

Nanotechnology is currently one of the most rapidly developing technologies, presenting great opportunities for the evolution of economic life worldwide. The mass use of nanomaterials in various areas of life, including the production of everyday products (cosmetics, clothes) as well as many industrial processes (electronics production, vehicle manufacture, food and beverage production, and agriculture) creates the risk of their uncontrolled release and accumulation in the environment. Despite many attempts to assess the risks associated with introducing nanoparticles into the environment and their toxicity to microorganisms, these issues are still insufficiently understood. It mainly results from the unique properties of nanomaterials compared to traditional materials, making it difficult to track their fate in the environment and to assess the current level of contamination.

Microorganisms inhabiting various environmental compartments are characterised by quick and complex responses to stress conditions, including the presence of nanomaterials. Data from recent case studies indicate that nanostructures can negatively affect the physiological processes of bacteria, which can consequently lead to a disruption in the functioning of ecosystems. Therefore, it seems crucial to conduct more intensified research in nanotoxicology to highlight the potential threats of nanomaterials to microbial communities and prevent the negative effects of the accumulation of these pollutants in the environment. Ecotoxicological studies using various strains of bacteria that play a key role in diverse ecosystems as well as model microorganisms are one of the basic steps to learn about the mechanisms of action of nanostructures.

Therefore, the objective of the project is a multifaceted analysis of the impact of inorganic nanoparticles (nAg, nCu, nTiO₂ and nZnO) on reference bacterial strains: *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus epidermidis* derived from the American Type Culture Collection (ATCC). The choice of strains from different families will allow bacterial responses to be compared in terms of their sensitivity or resistance to metal nanoparticles. It is worth noting that it will be a new approach combining the molecular mode of action of metallic nanoparticles with their genotoxicity and biochemical background. To explain the impact of nanoparticles on selected physiological processes of bacteria, appropriate biochemical, genetic and microbiological methods will be used. The scope of the work will include assessing the sensitivity of the strains to nanoparticles, measuring the antioxidant enzyme activity, studying alterations in the expression of selected stress genes, cell membrane permeability, fatty acid composition, cell morphology, and other intracellular parameters. To compare the toxicity of tested nanoparticles towards other environmental strains, the Microbial Assay for Risk Assessment will be performed. Additionally, the morphology of nAg, nCu, nTiO₂, and nZnO will be observed using scanning electron microscopy.

Since no specific regulations, protocols, or standardized methods are available to study nanoparticle toxicity towards microorganisms, it is necessary to conduct numerous experiments with various types of nanomaterials and microorganisms to determine potential nanoscale hazards. Such research is fundamental in the era of growing production and the increasing use of nanomaterials in various areas of life and assessing their potential threat to living organisms and the environment. The proposed project will provide new knowledge about the molecular action of metallic nanoparticles against microbial cells. Especially, a thorough analysis of the expression of bacterial stress genes and their products will guarantee new information about the biological activity of these nanostructures. Moreover, analyses of cellular fatty acid profiles and the development of new stress indicators seem to be milestones in nanotoxicological studies. It is also worth highlighting that the presented project aims to introduce an easily accessible and reproducible methodology for future research.