**Transfer analysis of pharmaceuticals, antibiotic resistance genes, microplastics and chemical pollutants from municipal wastewater to the catchment area using artificial neural networks and spatial modelling**

The transfer of emerging pollutants, such as pharmaceuticals and antibiotic resistance genes, microplastics, dioxins and ubiquitous nutrients, to the environment presents a new challenge for ensuring global water quality, with potentially very serious implications for human health and ecosystems. Generally, micropollutants, including pharmaceuticals, microplastics and dioxins and microbial contamination (antibiotic resistance bacteria - ARB and antibiotic resistance genes - ARGs) as well as nutrients, can enter the aquatic environment through wastewater treatment plants (WWTPs). Clearly, to halt the spread of these pollutants, there is a pressing need for deeper knowledge about the processes of their migration and transfer to the river system from WWTPs. The proposed research will be performed throughout the 9,258 km2 Pilica River catchment and will include 17 WWTPs, divided into three size categories, viz. small, medium-sized and large. The overall aim of the project is to more clearly determine the nature of micropollutant contamination in the catchment caused by pharmaceuticals, microplastics, dioxins and antibiotic-resistance genes. These contaminants present a severe threat to the aquatic environment, accompanied by a considerable threat to human health. According to EU directive 2008/105/EC, as a matter of priority, pollution sources should be identified and emissions dealt with at source.

**The main goal of the project** is to identify pharmaceutical consumption patterns and determine the transport of 23 pharmaceuticals, antibiotic resistance genes (ARG), five kinds of microplastic particles, dioxins and nutrients from wastewater to the Pilica river in the catchment scale; the project will also study the transformation of these compounds during the wastewater treatment process and determine their total toxicity and hormonal activity impact, which are currently very poorly understood. Pharmaceuticals represent a versatile group of compounds found in surface waters; however, although thousands have been approved for human or veterinary usage, only about 80–150 have been studied for their presence in the environment. It is believed that wastewater treated in WWTPs is the main anthropogenic source of antibiotics, ARB and ARGs released into the environment. Microplastics, i.e. particles with a diameter of 1 µm-5 mm, are created by the breakdown of plastic objects. They are ubiquitous in the environment and hence, are one of the most common and persistent pollutants. Microplastic water pollution may cause physical, chemical, and microbiological toxicity of organisms. Dioxins are also highly toxic and have been linked to cancer, liver damage, and various reproductive and developmental diseases.

**A novel aspect of the project** will be its interdisciplinary and comprehensive approach to research, aimed at determining the overall toxicity of water and sewage throughout the whole Pilica river basin. This is an important aspect, as the level of risk does not depend solely on the level of concentrations of individual pollutants, i.e. pharmaceuticals and their metabolites, microplastics, dioxins and nutrients, which demonstrate varied biological toxicities. To gain a better insight into the toxic effects of these chemicals, the project will determine the effects of any pharmacologically-active substances present in treated wastewater and river waters on the mortality, growth and reproduction of organisms; it will achieve this using a range of toxicity testing procedures, in conjunction with the new YES (estrogen) and YAS (androgen) hormone activity analysis methods. In addition, the microbial diversity of the river water and treated and untreated wastewater samples will be determined by genome sequencing. Another innovative element of the project is that it will use a wide range of statistical and mathematical data analysis methods i.a.: Kohonen artificial neural networks (ANN), and pattern recognition methods, as well as multivariate ordinations and spatial ArcGIS analysis and modeling.

**Research project methodology** - The project is intended to create an overall image of the regional consumption of pharmaceuticals (under Task 1 and 2), the burden caused by pharmaceuticals (Task 3), ARG levels and microbial diversity (Task 4 - 6), microplastics (Task 7), dioxins (Task 8), nutrients, Biological Oxygen demand (BOD) and Chemical Oxygen Demand (COD) (Task 9) released from selected wastewater treatment plants (WWTPs); it is also planned to study the total toxicity and hormonal activity of river water and treated wastewater (Task 10). All the results will be subjected to a detailed mathematical and statistical analysis along with spatial modelling of the examined processes (Task 11). **The importance of the project and expected results** – This project has huge potential to gain new knowledge in the field of a very poorly-studied process, i.e. the transfer of pharmaceuticals, drug resistance genes, microplastics and dioxins from wastewater to surface waters. Furthermore, its approach to interpreting and statistical analysis of environmental data is also innovative, and one which will generate new utilitarian tools for research in the Earth sciences and offer significant added value to Science. In this respect, the project is important for the international community. The garnered data will, in turn, contribute to the development of a holistic strategy for the reduction of contamination and degradation of water resources by micropollutants (pharmaceutics, microplastics, dioxins and antibiotic-resistant genes). As such, the innovative scope of the planned research fits perfectly into the demand for knowledge while supporting the European One Health Action Plan against Micropollutants and Antimicrobial Resistance, with the aim of building a safe future for society.