



**AVALIAÇÃO DA TOXICIDADE DOS POLUENTES PRESENTES NO RIO  
DOCE APÓS ROMPIMENTO DA BARRAGEM DO FUNDÃO (MG)  
ATRAVÉS DE DIFERENTES BIOMARCADORES EM PEIXES**

**Flávia Y. Yamamoto**

Denis M. S. Abessa

*Período de vigência:*  
01/09/2018 a 27/01/2023

# SEMINÁRIO

**1.**  
**Análises  
químicas água  
e sedimento**

**2.**  
**Biomarcadores  
bioquímicos nos  
peixes**

**3.**  
**Biomarcadores  
histológicos nos  
peixes**

**4.**  
**Toxicidade do  
elutriato em  
embriões de  
jundiás**

**5.**  
**Abordagem  
Metabolômica e  
análise de  
dados**

Atividades de mineração

Fe Mn

As



Contaminantes inorgânicos

Iron tailing spill

Rompimento da barragem (2015)

Alto RD

Poluição alta

Efluentes domésticos



Ressuspensão dos sedimentos

Efluentes industriais



Contaminantes orgânicos



Agricultura



DOCE RIVER BASIN

Médio e Baixo RD

Poluição leve-moderada

Estuário

Depósito de contaminantes

# ROMPIMENTO DA BARRAGEM DO FUNDÃO

Mariana, MG

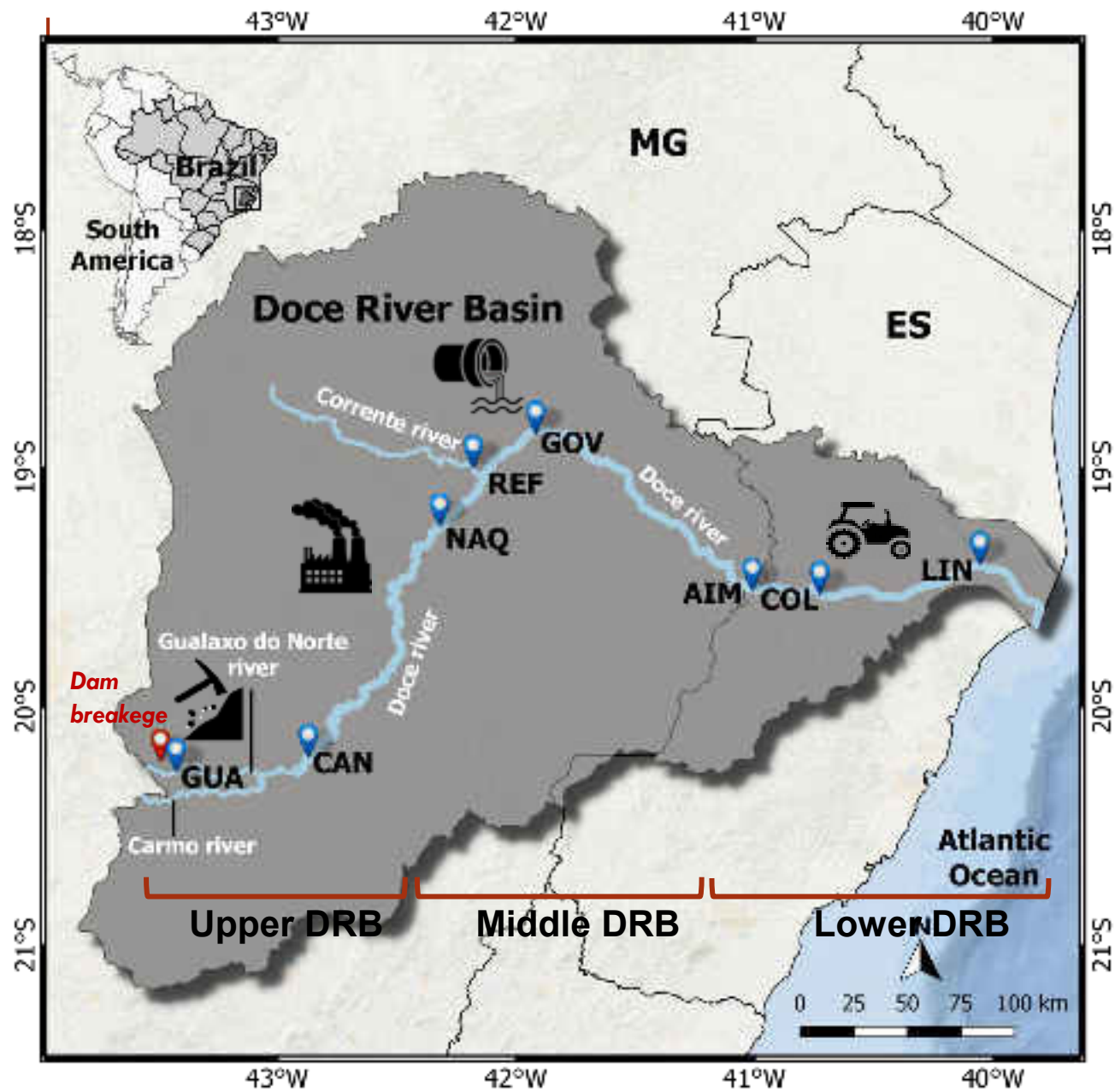
- 5 de Novembro 2015
- <40 milhões de m<sup>3</sup> de rejeitos de minério de Fe
- Mobilização de As, Pb, Hg
- Percurso de 600 km até a foz em Regência (ES)
- **Prejuízo socioeconômico**
- **Impacto ambiental**



ANA, 2015



# Área de estudo



## 7 – COLATINA (ES)





Contents lists available at ScienceDirect

## Journal of Hazardous Materials Advances

journal homepage: [www.elsevier.com/locate/hazadv](http://www.elsevier.com/locate/hazadv)



# Explaining the persistence of hazardous chemicals in the Doce River (Brazil) by multiple sources of contamination and a major environmental disaster



F.Y. Yamamoto<sup>a,\*</sup>, G.F.E. Pauly<sup>a</sup>, L.S. Nascimento<sup>b</sup>, G.M. Fernandes<sup>b</sup>, M.P. Santos<sup>c</sup>,  
R.C.L. Figueira<sup>d</sup>, R.M. Cavalcante<sup>b</sup>, M.T. Grassi<sup>c</sup>, D.M.S. Abessa<sup>a</sup>

<sup>a</sup> Institute of Biosciences, São Paulo State University, São Vicente, Brazil

<sup>b</sup> Marine Science Institute, Federal University of Ceará, Fortaleza, Brazil

<sup>c</sup> Chemistry Department, Federal University of Paraná, Curitiba, Brazil

<sup>d</sup> Oceanographic Institute, University of São Paulo, São Paulo, Brazil





Contents lists available at ScienceDirect

## Data in Brief

journal homepage: [www.elsevier.com/locate/dib](http://www.elsevier.com/locate/dib)



### Data Article

# Chemical data of contaminants in water and sediments from the Doce River four years after the mining dam collapse disaster



F.Y. Yamamoto<sup>a,1,\*</sup>, G.E. Pauly<sup>a,1</sup>, L.S. Nascimento<sup>b,2</sup>,  
G.M. Fernandes<sup>b,2</sup>, M.P. Santos<sup>c,3</sup>, B.S.M. Kim<sup>d,4</sup>, M.U. Carvalho<sup>a,1</sup>,  
R.C.L. Figueira<sup>d,4</sup>, R.M. Cavalcante<sup>b,2</sup>, M.T. Grassi<sup>c,3</sup>, D.M.S. Abessa<sup>a,1</sup>

<sup>a</sup> Institute of Biosciences, São Paulo State University, São Vicente, Brazil

