E105 enzyme with 1000 mg/kg nZnO, as well as the E39 enzyme with 200 mg/kg nZnO, also resulted in significant contaminant removal, indicating that these treatments can be highly effective in removing certain PAH compounds. Consequently, the combined application of E39 (catalase-peroxidase), E105 (gentisate 1,2 dioxygenase) enzymes and 200 mg/kg nZnO is recommended for the removal of PAH contaminants from polluted soils.
This innovative bioremediation approach using new enzymes could be an effective option for removing PAH compounds from nutrient-poor, low biological activity contaminated soils. However, further studies are needed to obtain a comprehensive understanding of the environmental impacts of nZnO, including its effects on organisms at multiple trophic levels, as well as to determine a narrower concentration range of nZnO that promotes degradation of PAH contaminants.

6. Thesis Points - New scientific findings of the dissertation

1. I was the first to carry out an environmental risk assessment for freshwater ecosystems in the case of graphene oxide nanomaterials. During the environmental risk assessment of nano graphene oxides, I demonstrated that the inhibition of the reproduction of the *Tetrahymena pyriformis* test organism is a highly sensitive method and can potentially be used as an early warning system when assessing the impact of graphene oxide-type pollutants. (2.)
    a. In the case of PM 995 nGO, I observed a significant inhibitory effect even at a concentration of 4 µg/L on the protozoan organism, which plays a crucial role in the food web and elemental cycling.
    b. I was the first to investigate the effects of graphene oxide nanoparticles on the *Tetrahymena pyriformis* test organism in the scientific literature.

2. I demonstrated that the tested nGO derivatives (AF 96/97 and PM 995) do not pose an environmental risk to freshwater ecosystems based on the comprehensive ecotoxicological risk assessment of nano graphene oxides (nGO) and our current knowledge of the environmental concentrations of graphene oxides.
   In order to assess the broad spectrum of effects, I developed a set of methods that can be used to evaluate the environmental risk of nano graphene oxides based on the examination of different endpoints of test organisms from various trophic levels. (2.)

3. Through a comprehensive analysis of experimental data obtained using the Biolog EcoPlate™ system, I proved that the combined use of metabolic activity and functional diversity indices is recommended for ecological studies of microbial communities during the assessment of the impacts of nanomaterials. The determination of the McIntosh index as a sensitive and representative diversity indicator is suggested as a complement to the commonly used endpoints in Biolog EcoPlate™ analyses. (1.)

4. During the development of enzyme-based bioremediation technology, I found that the addition of 20 mM calcium peroxide (CaO_2) as an oxidizing agent is advantageous for both monitoring and evaluating the efficiency of the technology.
   The addition of CaO_2 completely inhibited the activity of the natural microflora in the soil, allowing the degradation efficiency of various enzymes to be studied and described in the given environmental matrix without microbial influence.
   In the presence of 20 mM CaO_2 and metagenomically developed enzymes (catalase-peroxidase, gentisate 1,2-dioxygenase), the concentrations of anthracene and pyrene were significantly reduced compared to the control treatments, indicating that these new enzymes are capable of efficiently degrading recalcitrant PAH compounds. (3.)

5. I demonstrated that the efficiency of the innovative enzyme-based bioremediation technology developed for the removal of polycyclic aromatic hydrocarbons can be enhanced in soils with low microbial activity and unfavourable mechanical composition by the combined application of enzymes (catalase-peroxidase, gentisate 1,2-dioxygenase) and 200 mg/kg nZnO, especially for naphthalene, as well as the more recalcitrant phenanthrene and chrysene in moderately contaminated environments, without detecting any potential harmful effects. (4.)

7. Conclusion and Potential Applications

The results of the complex experiments conducted during my research may provide guidance for the responsible use of nanotechnological products, as well as for the development of environmental policies and regulations intended to promote the sustainable direction of nanotechnology development, as understanding the interactions between nanoparticles and living organisms is essential from the perspective of environmental sustainability and the protection of human health.

When examining the toxicity of graphene oxide nanoparticles (nGO), the inhibition of reproduction of the protozoan *Tetrahymena pyriformis* was found to be the most sensitive test system, where significant inhibitory effects were observed even at a concentration of 0.004 mg/L. These findings highlight the importance and necessity of applying *Tetrahymena pyriformis* in ecotoxicological research and testing methodologies. Furthermore, during the risk assessment applying the worst-case scenario, it was concluded that the examined GO nanoparticles do not pose an environmental risk. This conclusion was supported by the evaluation of the effects on aquatic microbial communities using Biolog EcoPlate™, during which concentration-dependent increases in microbial activity were observed within the examined concentration range of 0.8–100 mg/L.

Results from assessing the effects on the metabolic activity and functional diversity of freshwater microbial flora indicate that nZnO exerts higher inhibitory effect on microbial communities compared to $nTiO_2$, which is considerably influenced by the nanoparticle concentration and exposure time.

