

# Název: Metalothionein jako vhodný marker pro prostředí zasažené těžkými kovy

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World Environment Day  
5 June

Reg.č.projektu: CZ.1.07/2.3.00/20.0148

Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik



## GOAL

- focus on the application of electrochemical methods for studying of bacterial strains *Escherichia coli* and *Escherichia coli* cloned with human metallothionein gene (MT-3) like a model organism
- electrochemical detection before and after the application of cadmium and/or lead ions in four concentrations (25, 50, 75 and 150  $\mu\text{M}$ )

## INTRODUCTION

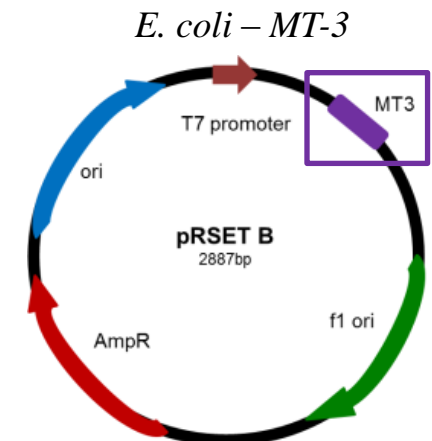
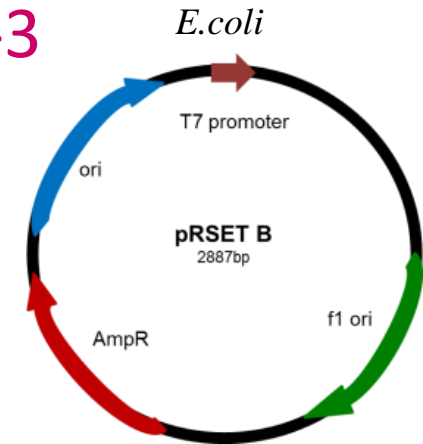
- One of the mostly used way how to enhance the ability of bacteria to withstand metal ions adverse effects is to clone gene for metallothioneins (MTs) or by over-expressing metal-binding peptides or proteins such as poly-histidines or poly-cysteines
- The special interest - **metallothioneins**, small polypeptides with high percentage (approximately 30%) of cysteine residue and with affinity to bind heavy metals
- Through multiple thiolate bonds of cysteines, metallothionein has the capacity to chelate seven atoms of Zn or Cd, or 12 atoms of Cu per molecule
- **They bind** essential (Zn, Ni, Cu) and also **toxic (Cd, Pb and Hg) metals**.
- Transporting and regulation of essential metals, protection against oxidative stress and protection against metal toxicity belong to their main intracellular functions.

*Adam et al.: An Effect of Cadmium and Lead Ions on Escherichia coli with the Cloned Gene for Metallothionein (MT-3) Revealed by Electrochemistry*

## CULTIVATION OF *E. COLI* AND *E. COLI* MT-3 RESISTANT STRAINS

Preparation of *Cd/Pb* resistant strains:

- Control bacterial culture of *E. coli* was cultivated in LB medium and *E. coli* with human MT-3 gene was cultivated in LB medium with ampicillin, chloramphenicol and IPTG for 24 hours with shaking
- In the microplate these cultures were mixed with cadmium and lead ions (25, 50, 75 and 150  $\mu\text{M}$ ) or strains without addition of heavy metal ions alone as a control for measurements.