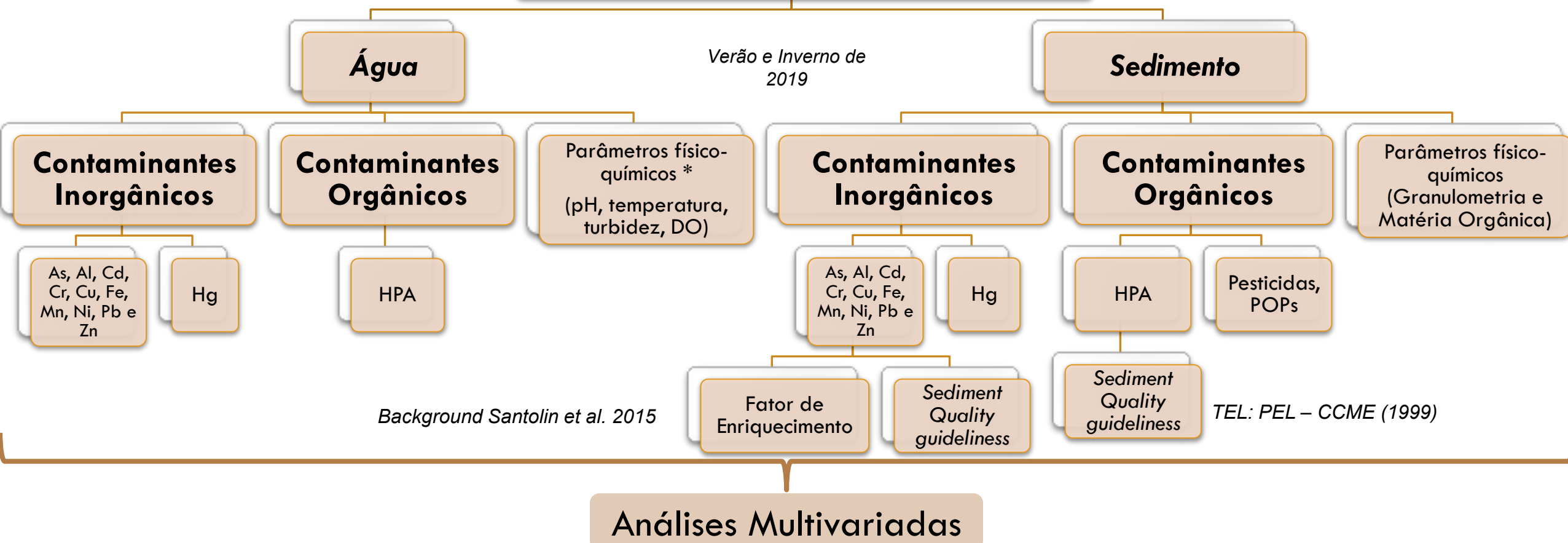
<sup>b</sup> Marine Science Institute, Federal University of Ceará, Fortaleza, Brazil

<sup>c</sup> Chemistry Department, Federal University of Paraná, Curitiba, Brazil

<sup>d</sup> Oceanographic Institute, University of São Paulo, São Paulo, Brazil



# ANÁLISES QUÍMICAS





# RESULTADOS

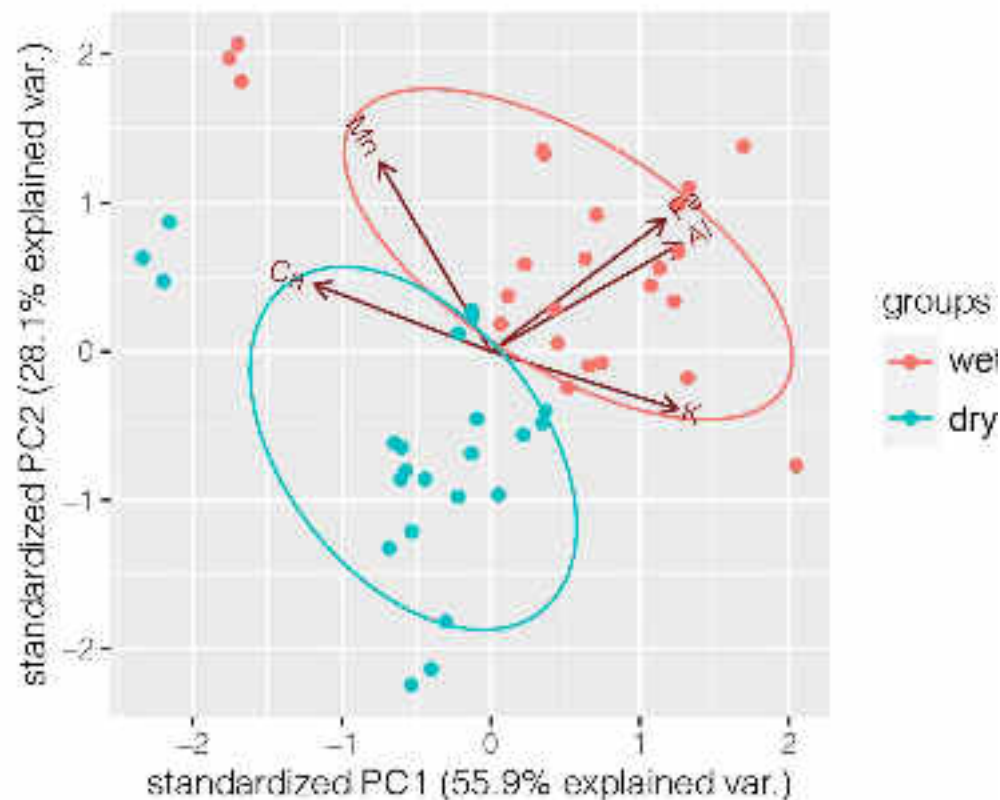
## Fator de Enriquecimento e SQG

|     |     | ENRICHMENT FACTOR |       |       |       |       |       |       | SEDIMENT QUALITY GUIDELINESS |          |      |      |           |       |
|-----|-----|-------------------|-------|-------|-------|-------|-------|-------|------------------------------|----------|------|------|-----------|-------|
|     |     |                   |       |       |       |       |       |       | INORGANICS                   |          |      | PAHs |           |       |
|     |     | As                | Cd    | Cu    | Fe    | Hg    | Mn    | V     | >TEL                         | >PEL     | SQGq | ΣPAH | >TEL      | SQGq  |
| GUA | Sum | 20.44             | 17.79 | 10.90 | 3.84  | 1.75  | 44.19 | 6.80  | 8 (38%)                      | 2 (9.5%) | 0.39 | 0,00 | 1 (7.7%)  | 0.003 |
|     | Win | 32.04             | 56.79 | 5.59  | 16.41 | 49.63 | 56.09 | 11.41 | 6 (28%)                      | 5 (23%)  | 1.03 | 0,00 | 1 (7.7%)  | 0.000 |
| CAN | Sum | 3.38              | 6.40  | 1.78  | 3.24  | 3.37  | 1.54  | 10.75 | 4 (19%)                      | 0        | 0.16 | 0,01 | 1 (7.7%)  | 0.003 |
|     | Win | 8.25              | 6.66  | 3.24  | 3.04  | 4.81  | 3.58  | 16.82 | 8 (38%)                      | 2 (9.5%) | 0.45 | 0,00 | 1 (7.7%)  | 0.003 |
| NAQ | Sum | 5.95              | 9.01  | 1.81  | 6.28  | 4.76  | 2.42  | 11.40 | 5 (23%)                      | 0        | 0.20 | 0,39 | 6 (46%)   | 0.285 |
|     | Win | 4.62              | 6.81  | 1.54  | 4.52  | 2.36  | 2.94  | 9.98  | 9 (42%)                      | 2 (9.5%) | 0.40 | 0,11 | 5 (38.5%) | 0.063 |
| REF | Sum | 0.75              | 3.54  | 1.21  | 3.14  | 1.71  | 4.05  | 9.09  | 3 (14%)                      | 0        | 0.22 | 0,02 | 1 (7.7%)  | 0.006 |
|     | Win | 0.77              | 3.39  | 1.26  | 3.36  | 8.22  | 4.07  | 9.11  | 6 (28%)                      | 0        | 0.34 | 0,02 | 1 (7.7%)  | 0.001 |
| GOV | Sum | 1.96              | 5.43  | 2.14  | 1.88  | 5.19  | 0.93  | 10.29 | 2 (10%)                      | 0        | 0.13 | 0,17 | 6 (46%)   | 0.105 |
|     | Win | 1.94              | 4.66  | 2.06  | 2.05  | 4.11  | 0.98  | 8.87  | 10 (47%)                     | 2 (9,5%) | 0.47 | 0,11 | 4 (30.8%) | 0.04  |
| AIM | Sum | 0.49              | 2.06  | 1.39  | 1.79  | 4.47  | 1.14  | 7.67  | 2 (9.5%)                     | 0        | 0.15 | 0,02 | 1 (7.7%)  | 0.058 |
|     | Win | 0.88              | 1.71  | 1.20  | 1.62  | 13.08 | 0.52  | 6.03  | 1 (4.7%)                     | 0        | 0.17 | 0,03 | 1 (7.7%)  | 0.011 |
| COL | Sum | 1.83              | 2.43  | 1.48  | 2.27  | 5.30  | 0.86  | 7.37  | 0                            | 0        | 0.11 | 0,21 | 6 (46%)   | 0.100 |
|     | Win | 0.80              | 1.40  | 1.18  | 1.26  | 2.39  | 0.43  | 4.97  | 7 (33%)                      | 0        | 0.26 | 0,53 | 6 (46%)   | 0.313 |
| LIN | Sum | 1.61              | 2.12  | 1.58  | 2.02  | 4.78  | 1.26  | 7.38  | 0                            | 0        | 0.11 | 0,24 | 6 (46%)   | 0.150 |
|     | Win | 3.28              | 2.62  | 1.34  | 2.71  | 8.84  | 1.06  | 5.58  | 0                            | 0        | 0.11 | 0,16 | 6 (46%)   | 0.092 |

