First RECETOX Doctoral Conference
25-28.5.2020

MUNI | RECETOX
SCI

Ecotoxicity Evaluation of Silver-based Antimicrobial Systems

Ph.D. Student: Mgr. Markéta Hůlková **Supervisor: prof. Ing. Blahoslav Maršálek, CSc.**

BOTANICKÝ ÚSTAV AV ČR v.v.i. | MONTANA STATE UNIVERSITY | UFZ HELMHOLTZ Centre for Environmental Research | FULBRIGHT

Introduction

Silver nanoparticles (AgNPs) are promising antimicrobial agents with broad-spectrum activity at low doses, a low incidence of bacterial resistance compared to traditional antibiotics and a low toxicity to humans. Nanosilver in various forms has thus become an active component of many products and technologies aimed at controlling unwanted bacteria, fungi and algae. However, our knowledge of the technical qualities of these systems lags behind the understanding of their potential impacts on environmental health. The presented **studies I - II** fill a gap in the literature by performing efficacy and ecotoxicity analysis of two types of recently developed nanosilver-based antimicrobial systems: the multicomponent hybrid nanocomposites of silver and magnetic iron oxide (**Study I**), which are essentially magnetically-guided antimicrobial agents, and of surface-immobilized AgNPs (**Study II**), that are targeted for preventing biofilm formation. The first system design allows nanocomposite recovery from a media after disinfection and recycling, and both systems allow sustainable use of resources, reduce silver emissions, and the risk of environmental contamination. **Study III** assessed ecotoxicity of nanoparticle surface coating agents, which are a component of nanoparticles that significantly contributes to their reactivity, behavior in environmental and biological media, and overall toxicity.

Methodology

Drip flow biofilm reactor experiments, microbiology testing, confocal scanning laser microscopy (CSLM), epifluorescence microscopy, ecotoxicological bioassays incl. fish embryotoxicity testing, etc.

Conclusions

AgNPs have considerable potential to be the first choice for future antimicrobial applications but require robust testing and validation to determine where they will be, and where they will not be, a successful strategy. **Studies I - III** expand our knowledge of the ecotoxicological effects of nanosilver-based systems and improves our understanding of the mechanisms and factors (i.e. nanoparticle surface coating, silver ion release) affecting them, so that we can better predict and prevent their impacts on the environment. The acquired knowledge will serve for designing AgNP-based systems with enhanced antimicrobial efficacy, stability, specificity, and reduced potential side effects. The information provided will contribute to a reliable risk assessment and to the development of preventive measures for a safe and sustainable use of AgNP-based antimicrobial systems.

