Fungi are the second largest group of eukaryotes. The widespread presence in the environment and food chain of some species, the so-called fungal pathogens, can, however, make them dangerous to humans. Mycotoxins are low-molecular-weight secondary metabolites produced by pathogens fungal pathogens such as *Aspergillus*, *Fusarium* and *Penicillium* and pose a serious threat to the safety of food and consumer health. Some commonly produced mycotoxins, such as afla- and ochratoxins, zearalenone (ZEN), deoxynivalenol (DON) and patulin (PAT) can cause disease and death in humans and other animals. For this reason, mycotoxin contamination is responsible for wasting about 1.3 billion tons of food every year. About 25% of the world's grain products are contaminated with mycotoxins, which is also demonstrated by the recent situation in Poland, when imported grain often contained harmful substances. In view of this, monitoring the quality of food and feed is very important before they enter the consumer market to ensure a sustainable food supply. Therefore, there is a need to develop readily available and reliable tools for the detection of contaminants mycotoxins and the fungal pathogens that are their source.

Electrochemical DNA biosensors, due to their low manufacturing cost, simplicity of signal readout and the capabilities they offer for analysis directly in complex samples, are valuable tools of growing importance in genetic diagnostics and environmental analysis. They owe their versatility and broad applicability to the unique receptor properties of single-stranded oligonucleotide sequences. Their appropriate and rational design can result in obtaining DNA probes capable of selective interactions with both complementary DNA sequence (genosensors) as well as with other analytes, such as organic toxins (aptasensors). This makes them potentially attractive tools for rapid detection of food safety hazards. Classical affinity biosensors, which also include aptasensors and genosensors, operate based on the heterogeneous mechanism, that employs receptors responsible for the binding of the detected substances in an immobilized form on a solid substrate. This approach facilitates subsequent separation of unbound sample components and signal readout. However, the restriction of the freedom to change the conformation of the receptor DNA sequence, i.e. to match its shape to the molecule to be detected, which results from surface tethering, can significantly weaken the affinity of the receptor. Such a phenomenon is unfavorable and compromises the analytical performance of such biosensor.

The reported project aims to study in depth the significance of the effect of the oligonucleotide receptor form (free *vs.* surface-bound) on its ability to bind various analytes, on the example of a small organic molecule - mycotoxin DON and biomacromolecule - specific genetic sequence of *Fusarium ssp*. (a genetic biomarker obtained by PCR amplification reaction). To demonstrate the potential advantages of using receptors in an unbound form (homogeneous mechanism), the study will develop the design of two variants of the DNA biosensor (aptasensor and genosensor) for detection of the above-mentioned model analytes, which are important for food security. The proposed solutions are based on the molecular recognition of DON toxin and *Fusarium* gene by the DNA receptor present in the solution, specific labeling by magnetic nanoparticles and conjugates of the enzyme alkaline phosphatase, magnetic separation of the labeled complexes, and electrochemical detection of the enzymatic reaction products. We believe the developed biosensing strategy will combine the advantages of: i) high sensitivity - due to unleashing of previously constrained oligonucleotide receptors binding capacity, ii) the versatility of labeling with ALP-DNA conjugates and magnetic separation, and iii) the simplicity of electrochemical detection using non-modified electrodes.