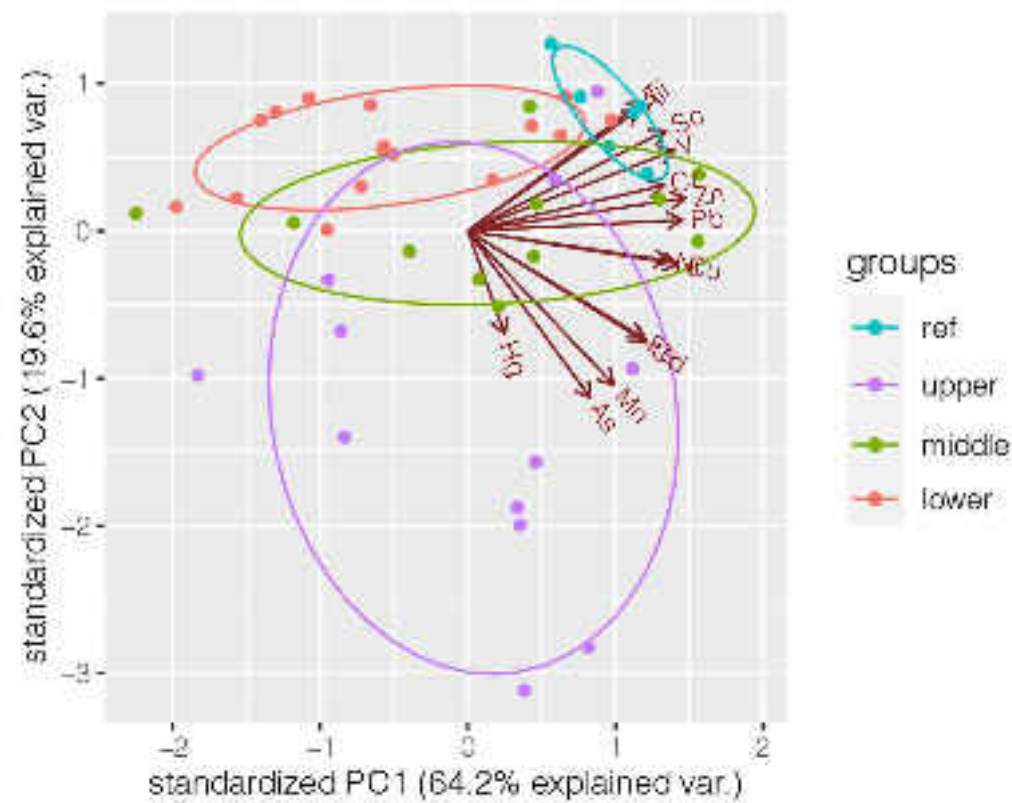
GUA – Maior quociente SQG – pior cenário da poluição do sedimento

# PCA – CONTAMINANTES INORGÂNICOS

# Água



# Sedimento

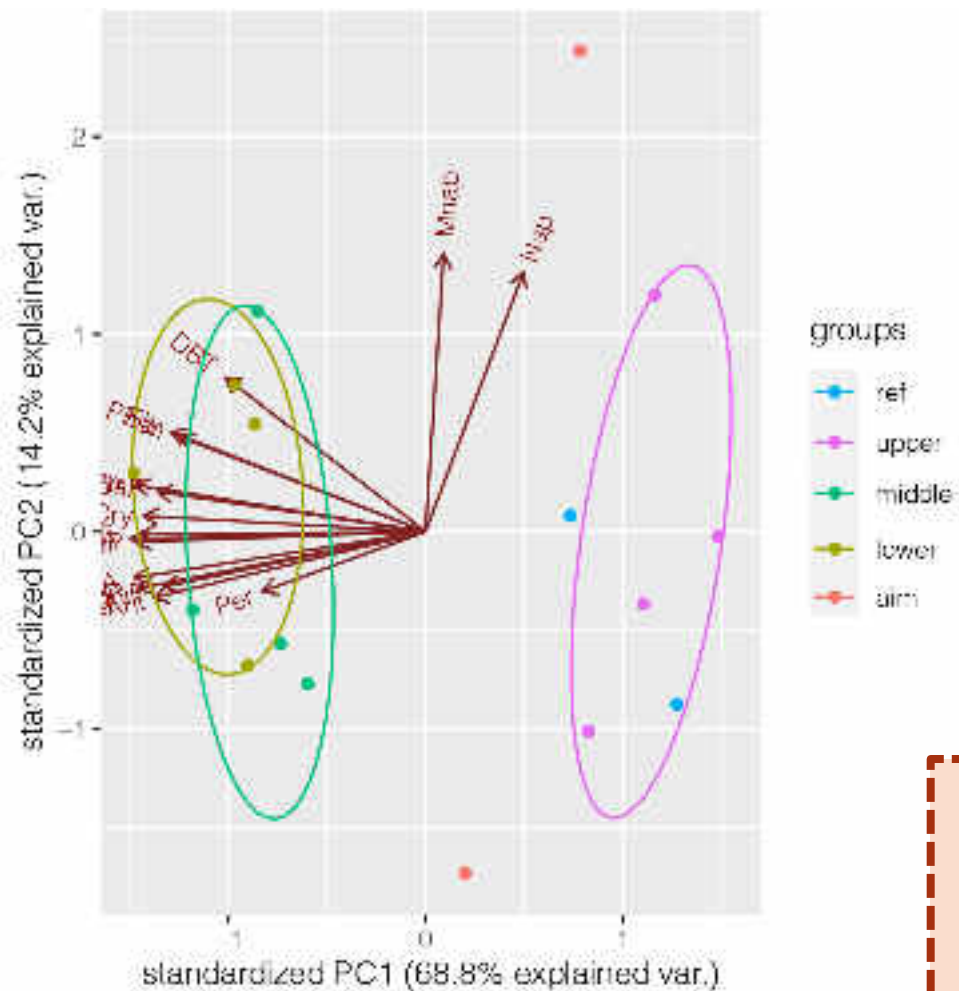


1º Comp. – agrupou Al, Fe, K (+), maiores no baixo RD de Mn e Ca (-), maiores no alto RD

