Determination of biochemical biomarkers in biota – useful ecotoxicological tools of environmental contamination

Zuzana Redžović
Ruđer Bošković Institute
zuzana.redzovic@irb.hr

KICK-OFF MEETING

Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zagreb, 11th October 2021

HrZZ Croatian Science Foundation

RB

ANDRIJA STAMPAR TEACHING INSTITUTE OF PUBLIC HEALTH

WELLFISH DIAGNOSTICS

MONTAN UNIVERSITÄT

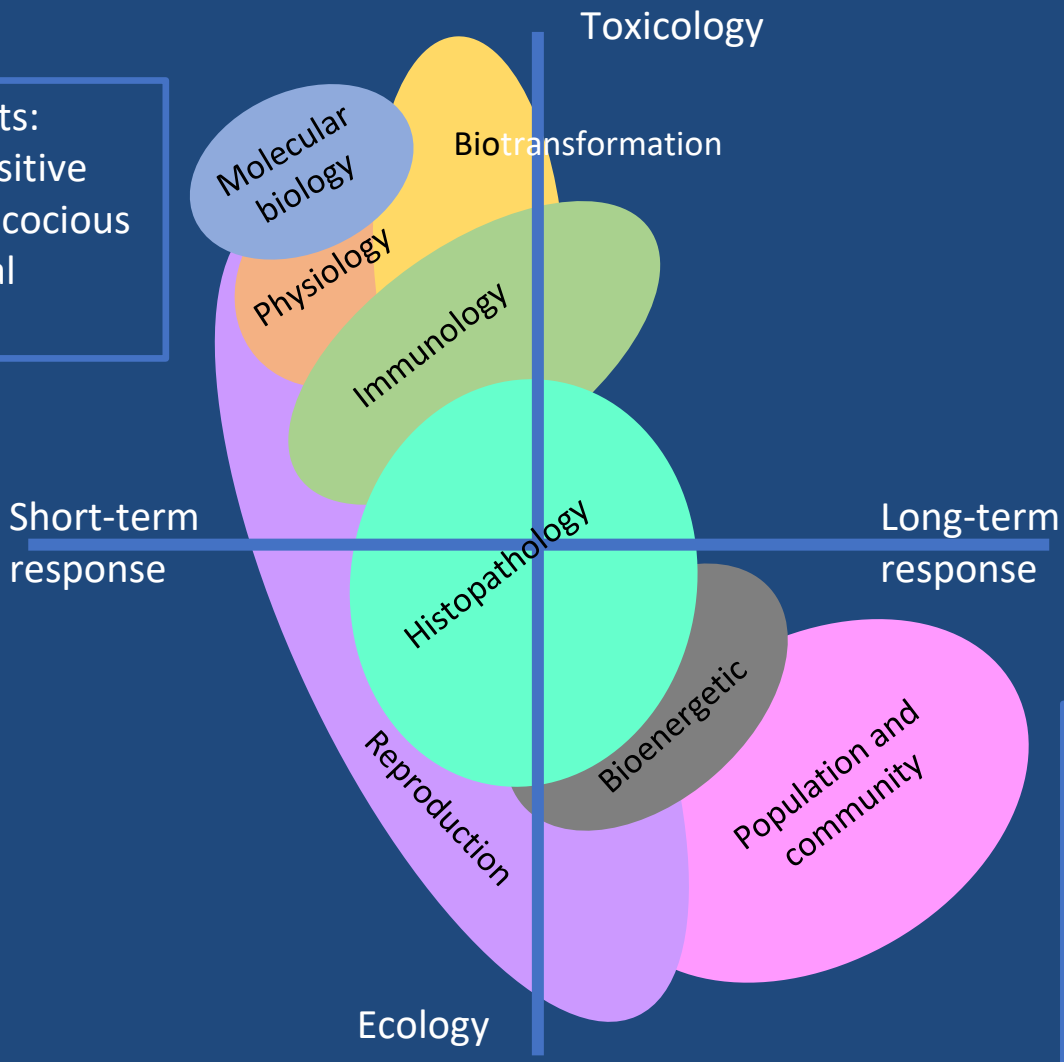
FFoQSI

BIOTOXMET

INTRODUCTION

Biological relevance of biomarkers

Molecular effects:
- the most sensitive and most precocious
- low ecological relevance



