Diagonal project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement Nº 953152.

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DEVELOPMENT OF A METHODOLOGY TO PERFORM TOXICOKINETICS AND TOXICODYNAMICS STUDIES IN BIOFILMS

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INTRODUCTION

Environmental research has focussed on elucidating and modelling of **toxicokinetics** and **toxicodynamics** of different types of nanomaterials in soil and aquatic organisms, and also in biofilms.

The first steps towards the developing of a protocol to analyse **Multi-Component NanoMaterials (MCNMs)** and **High Aspect Ratio Nanoparticles (HARNs)** tokicokinetics and toxicodynamics in biofilms are described here, using *P. putida* as model organism.

This study is part of a task included in the project DIAGONAL (H2020, NMBP-16-2020).

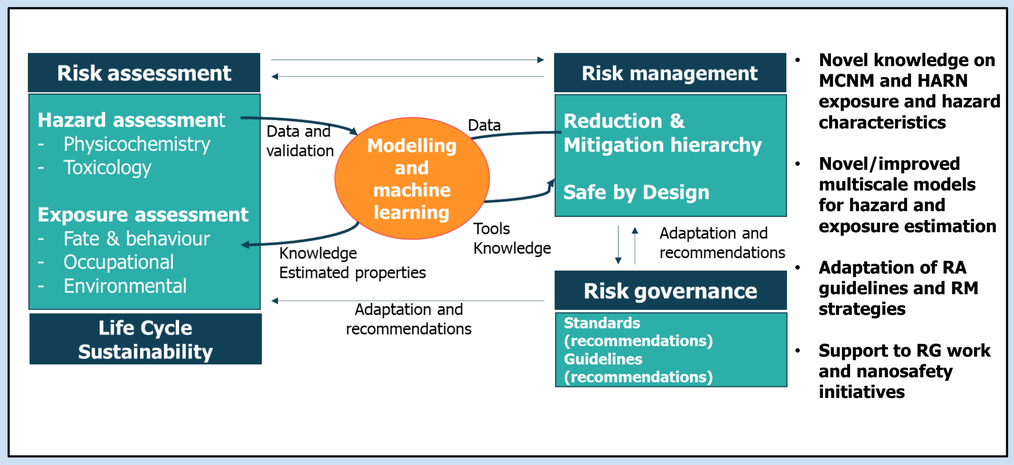


Figure 1: Project concept of DIAGONAL.

TOXICOKINETICS

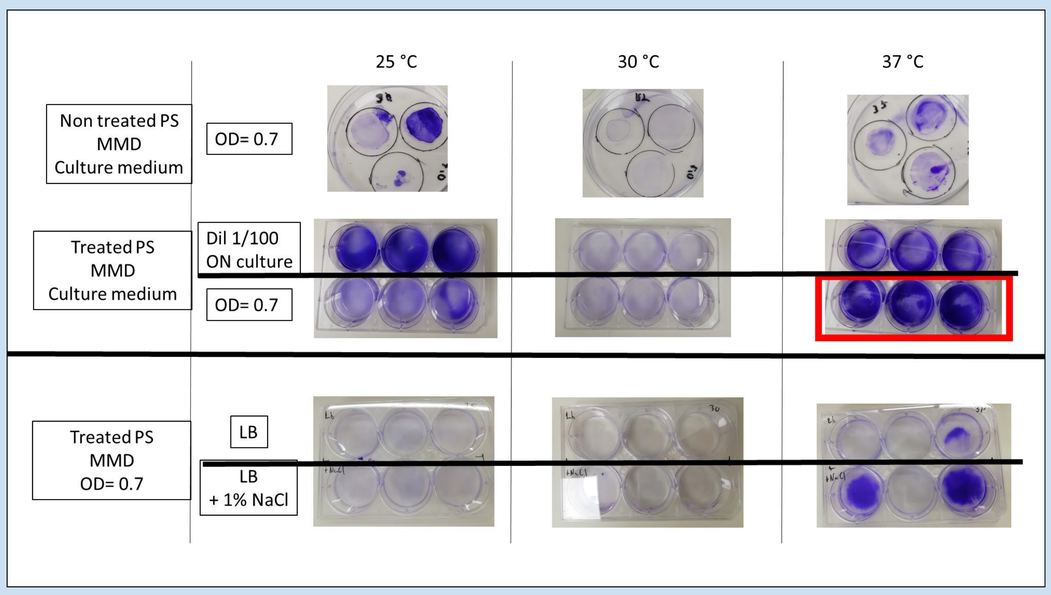
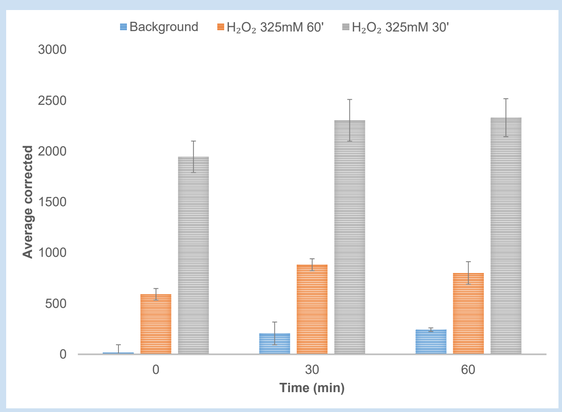


Figure 2: Artificial biofilm development: different conditions (T°, culture medium, initial inoculum, surface) were tested in order to select those more appropriate for biofilm formation.

TOXICODYNAMICS



Ratio		
Time	H_2O_2 30'	H_2O_2 60'
30 min	11,190	4,288
60 min	9,610	3,303

Figure 3: ROS levels in planktonic cells of *P. putida* after exposure to H_2O_2 during 30 and 60 minutes (left). Table on the right represents the ratios obtained (relative fluorescence value to the control (untreated cells) which was assigned a value of 1). Cells were stained with CM-H2DCFDA.

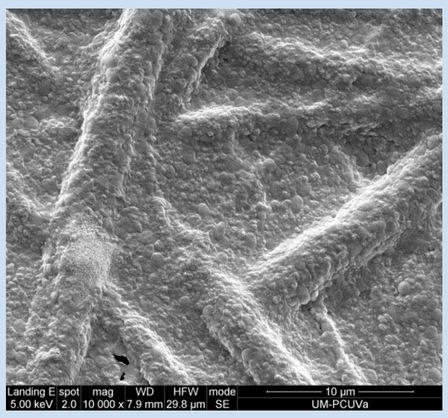


Figure 4: *P. putida* biofilms visualized by SEM (left) and graphical abstract (right).

Preliminary protocol for toxicokinetics and toxicodynamics of metallic nanoparticles in biofilms:

Biofilm formation:

- Initial inoculum: OD: 0,7 (≈ 3-7 x 10^7 bacteria/mL)
- Culture medium: MMD
- Tº: 37 ºC
- Incubation time: 96 h
- Surface: cell culture treated PS well plates

Toxicokinetics:

- Biofilm exposure to nanoparticles at different times.
- At each time point:
  - Medium is recovered (Fraction 1)
  - Biofilms are washed with water (Fraction 2)
  - Biofilms are recovered with cell scrappers and weighed (Fraction 3)
- ICP-MS analysis of each fraction
- Application of the appropriate model

Toxicodynamics:

- Biofilms recovering with cell scrappers.
- CM-H2DCFDA staining in tubes (2h, 30 °C, 120 rpm)
- Wash with PBS
- Bacteria are resuspended in MMD, transferred to tubes, and fluorescence is measured at 30 and 60 minutes (30 ºC, 120 rpm)