2º Comp. – discriminou verão (maiores níveis de Fe, Al, Mn) e inverno na água

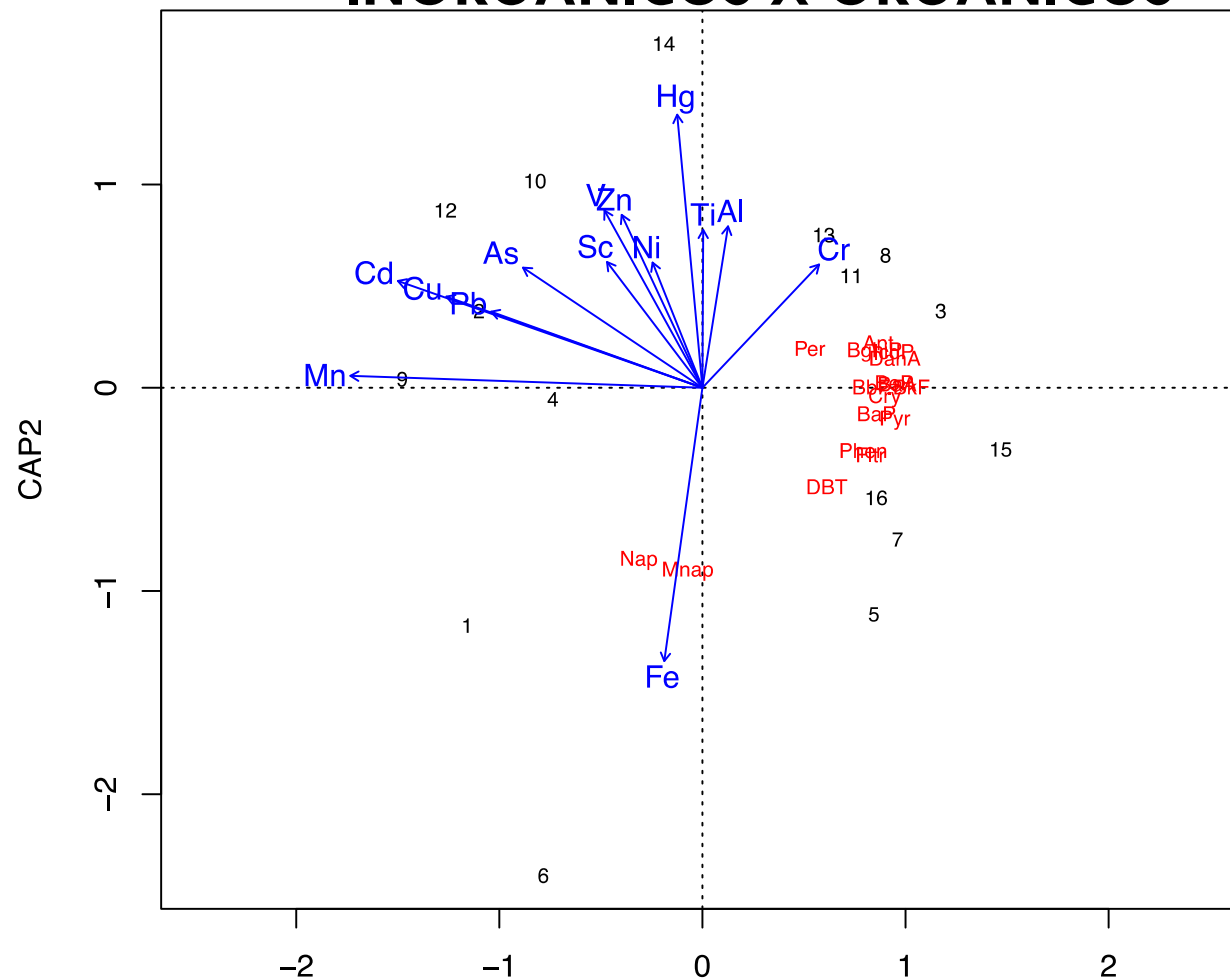
# PCA – ORGÂNICOS

## Sedimento



## RDA

### INORGÂNICOS X ORGÂNICOS

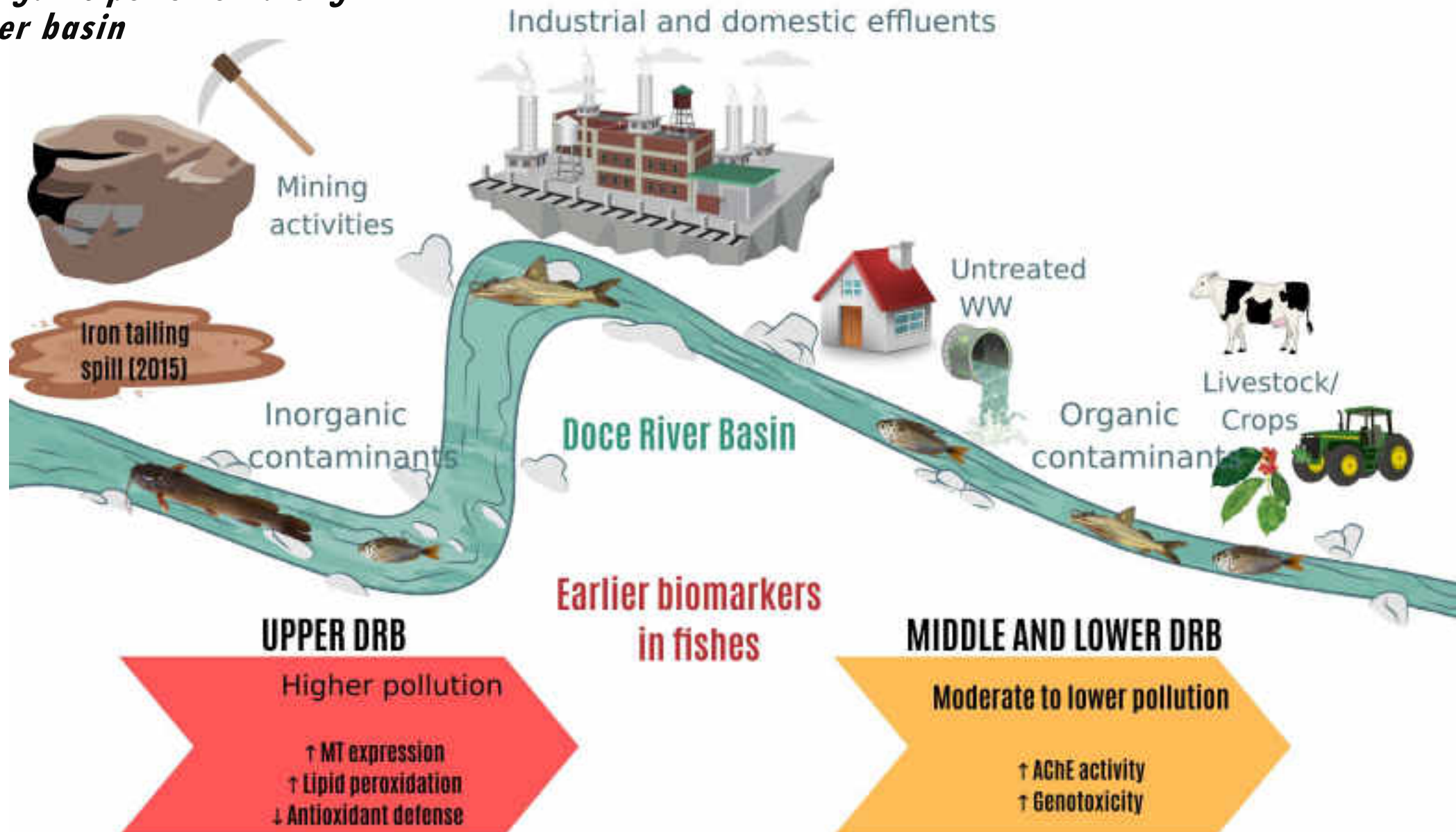


**RDA** – Indicou baixa correlação entre os contaminantes inorgânicos e orgânicos (com exceção do Cr), sugerindo suas diferentes fontes de contaminação e padrões de distribuição



***Earlier biomarkers in fish evidencing stress responses and tolerance to metal and organic pollution along the doce river basin***

**Continuous exposure to multiple pollutants**



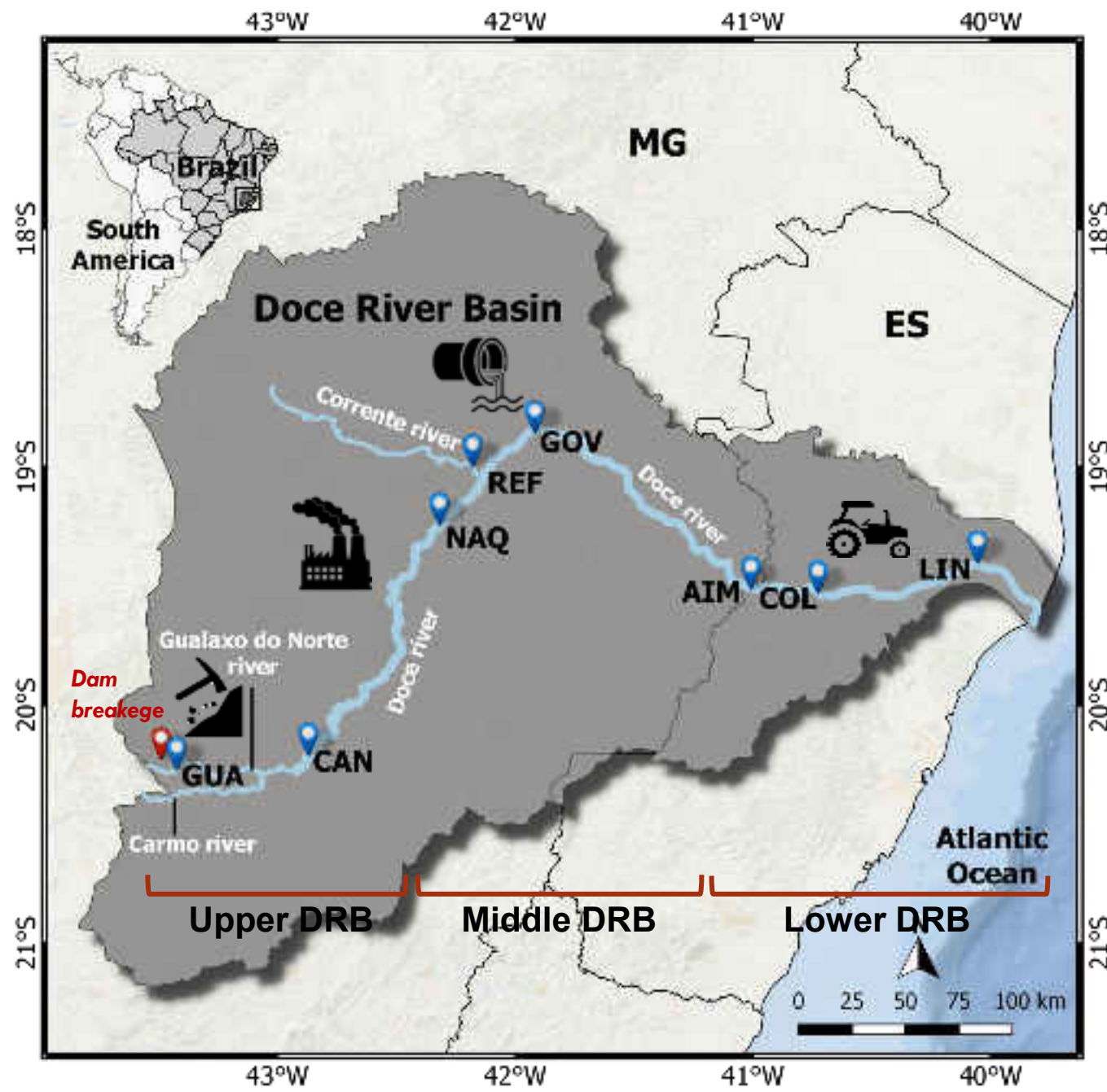
## Materiais e Métodos

**Coleta das amostras**

**8 pontos de coleta**

**Verão e inverno de 2019**

15 redes de 10m (2 dias por ponto)