Population responses:
- relevant to assess the "good ecological status"
- effects become significant only after severe environmental degradation

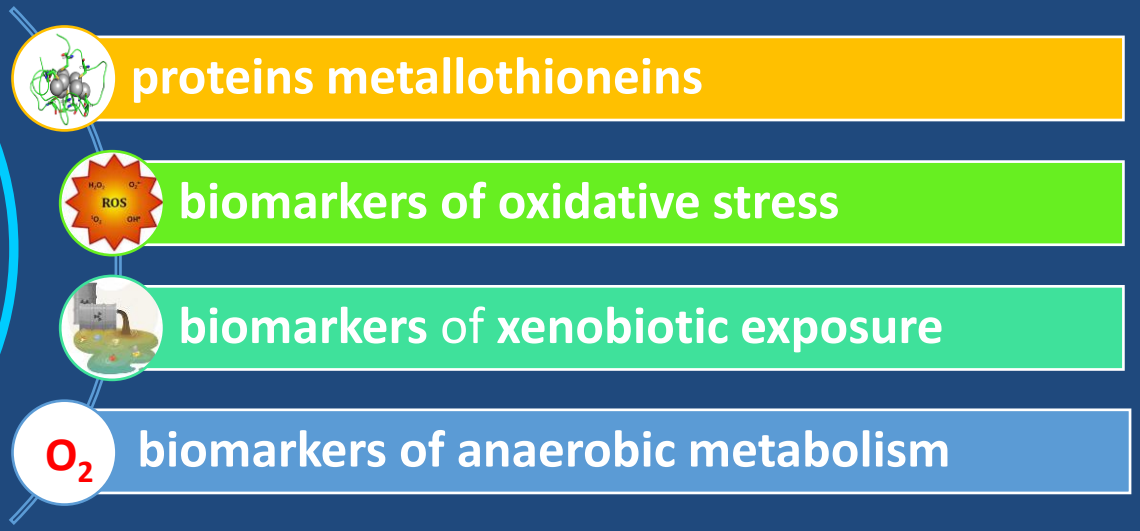
After: Amiard-Triquet et al., 2013

INTRODUCTION

Multibiomarker approach

BIOMARKERS – changes in cellular structures or functions that reflect an interaction between a biological system and a potential chemical, biological or physical harmful factor

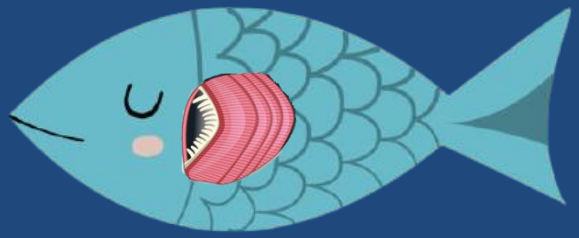
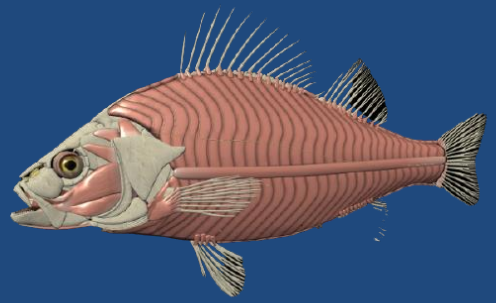
MULTIBIOMARKER APPROACH – necessary in environments exposed to **different groups of pollutants** → to assess the biological responses of the organisms that inhabit them