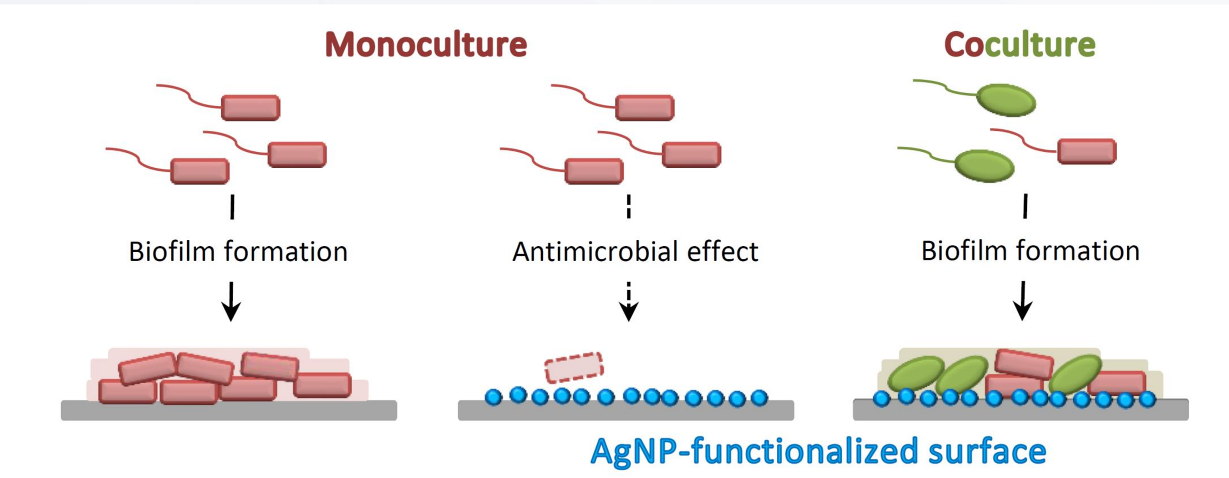

Fig 3: Graphical abstract of study II

Study I

Study I is the first to assess the toxic potency of silver-magnetite nanocomposite against ten model organisms representing different trophic levels and environments, including bacteria, algae, plants, invertebrates and a vertebrate. The effect of aging on the nanocomposite toxicity was also analyzed. Evidence is provided (Fig.1) identifying the nanocomposite as potentially hazardous at very low concentrations (< 0.1 mg of silver /L). Planktonic crustaceans, fish embryos and cyanobacteria were extremely sensitive, with the EC50 values equal or less than 0.1 mg/L. Although bacteria and algae are the target groups for the nanocomposite application, the range of effective concentrations spanned nearly 4 orders of magnitude, from 0.02 to 8.6 mg/L, with some bacterial strains being among the least sensitive organisms of all tested. The obtained species sensitivity data show that the effective concentrations of the silver nanocomposite for target and non-target organisms overlap. Any leakage into surface waters can thus pose a threat to aquatic species and environmental health. In spite of that fact, aged nanocomposite was almost 5-times less toxic than the pristine form.