## Coleta das amostras biológicas

- Peixes anestesiados com Benzocaína 0,02%
- Coleta das amostras no campo para redução do estresse e manutenção dos peixes vivos



# Peixes do Rio Doce

- Aproximadamente 420 peixes:
- Diferentes **Níveis tróficos e nichos** (pelagico e demersal)

**Lambari (*Astyanax lacustris*) – PELÁGICO**  
**O mais abundante**



**Cascudo (*Hypostomus affinis*) - DEMERSAL**



**Jundiá (*Rhamdia quelen*) - DEMERSAL**



**Mandi (*Pimelodus maculatus*) - DEMERSAL**





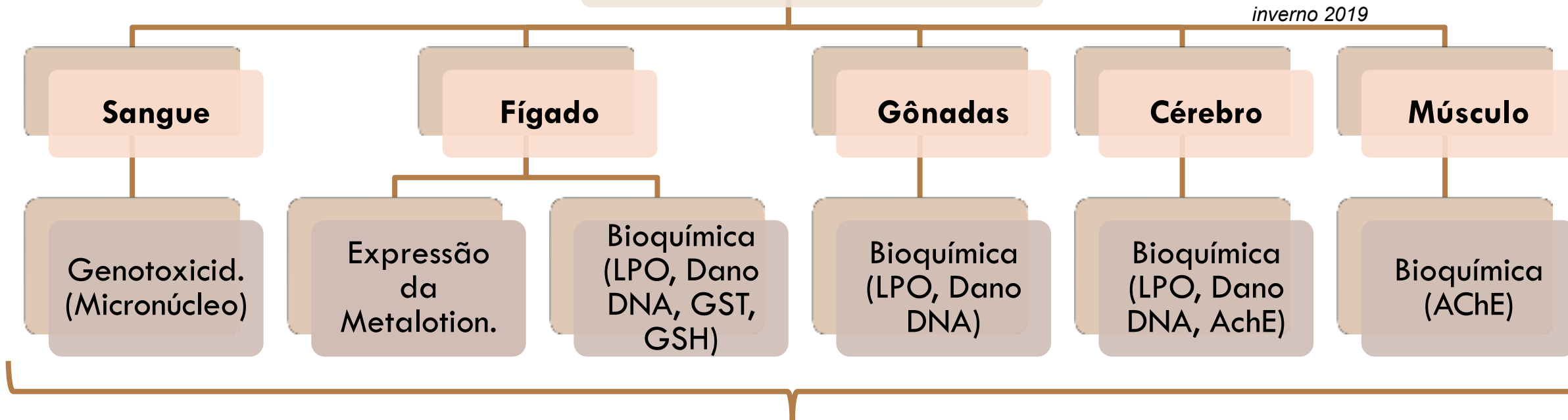


*~15 indivíduos por ponto e sp.*



## ANÁLISES BIOLÓGICAS (BIOMARCADORES) 1

*Amostras verão e  
inverno 2019*

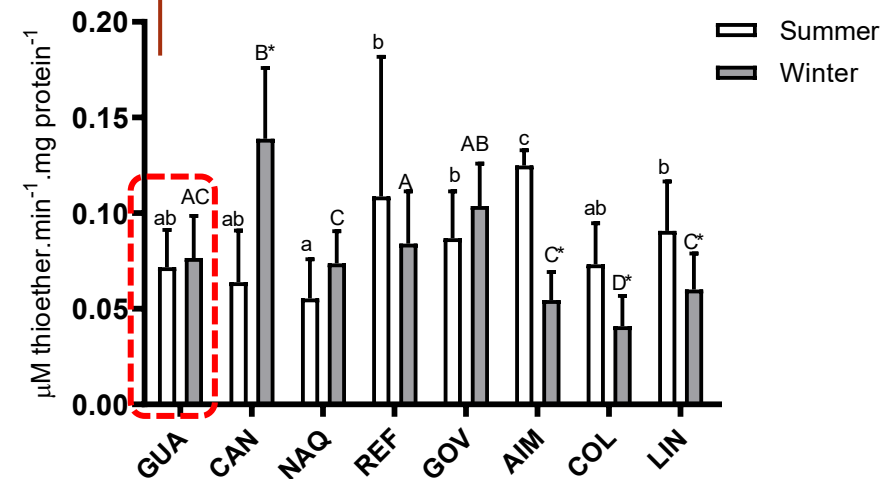


**Análises Estatísticas Multivariadas (PCA)  
e integração com os dados químicos**

# Resultados

# Biomarcadores

**Catfish GST**



## Peixes GUA (Alto RD)

↓ Atividade da GST e depleção GSH

↑ LPO e MT

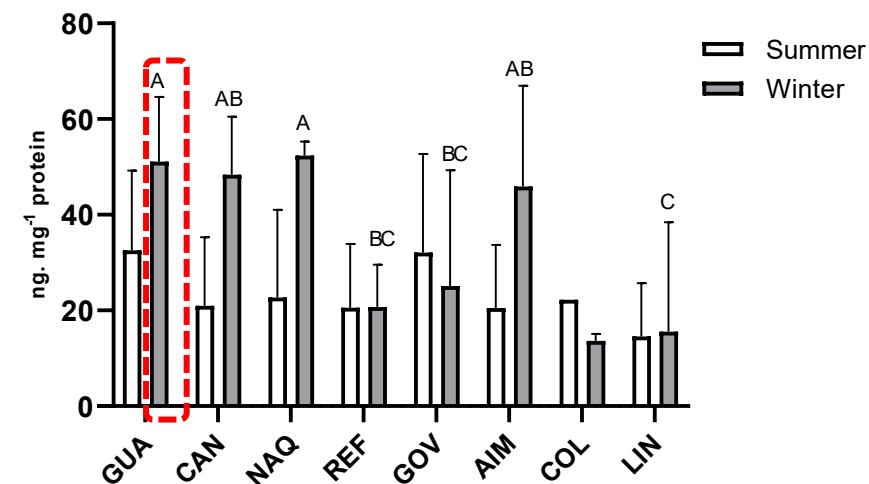
## GOV (Médio RD)