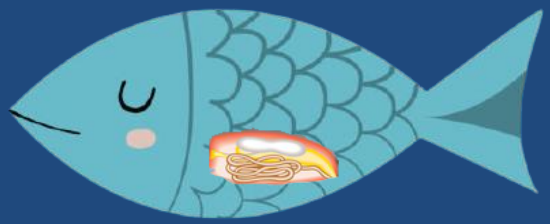
MEASUREMENTS

Biomarkers in the fish intestine

- most commonly used bioindicator tissues in biomonitoring studies are liver, gills and muscle (Filipović Marijić and Raspor 2006, Barišić et al. 2015)

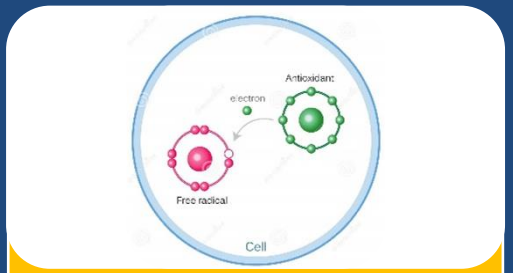
- the intestine is less used → the **data** on **biochemical biomarkers** are **rare** → rare data on biomarker responses to changes in environmental conditions and exposure to pollutants



BIOTOXMET PROJECT WILL INVOLVE:

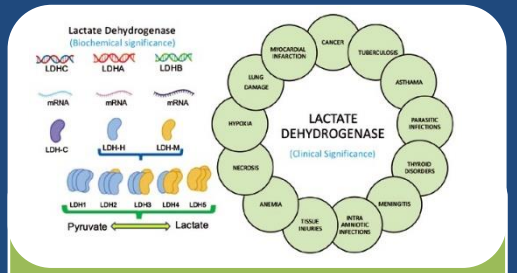
Biomarkers of metal exposure

- proteins metallothioneins (MT)



Biomarkers of antioxidant defence

- SOD, CAT, GPx
- GR, GSH, GST

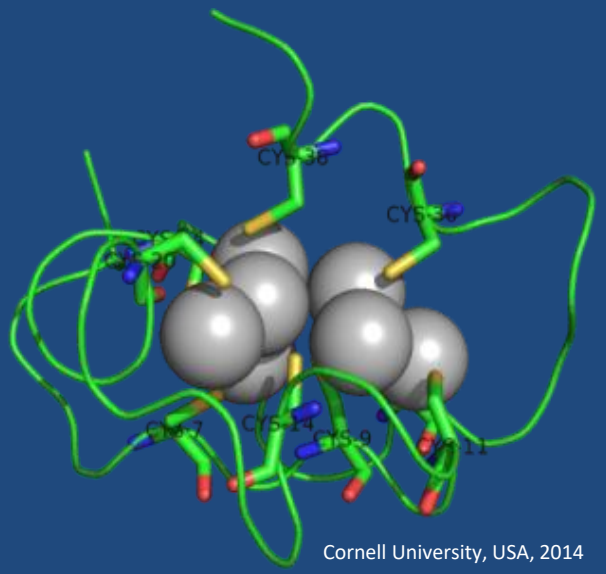


Biomarkers of tissue metabolic activity

- LDH

METALLOTHIONEINS

- a specific biomarker for **heavy metal contamination**
- soluble, thermostable low molecular weight proteins (6-7 kDa)
- high affinity for heavy metal cations (**Cd**, **Cu**, **Hg**, **Zn**, etc.)
- rich **-SH group** to which they bind metals or free radicals
- present in the normal homeostasis of essential metals

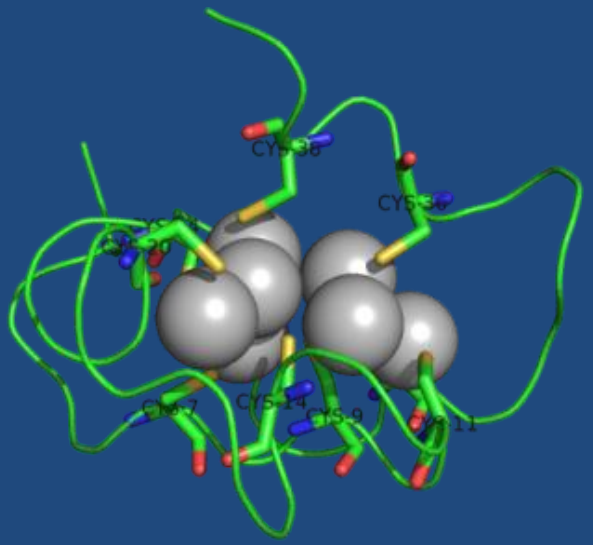


Cornell University, USA, 2014

- **increased metal conc.** → **MT induction**
- regulate the concentration of free metal cations in the cell and / or free radicals and thus protect cell structures from their toxic effect