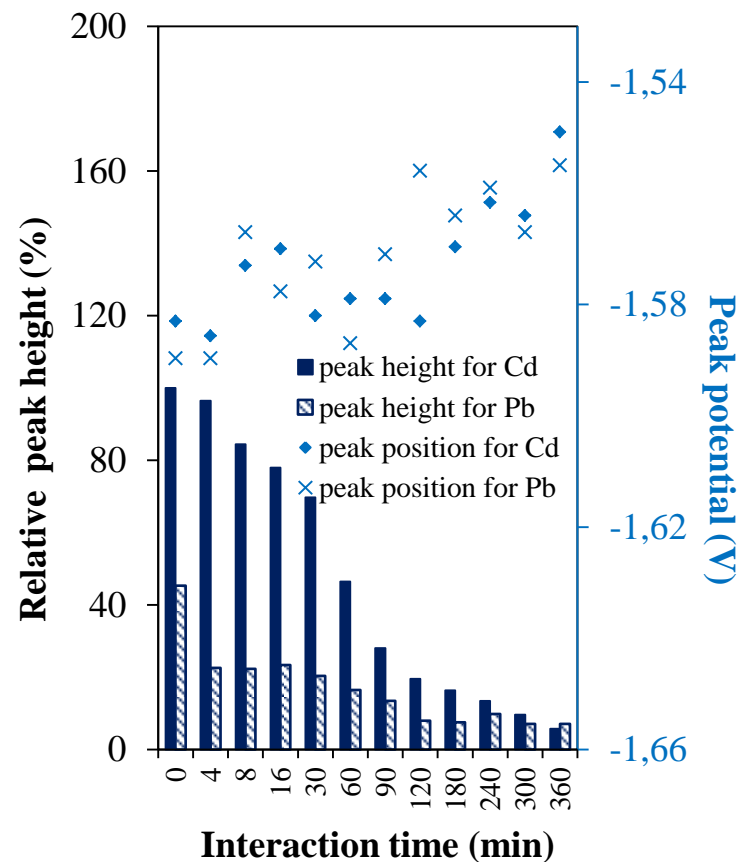
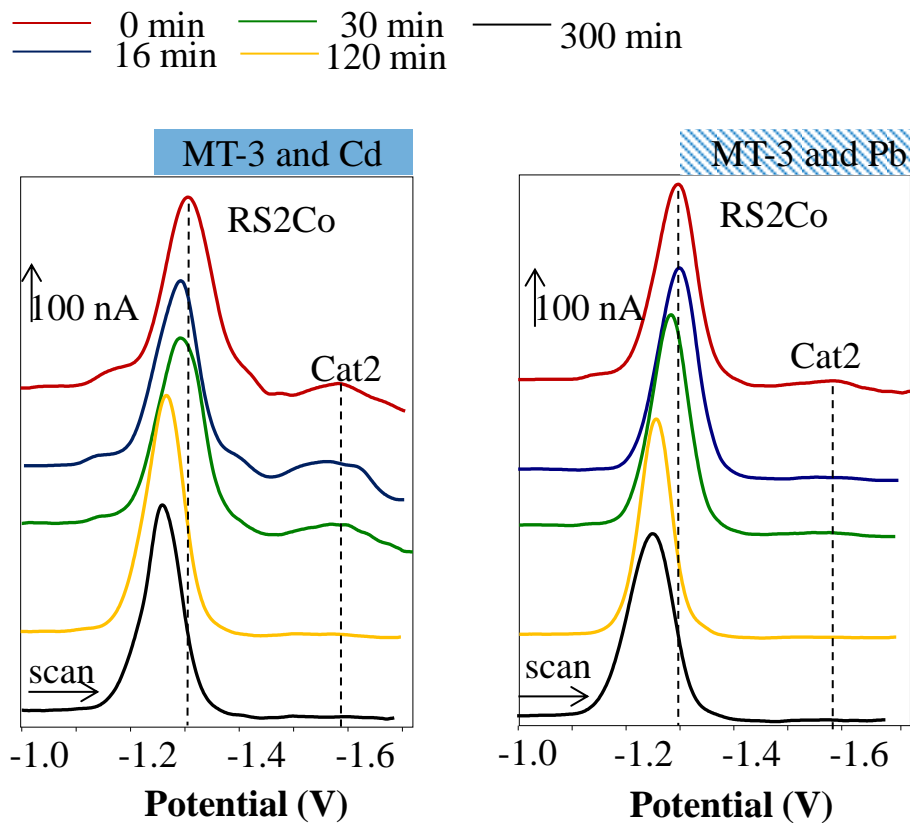


## ELECTROCHEMICAL DETECTION OF *E. COLI* MT-3 WITH METALS AND RESISTANT STRAINS

- DPV, in the electrochemical cell
- three electrodes set-up: working mercury drop electrode (HMDE), reference silver-chloride electrode Ag/AgCl/3M KCl and auxiliary carbon electrode
- Brdicka electrolyte: 1980  $\mu\text{l}$ /1900  $\mu\text{l}$
- Dosage sample volume: 10 and 10  $\mu\text{l}$  /100  $\mu\text{l}$
- The analysed samples were deoxidized by argon for 120 seconds
- The parameters: initial potential -0.7 V, final potential -1.75 V, the time interval 0.2 s, step potential 2 mV, amplitude -250 mV