↑ níveis GSH e dano no DNA

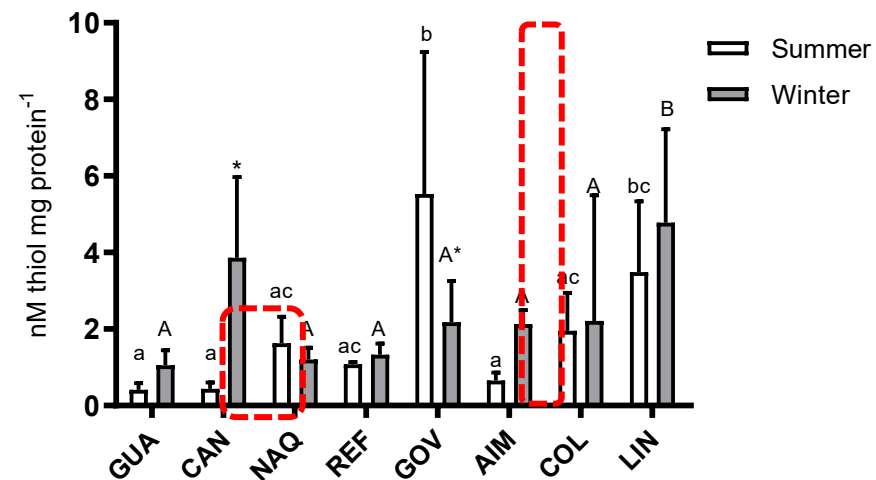
## Baixo RD

↑ AChE no cérebro e músculo

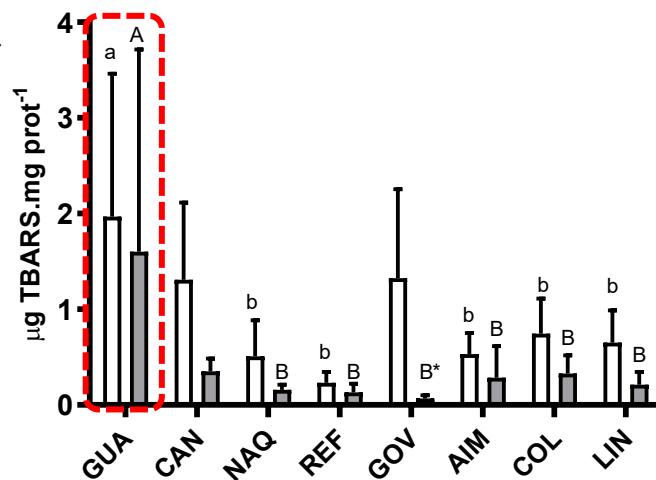
**Lambari MT**



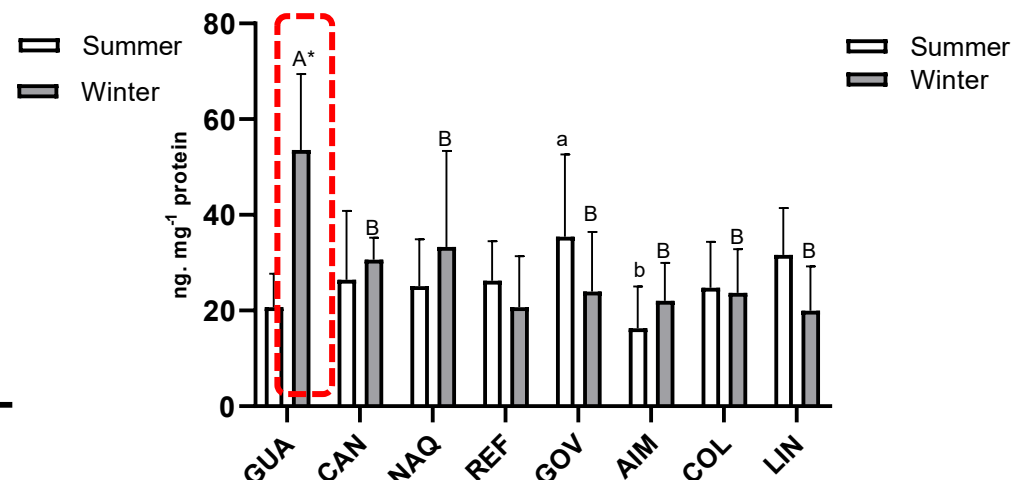
**Catfish GSH**

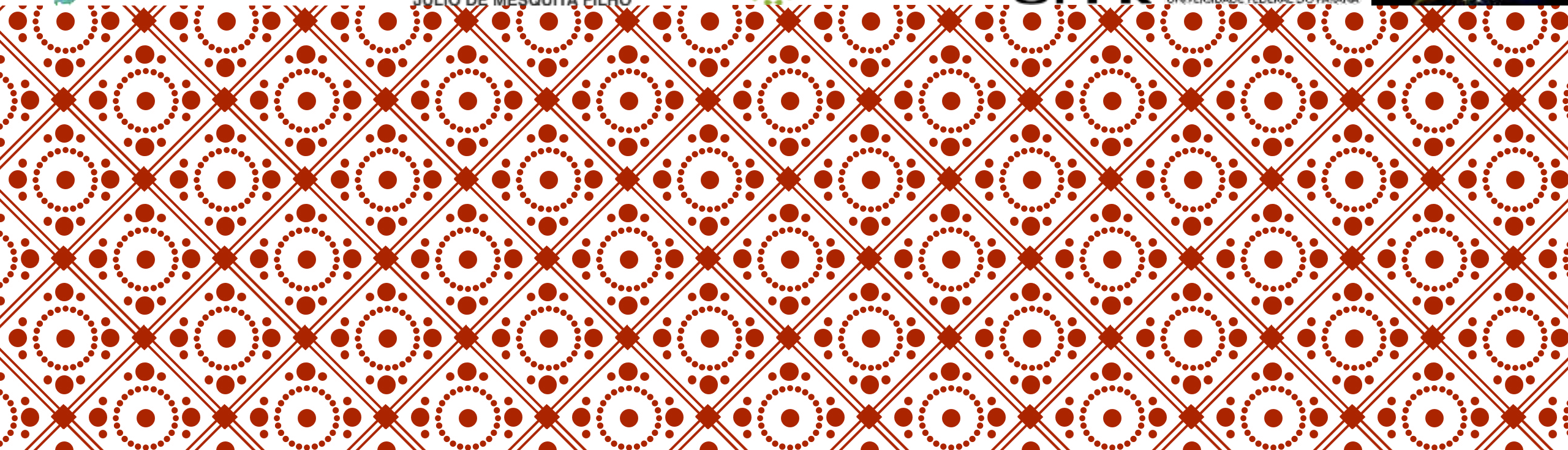


**Catfish liver LPO**



**Catfish MT**





# ***Distinct histopathological lesions in fishes from the doce river basin under influence of multiple pollutants and environmental factors***

Flavia Yoshie Yamamoto<sup>a\*</sup>, Kaori Onishi<sup>a</sup>, Cláudia Neves Correa<sup>a</sup>, Gabriela Pustiglione Marinsek<sup>a</sup>, Cláudia Feijó Ortolani-Machado<sup>b</sup>, Denis Moledo de Souza Abessa<sup>a</sup>





## ANÁLISES BIOLÓGICAS (BIOMARCADORES) 2

*Amostras verão e  
inverno 2019*

**Sangue**

Expressão da  
Vitelogenina

Dosagem  
Estradiol

**Fígado**

Histologia (ML)

**Gônadas**

Histologia (ML)

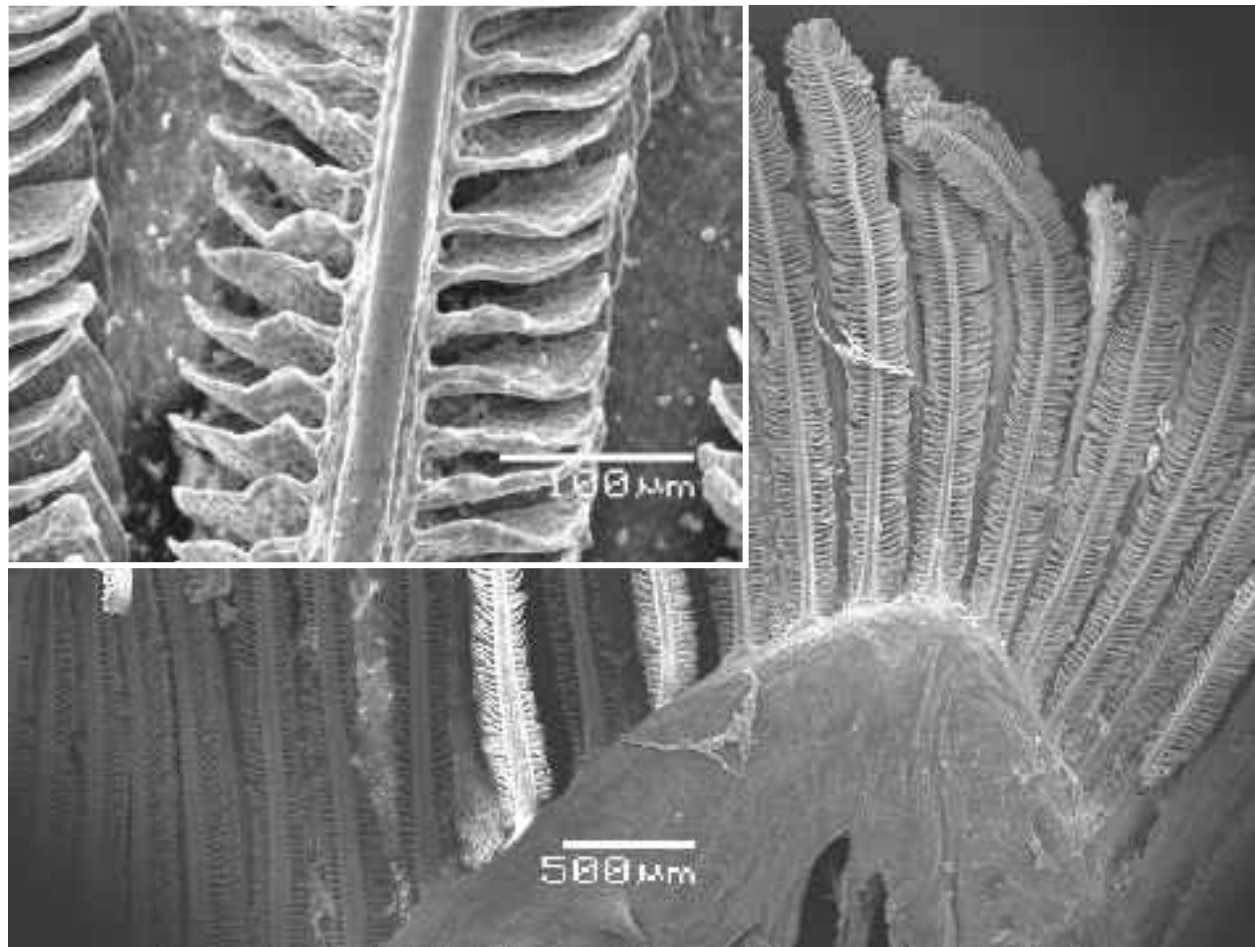
**Brânquias**

Histologia  
(MEV)