Significant effects were detected even at low concentrations (0.8 mg/L) using the Biolog EcoPlate™ system, in which various endpoints were determined, showing different levels of sensitivity.

Based on our results, the McIntosh index proved to be one of the most sensitive metrics that most representatively reflects the changes in microbial activity and functional diversity; thus, its use is recommended in the future studies.

However, it is important to note that examining other endpoints simultaneously is advisable, as they often provide different and complementary information. Overall, these findings demonstrate the suitability of Biolog EcoPlate^{TM} technique for assessing the effects of nanoparticles on aquatic microbial communities.

The examined nanoparticles were found to possess significant potential to influence processes regulated by bacterial communication, the advantage of which can be exploited in numerous areas (e.g., prevention of diseases associated with biofilm formation, wastewater treatment, production of dyes based on pigment production), but defining the parameters for their application requires further research.

Based on our results, catalase–peroxidase and gentisate 1,2-dioxygenase enzymes designed through metagenomic approaches can be effectively applied for the remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). According to the findings obtained from the evaluation of these enzymes' efficiency, treatment combined with calcium peroxide (CaO_2) may serve as a suitable method for demonstrating the activity of various enzymes.

According to our results, for the remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs), new types of catalase-peroxidase and gentisate 1,2-dioxygenase enzymes designed by metagenomic methods can be effectively applied. Testing the efficiency of these enzymes revealed that calcium peroxide (CaO_2) treatment may may serve as an appropriate method for detecting the activity of various enzymes under relevant environmental conditions.

The results of the combined treatments with nZnO and enzymes also demonstrated that the application of 200 mg/kg nZnO may be advantageous in the remediation of PAH-contaminated soils, which can serve as a basis for the development of new nano-bioremediation strategies.

It can be concluded that, in the course of the research, the toxic effects of nanoparticles were assessed and characterised, while their beneficial effects were also examined in order to establish further application areas.

8. Publications

8.1. Publications related to the topic of the dissertation

Publications published in peer-reviewed international journals (impact factor, quartile, independent citation)

1. **Németh, I.**, Molnár, S., Vaszita, E., Molnár, M. (2021). The Biolog EcoPlate™ technique for assessing the effect of metal oxide nanoparticles on freshwater microbial communities. *Nanomaterials,* 11(7), 1777. https://doi.org/10.3390/nano11071777 IF: 5,719; Q1; IC: 50
2. **Németh, I.**, László, K., Bulátkó, A., Vaszita, E., Molnár, M. (2023). Ecotoxicity assessment of graphene oxides using test organisms from three hierarchical trophic levels to evaluate their potential environmental risk. *Nanomaterials*, 13(21), 2858. https://doi.org/10.3390/nano13212858 IF: 4,4; Q1; IC: 2
3. Nagy, K. K., Takács, K., **Németh, I.,** Varga, B., Grolmusz, V., Molnár, M., Vértessy, G. B. (2024). Novel enzymes for biodegradation of polycyclic aromatic hydrocarbons identified by metagenomics and functional analysis in short-term soil microcosm experiments. *Scientific Reports*, 14, 11608. https://doi.org/10.1038/s41598-024-61566-6 IF: 3,9; D1; IC: 10

4. **Németh, I.**, Vaszita, E., Molnár, M. (2025). Influence of nZnO on enzyme-mediated PAH-removal from contaminated soil. *Periodica Polytechnica Chemical Engineering*, https://doi.org/10.3311/PPch.41649 IF: 1,8; Q3.

Manuscripts under review

5. **Németh, I.**, Vaszita, E., Molnár, M. (2025). Nanoparticle-induced modulation of biofilm formation and pyoverdine production in *Pseudomonas aeruginosa*: A comparative study of ZnO and TiO_2 nanoparticles. *Environmental Toxicology*, IF: 3,9; Q1. (under review, *major revision* in progress)
6. Law, C.Y.K., Wang, X., Molnár, M., Folens, K., Peñacoba-Antona, L., **Németh, I.**, De Vrieze, J., Rabaey, K., Esteve-Núñez, A., De Gusseme, B., Hofmann, R., Boon, N. (2025). Removal of fluorinated micropollutants with biogenic nanoparticles in a continuously operated membrane reactor. *Biotechnology Reports*, IF: 3,4; Q1. (under review, *major revision* in progress)

Peer-reviewed conference publications

7. **Németh, I.**, Szikszai, S., Molnár, M. (2022). Cink-oxid és titán-dioxid nanorészecskék hatása *Pseudomonas aeruginosa* baktérium életjelenségeire. *XXIII. Nemzetközi Tudományos PhD-Konferencia elektronikus könyve,* Professzorok az Európai Magyarországért Egyesület, Budapest. ISBN 978-615-5709-16-6

Publications published in non-peer-reviewed popular science journals

8. Fekete-Kertész, I., Nagyné, L. K., **Németh, I.**, Molnár, M. (2022). Bolhából elefántot? - Nanorészecskék környezettoxikológiája. *Élet és Tudomány*, 77(26), 850–852.