METALLOTHIONEINS

- **ethanol precipitation** → **spectrophotometric determination of the content of free -SH groups**
- differential fractional protein precipitation occurs



Cornell University, USA, 2014

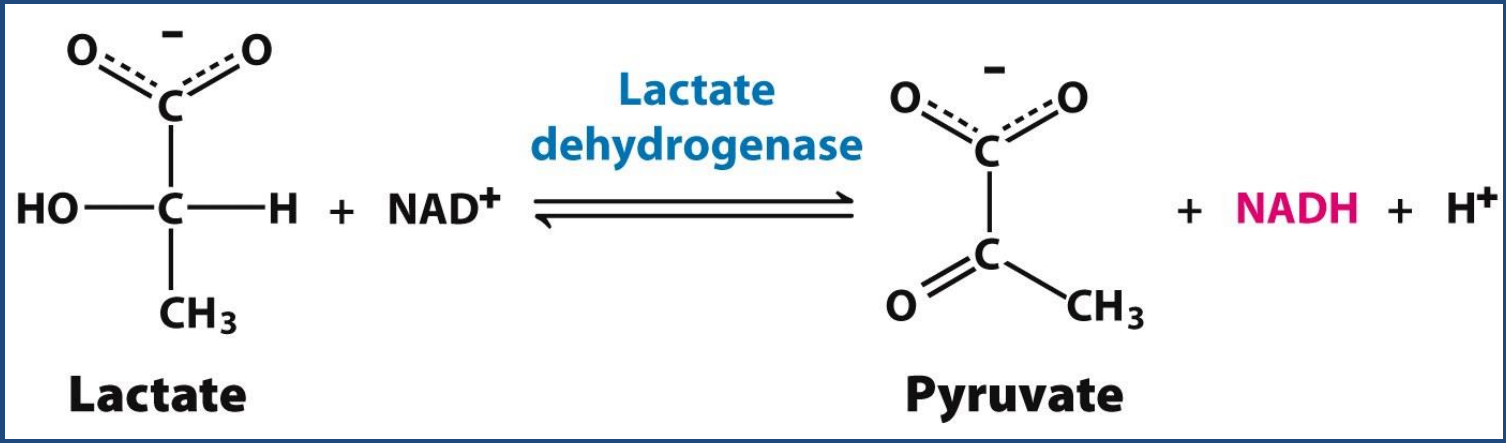
- **MT concentrations** will be determined **at 412 nm** wavelength and expressed in **µg MT per mg protein**

Viarengo et al., 1997

Metabolic enzymes

1. Catalase (CAT)	• ROS detoxification Claiborne (1985)
2. Total glutathione (GSH)	• antioxidant enzyme Tietze (1969); Rahman et al. (2006)
3. Lactate dehydrogenase (LDH)	• anaerobic metabolism, functional and environmental anaerobiosis Bergmeyer and Bernt (1974)
4. Superoxide dismutase (SOD)	• antioxidant enzyme, anthropogenic pollution Marklund and Marklund (1974)
5. Glutathione reductase (GR)	• antioxidant enzyme Mavis and Stellwagen (1968)
6. Glutathione S-transferase (GST)	• marker of detoxification processes Habig et al. (1974)
7. Glutathione peroxidase (GPx)	• ROS detoxification Flohe et al. (1973)