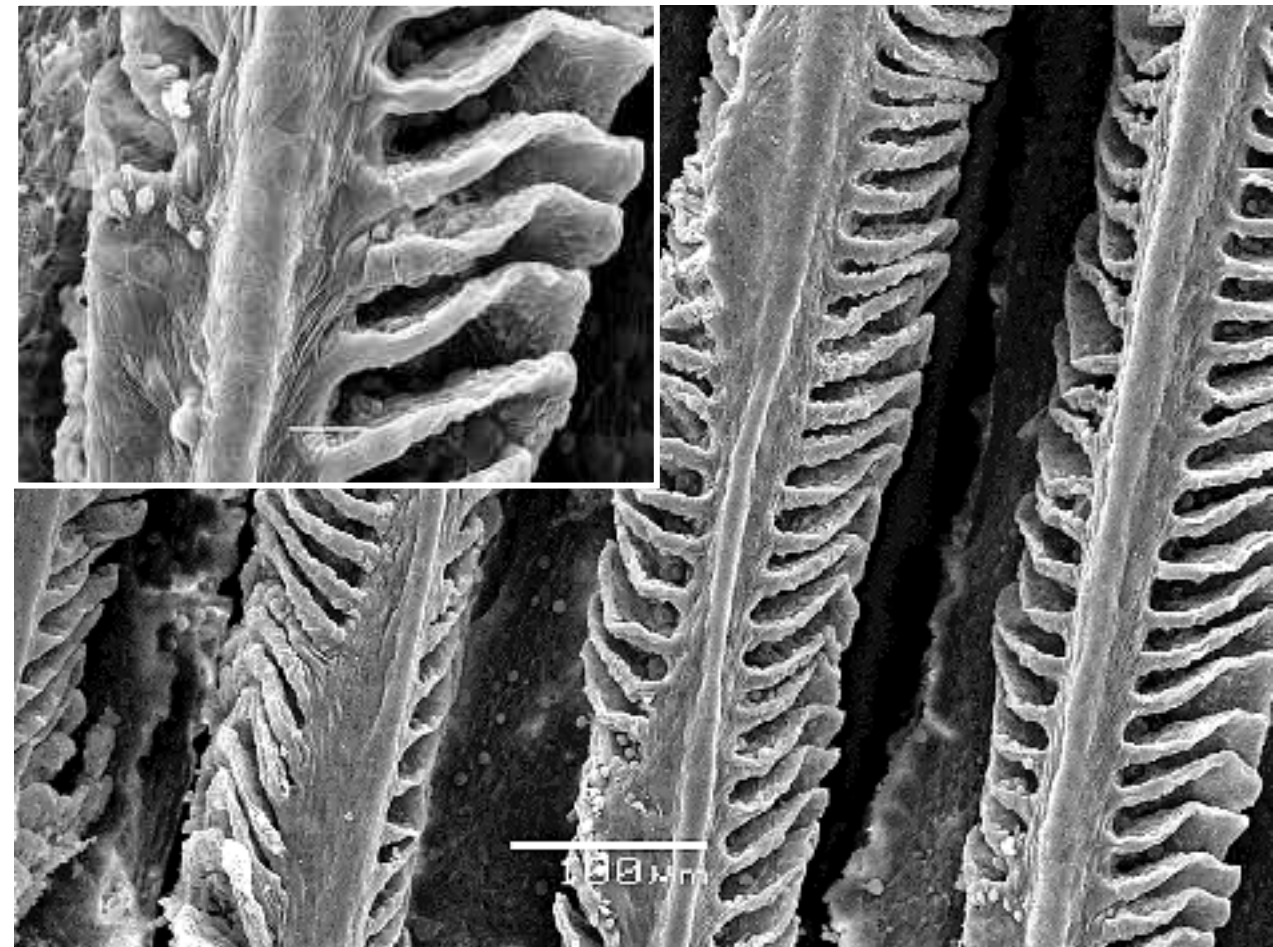
Análises Estatísticas Multivariadas (PCA) e  
integração com os dados químicos

# Histopatologia - brânquias

Brânquias lambaris sem alterações

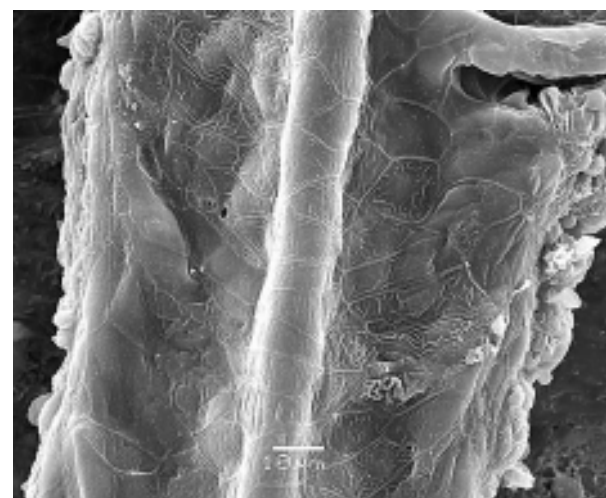
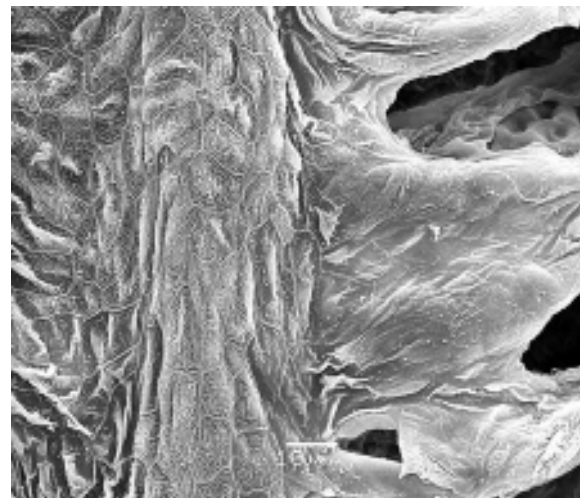
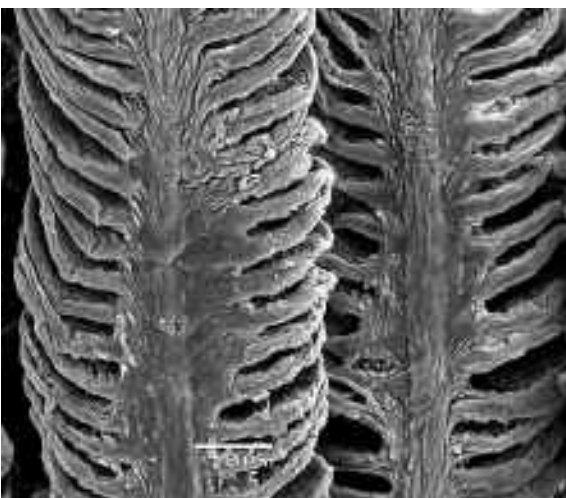
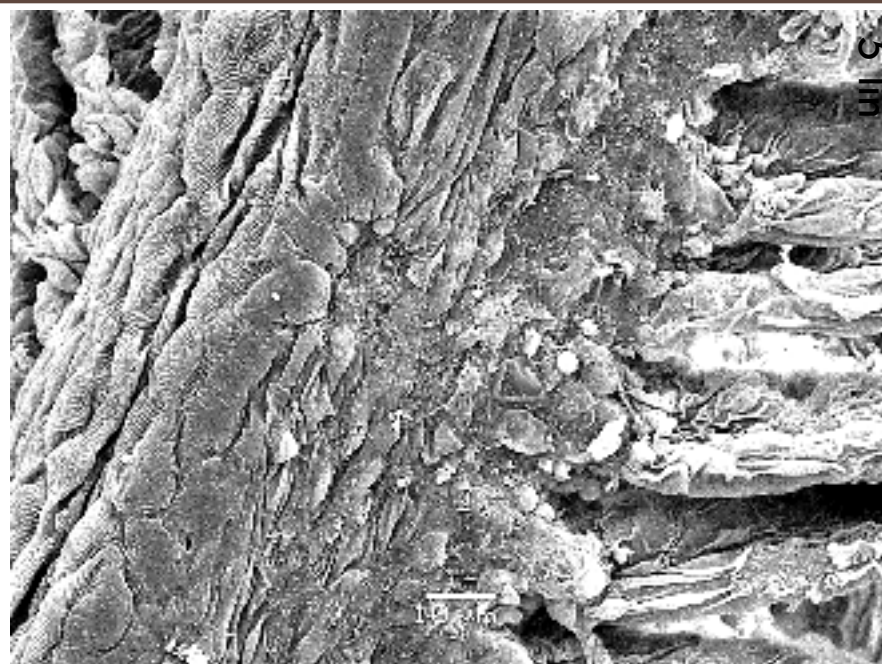
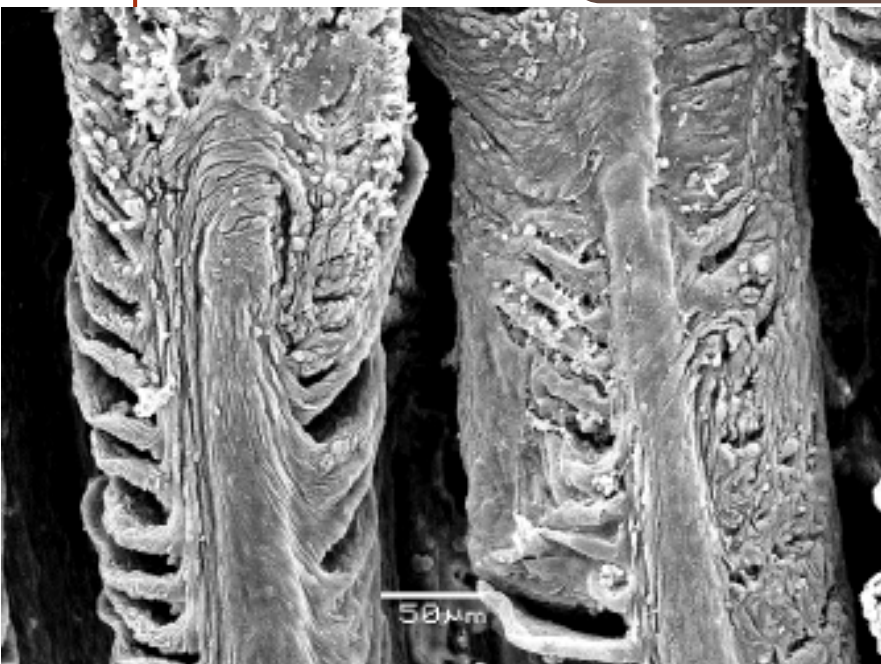