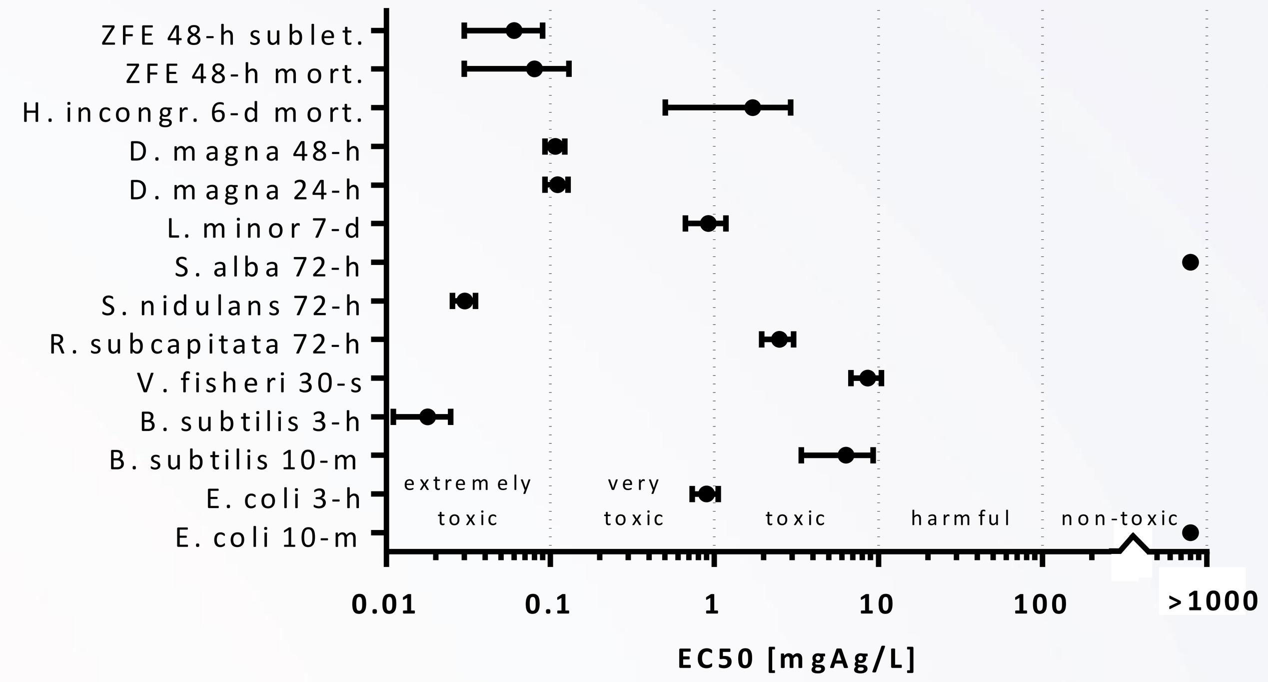

Fig 1. Toxicity of smPEI to different organisms expressed by mean $L(E)C_{50}$ values and the respective variation scale, and the respective hazard classification. Hazard potential is reported according to the EU-directive 93/67/EEC classification scheme. Note the logarithmic scale of x-axis.

Study II

Study II is among the first to analyze anti-biofilm efficacy of surfaces functionalized with immobilized AgNPs against multi-species microbial consortia. It was shown that polyethylene surfaces modified with the AgNPs were highly effective against *Pseudomonas aeruginosa* biofilms reducing viable cell counts by 99.8% as compared to controls (Fig. 2). However, the efficacy of the AgNP-modified surface was compromised when *P. aeruginosa* was cocultured with *Candida albicans* (Fig 3). As most microorganisms grow in complex multi-species communities, interspecies interactions can enhance biofilm stress tolerance and strongly influence the efficacy of anti-fouling AgNP-coatings. Besides, confocal microscopy imaging of the biofilm on immobilized AgNP-modified surfaces refutes the hypothesis that microbial surface colonization can be inhibited by contact-mediated bacterial killing, as is often reported in literature. Study II highlights the limited efficacy of nanosilver applications for the control of microbial biofilms, and the need to assess their efficiency critically, under application relevant conditions, and against multispecies biofilm communities.

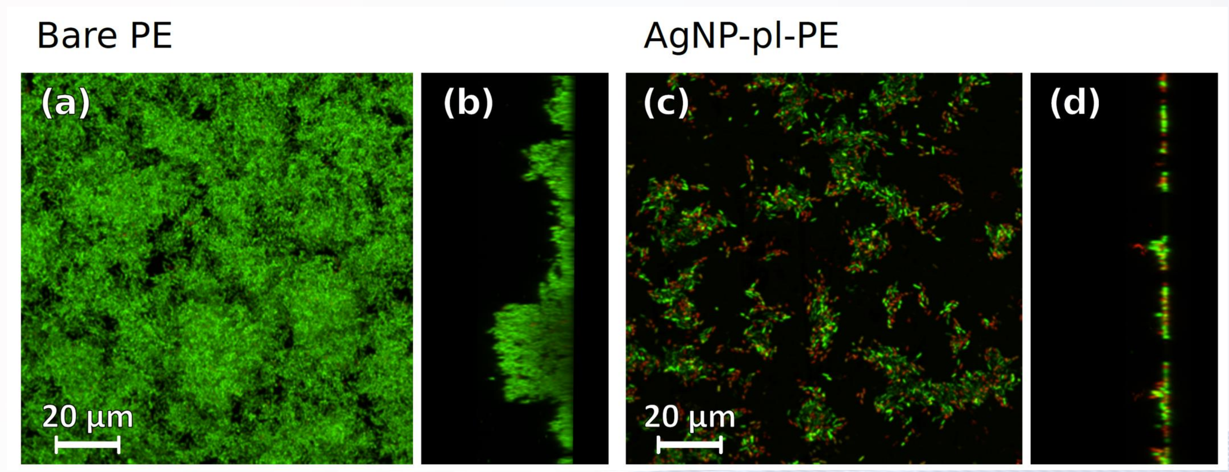

Fig 2. Confocal images of *P. aeruginosa* monospecies biofilms stained with LIVE/DEAD® BacLight Bacterial Viability Kit. Cells with intact membranes are stained fluorescent green with SYTO9 nucleic acid stain, and cells with damaged membranes are stained fluorescent red with propidium iodide nucleic acid stain. Subfigures a, c show projected top views and b, d vertical CLSM optical sections of biofilms grown in the bottom region of silver-free control (a, b) and AgNP-functionalized (c, d) polyethylene coupons. The scale bar of 20 µm applies to all images. Viable cell counts quantified a 99.8% reduction in *P. aeruginosa* viable cells relative to the control condition.