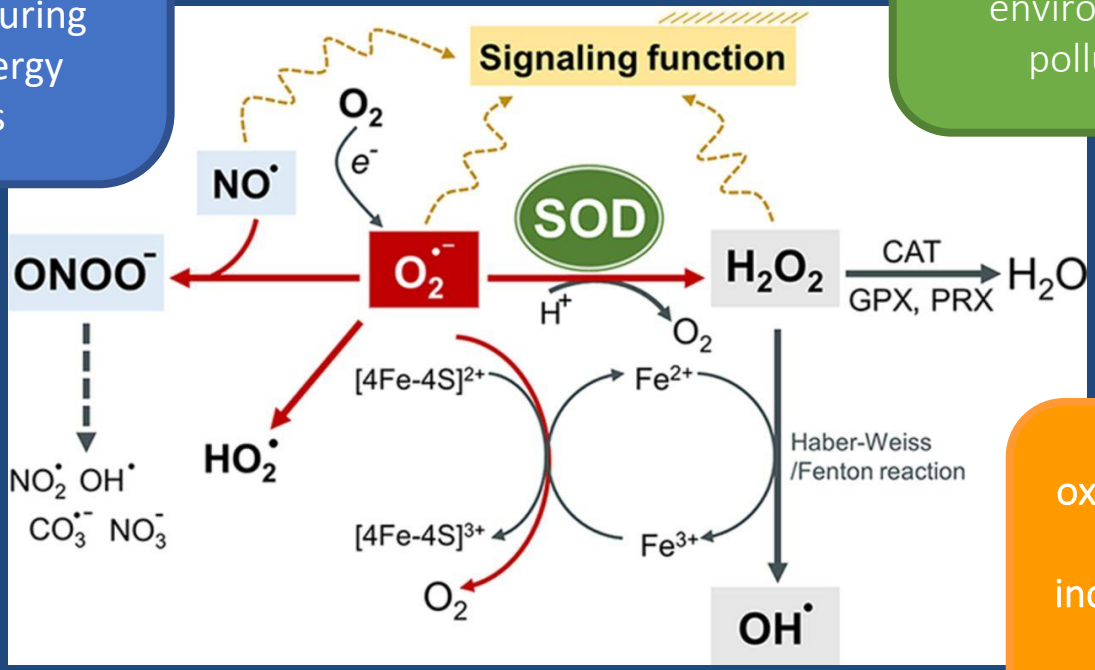
Lactate dehydrogenase (LDH)



- catalyzes the reversible conversion of pyruvate to lactate
- the conversion occurs when O2 is absent or very low
- a key enzyme in the anaerobic metabolic processes
- **increased LDH activity → increased anaerobic capacity**

Superoxide dismutase (SOD)

catalyzes the **dismutation** of the **superoxide ion** (O_2^-) by producing **H_2O_2** and the **O_2** molecule during oxidative energy processes