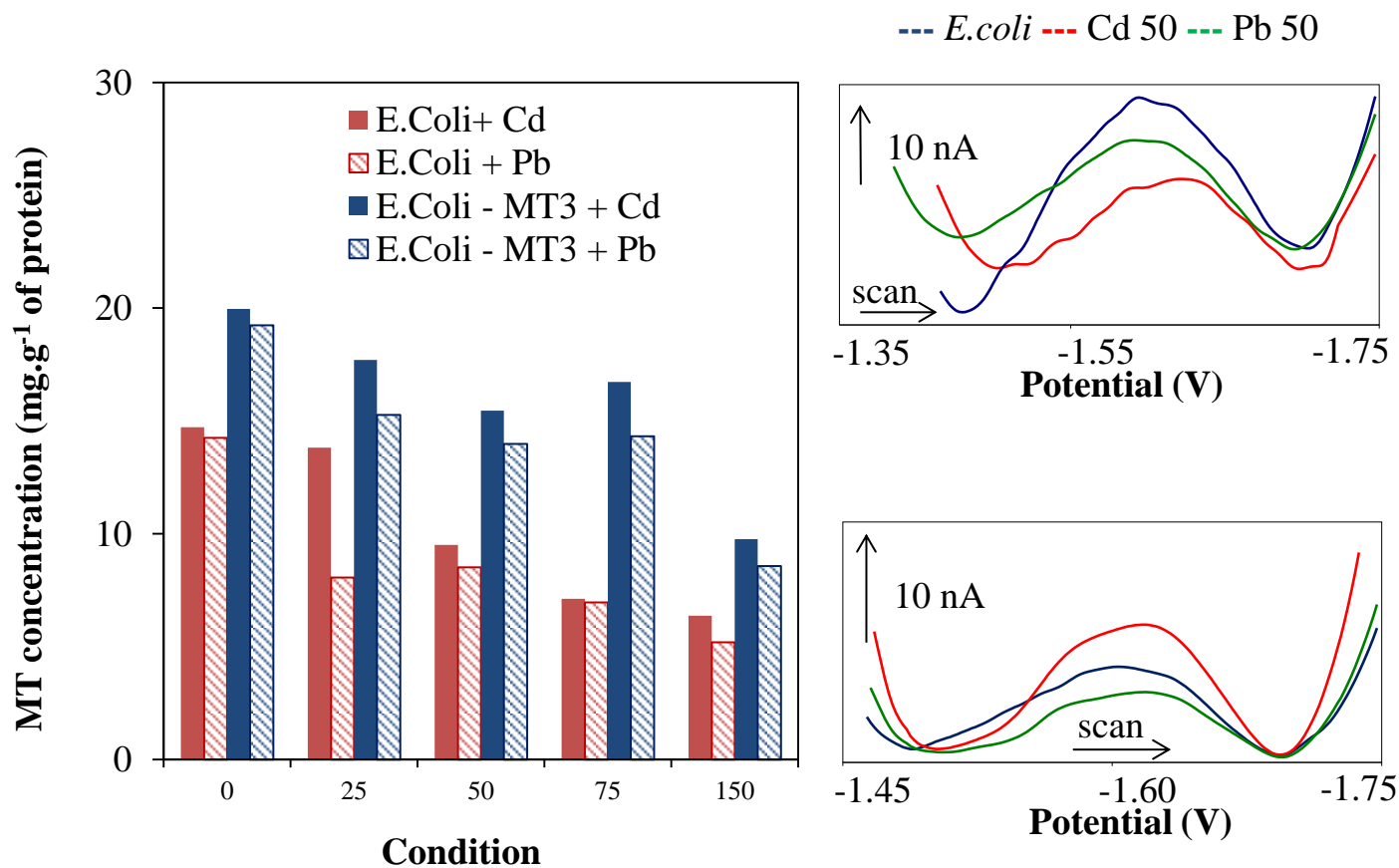
## E. COLI MT-3 WITH METALS

MT-3 ( $13 \mu\text{g}\cdot\text{ml}^{-1}$ ) with  
200  $\mu\text{M}$  Cd and/or Pb ions



## RESISTANT STRAINS

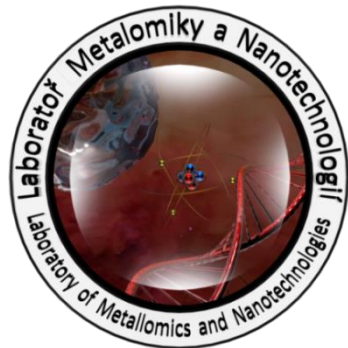
cadmium and lead ions  
(25, 50, 75 and 150  $\mu\text{M}$ )



## SUMMARY

- The changes in electrochemical records due to the interactions of metallothionein MT-3 with cadmium and lead ions showed decline of Cat2 signal of MT with the increasing interaction time because of metal ions binding to cysteines.
- Electrochemical determination also revealed that Cd(II) remains in *E. coli* cells in the higher amount than Pb (II). Opposite situation was found at *E. coli* – MT-3 strain.





# Děkuji za pozornost!

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