Study III

Study III is among the first to assess and compare the ecotoxicity of eight relatively inexpensive, commercially available, and frequently used nanoparticle surface coating agents. It was shown (Fig. 4) that polyethylenimine (PEI) was very toxic to primary producers (EC50 < 1 mg/L). Sodium dodecyl sulphate (SDS) and polyethylene glycol (PEG) were classified as toxic and harmful to aquatic species, respectively. Carboxymethyl cellulose (CMC), dextran (DEX), polyacrylic acid (PAA), polystyrene sulphonate (PSS), and polyvinyl pyrrolidone (PVP) were found to be non-toxic to all tested species.

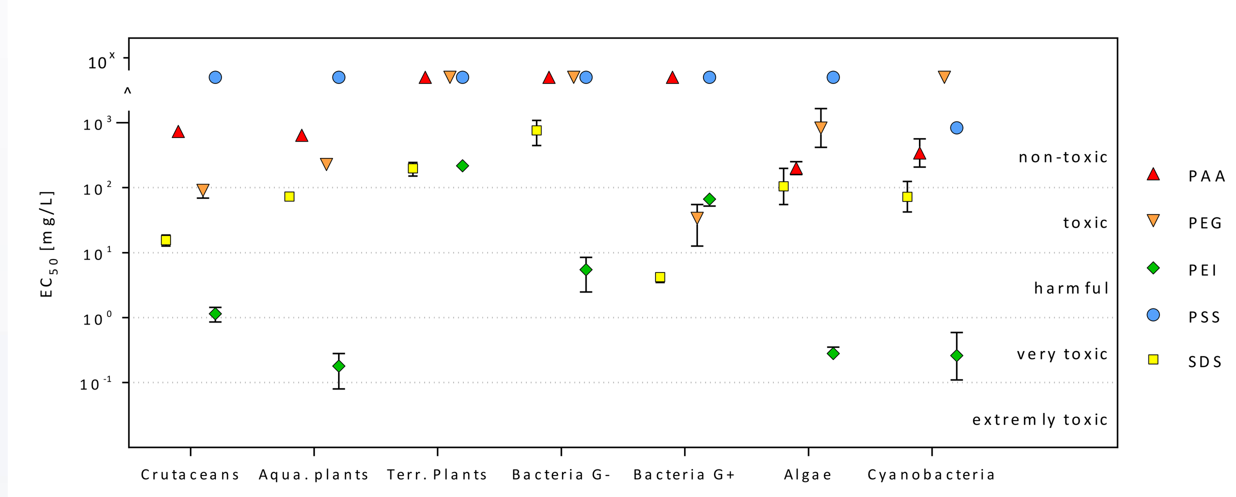

Fig 4. Toxicity of the surface coating agents against the different test organisms. Toxicity of SCAs expressed as 50% effect concentration (EC50) was ranked to different hazard categories according to the lowest mean EC50 values based on CEC (1996) classification as modified in Blaise (2008). DEX, CMC and PVP were not plotted in the graph for the sake of clarity as all their EC50 values were high above 1g/L.

Blaise, C. *et al.* (2008) 'Ecotoxicity of Selected Nano-Materials to Aquatic Organisms', *Environmental Toxicology*, 23(5), pp. 591–8. doi: 10.1002/tox.

CEC (1996) 'CEC (Commission of the European Communities) technical guidance document in support of commission directive 93/67/EEC on risk assessment for new notified substances. Part II, Environmental Risk Assessment.' Luxembourg.