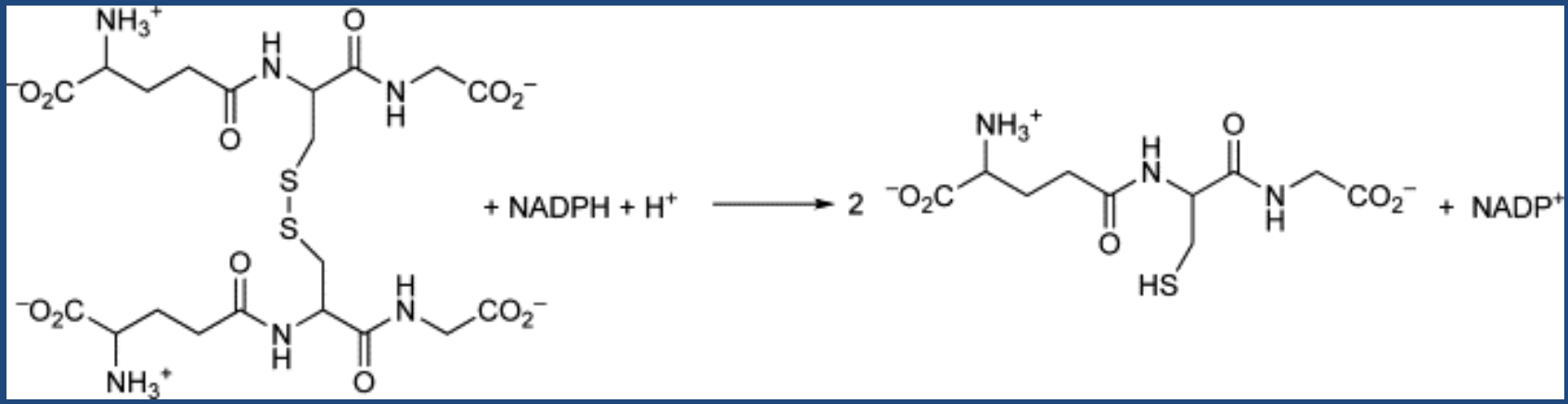


protection of the **cell** from potentially **toxic effects** of environmental pollutants

oxidative stress causes an **increase** in **SOD activity**

Glutathione reductase (GR)

- antioxidant enzyme
- potential biomarkers for synergistic intoxication of pesticides in fish



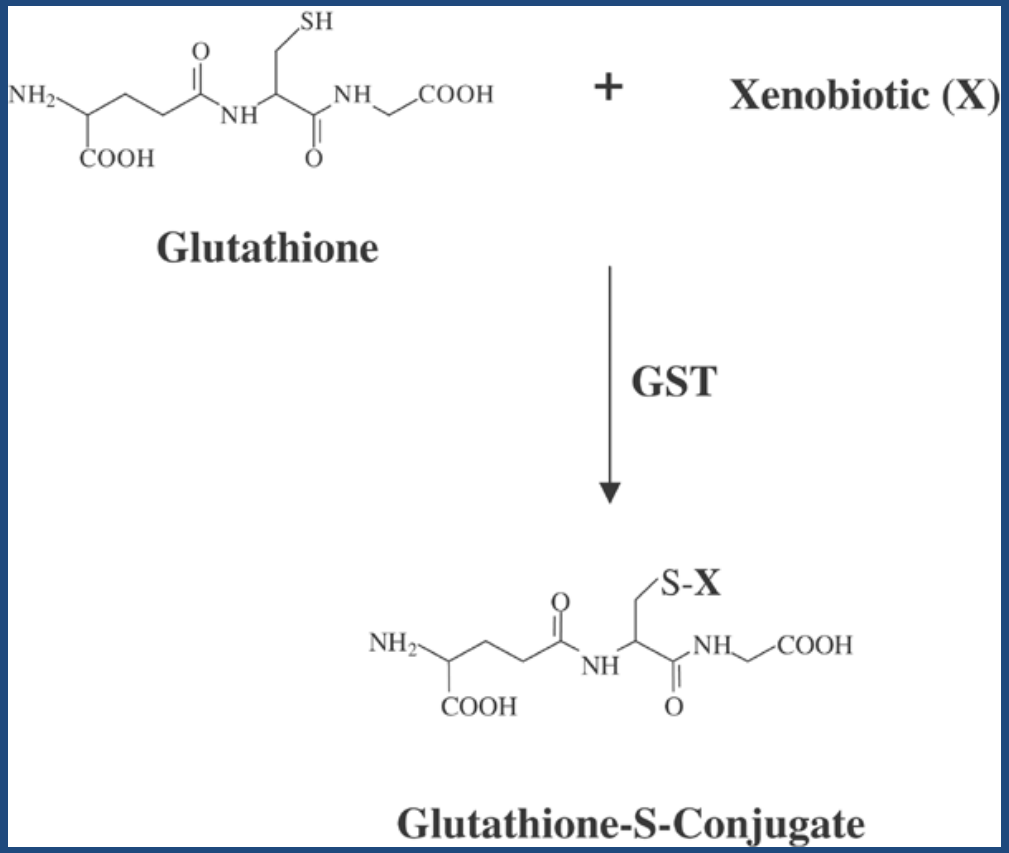
Glutathione Disulfide (GSSH)