Conference participations with oral and/or poster presentations

Oral presentations:

9. **Németh, I.**, Szikszai, S., Molnár, M. (2022). Cink-oxid és titán-dioxid nanorészecskék hatása *Pseudomonas aeruginosa* baktérium életjelenségeire. *XXIII. Nemzetközi Tudományos PhD-Konferencia,* Professzorok az Európai Magyarországért Egyesület, Budapest. 2022.04.28.
10. **Németh, I.**, Bodó, F., Szikszai, S., Molnár, M. (2020). Effects of zinc oxide and titanium dioxide nanoparticles on *Pseudomonas aeruginosa* and *Serratia marcescens* biofilm formation. *4th National Conference of Young Biotechnologists*, Debrecen, Hungary. 2020.12.19.
11. **Németh, I.**, (2022). Nanorészecskék hatásának felmérése a környezetre és bioremediációs eljárások hatékonyságára. *5. ABÉT Tanszéki Doktoráns Konferencia*, Budapest. 2022.01.31.
12. **Németh, I.**, Molnár, M. (2022). Nanorészecskék ökotoxikológiai vizsgálata eltérő tesztrendszerekkel. *XXV. Tavaszi Szél Konferencia*, Pécs. 2022.05.06.

Poster presentations:

13. **Németh, I.**, Szikszai, S., Molnár, M. (2022). Cink-oxid és titán-dioxid nanorészecskék hatása *Pseudomonas aeruginosa* baktérium életjelenségeire. *XXIII. Nemzetközi Tudományos PhD-Konferencia,* Professzorok az Európai Magyarországért Egyesület, Budapest. 2022.04.28.
14. **Németh, I.**, Bodó, F., Szikszai, S., Molnár, M. (2020). Effects of zinc oxide and titanium dioxide nanoparticles on *Pseudomonas aeruginosa* and *Serratia marcescens* biofilm formation. *4th National Conference of Young Biotechnologists*, Debrecen, Hungary. 2020.12.19.

8.2. Publications not closely related to the topic of the dissertation

Publications published in peer-reviewed international journals (impact factor, quartile, independent citation)

15. Molnár, M., Fenyvesi, E., Berkl, Zs., **Németh, I.**, Fekete-Kertész, I., Márton, R., Vaszita, E., Varga, E., Ujj, D., Szente, L. (2021). Cyclodextrin-mediated quorum quenching in the *Aliivibrio fischeri* bioluminescence model system – Modulation of bacterial communication. *International Journal of Pharmaceutics*, 594, 120150. https://doi.org/10.1016/j.ijpharm.2020.120150 IF: 5,720; D1; IC: 28
16. Dell'Armi, E., Zeppilli, M., Di Franca, M. L., Matturro, B., Feigl, V., Molnár, M., Berkl, Zs., **Németh, I.**, Yaqoubi, H., Rossetti, S., Papini, M. P., Majone, M. (2022). Evaluation of a bioelectrochemical reductive/oxidative sequential process for chlorinated aliphatic hydrocarbons (CAHs) removal from a real contaminated groundwater. *Journal of Water Process Engineering,* https://doi.org/10.1016/j.jwpe.2022.103101 IF: 5,485; Q1; IC: 17

Peer-reviewed conference publications

17. Berkl, Zs., Molnár, M., Fenyvesi, É., **Németh, I.**, Buda, K., Fekete-Kertész, I., Márton, R., Szente, L. (2020). Cyclodextrin-mediated quorum quenching in *Aliivibrio fischeri* model system. *4th National Conference of Young Biotechnologists*, Debrecen, Hungary. 2020.12.19.

Other conference participations

Oral presentations:

18. Berkl, Zs., Molnár, M., Fenyvesi, É., **Németh, I.**, Buda, K., Fekete-Kertész, I., Márton, R., Szente, L. (2020). Cyclodextrin-mediated quorum quenching in *Aliivibrio fischeri* model system. *4th National Conference of Young Biotechnologists*, Debrecen, Hungary. 2020.12.19.

Poster presentations:

19. Berkl, Zs., Molnár, M., Bordohányi, Á., Fekete-Kertész, I., **Németh, I.**, Fenyvesi, É., Szente, L. (2019). The effect of cyclodextrins on the biofilm formation of *Pseudomonas aeruginosa* – Modulation of quorum sensing. *6th European Conference on Cyclodextrins*. Santiago de Compostela, Spain.
20. Molnár, M., Berkl, Zs., **Németh, I.**, Fekete-Kertész, I., Márton, R., Timár, B., Fenyvesi, É., Szente, L. (2019). Cyclodextrin-mediated quorum quenching in the *Aliivibrio fischeri* bioluminescence model system – modulation of bacterial communication. *6th European Conference on Cyclodextrins*. Santiago de Compostela, Spain.