Glutathione (GSH)

oxidative stress causes an **increase** in **GR activity**

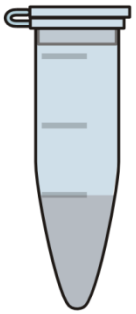
Glutathione S-transferase (GST)

- marker of detoxification processes

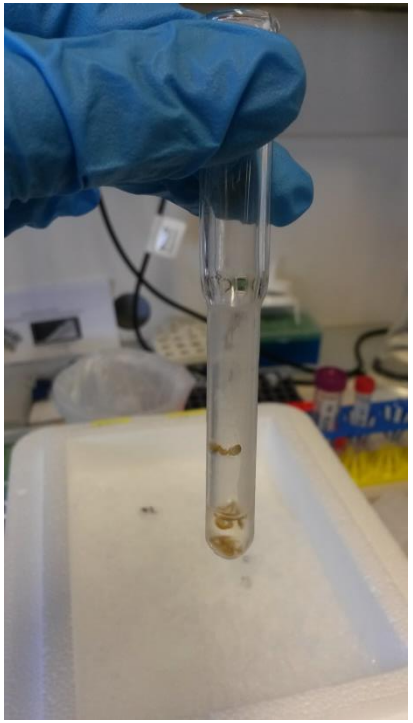


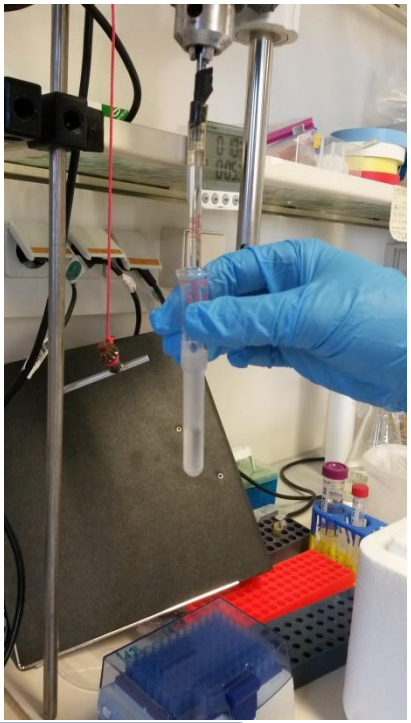
- catalyzes the conjugation of reduced glutathione (GSH) to xenobiotic substrates
- indicates potential **organic contamination**
- an **increase** in **GST activity** indicates stimulation of **detoxification processes**

Laboratory procedure



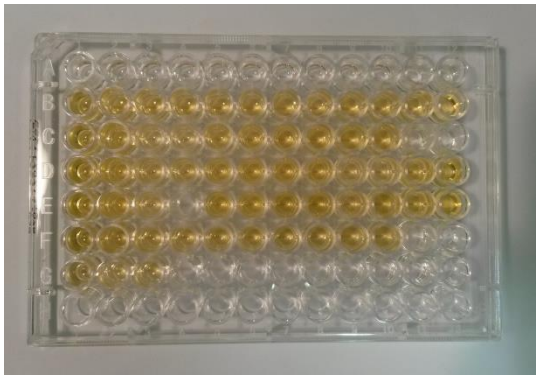
Fish tissue





Homogenisation

Centrifugation



Microplate method

Spectrophoto metric measurement

Metabolic enzymes

- spectrophotometric methods
- measurements at a certain wavelength (340 and 420 nm)

- enzyme activity is calculated from the slope (Vmax = miliUnits / min) of the curve

Tecan Infinite M200, ICAR, 2021.

Enzyme activity

U g^{-1}

U =µmol/min

One enzyme unit (U) is the activity of a particular enzyme that catalyzes a change of 1 µmole of substrate in one minute

n/pKat g^{-1}

Katal = mol/sec

One katal corresponds to the activity of an enzyme that catalyzes the change of one mole of substrate in one second

THANK YOU !