Brânquias lambaris alteradas





# Histopatologia - brânquias

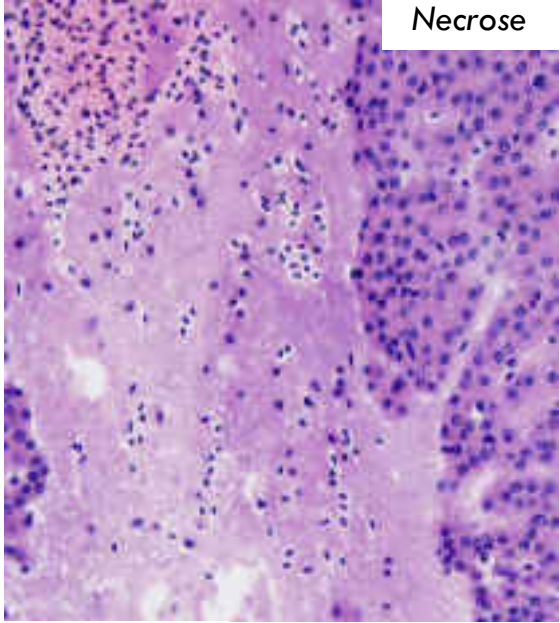


*A. bifasciatus* da estação chuvosa apresentou maior incidência de fusão de lamelas (COL e LIN > 70%), alterações estruturais nas lamelas primeira e secundária, neoplasia de células pavimentosas (100 % em GUA, COL) e perda de microdigitações.

Em geral, essas alterações foram mais frequentes em lambaris do que em bagres

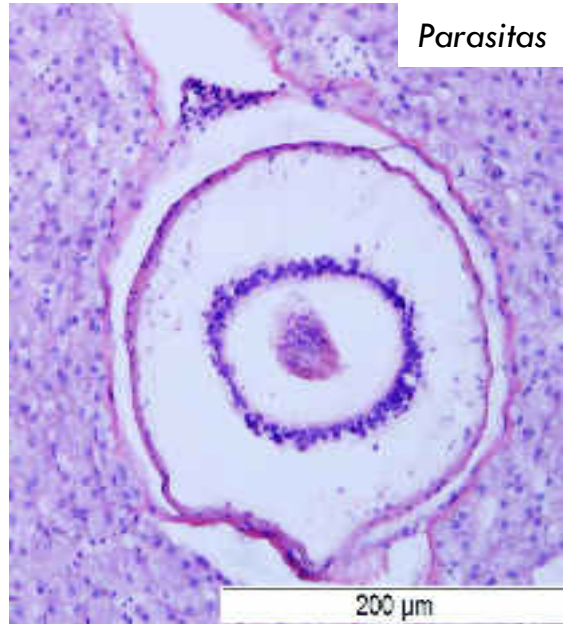
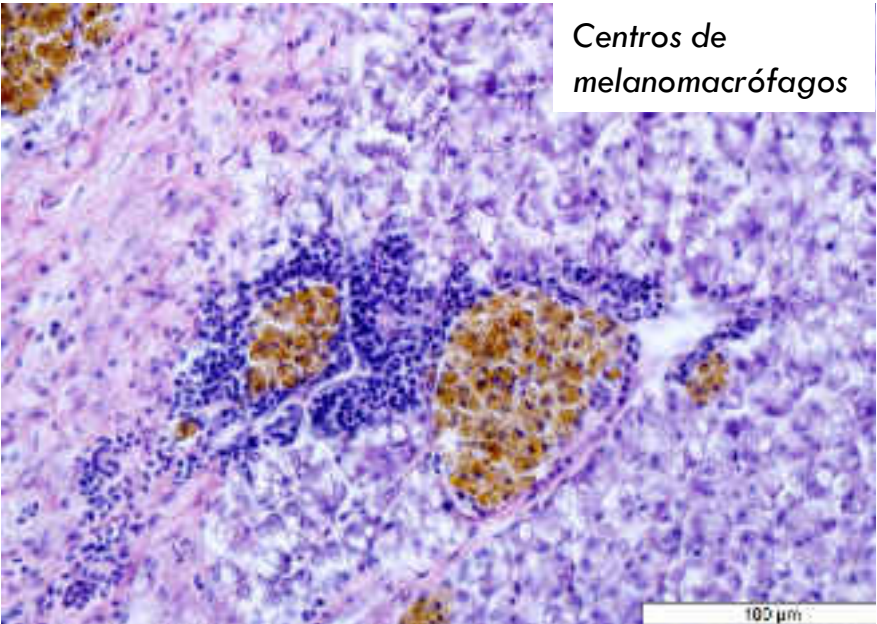
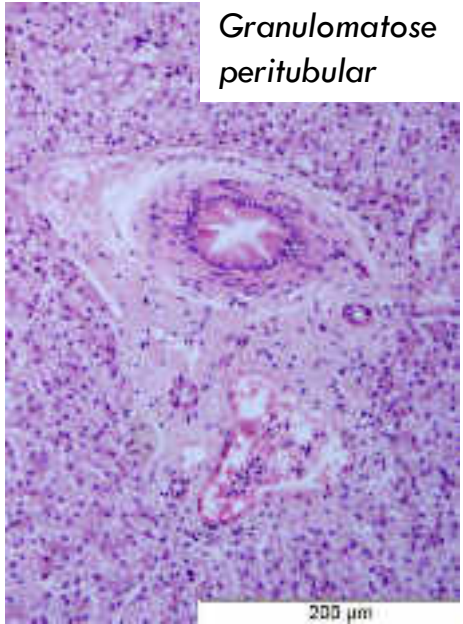
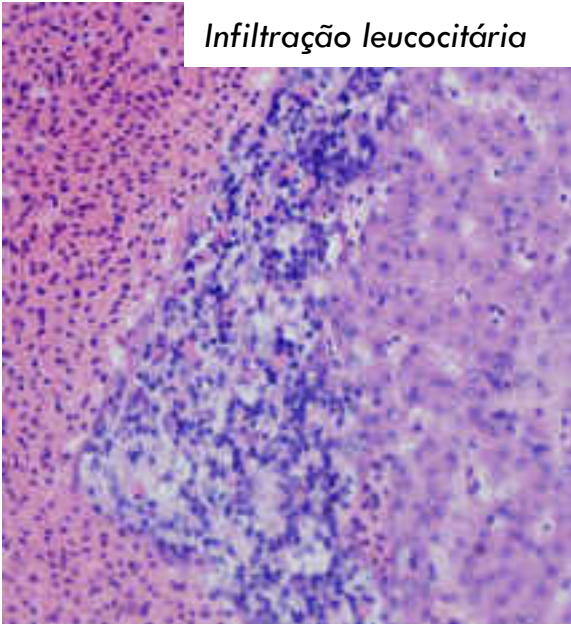


# Histopatologia - Fígado



➤ Maior incidência de alterações do **sistema circulatório** em peixes do Alto Rio Doce (Rhamdia quelen)

➤ Peixes do Médio e Baixo demonstraram maior frequência de alterações associadas ao **sistema imune**, principalmente durante o inverno





Contents lists available at ScienceDirect

## Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)



# From molecular endpoints to modeling longer-term effects in fish embryos exposed to the elutriate from Doce River



Flávia Yoshie Yamamoto <sup>a,\*</sup>, Angie Thaisa Costa Souza <sup>b</sup>, Vinicius de Carvalho Soares de Paula <sup>c</sup>, Isabella Beverari <sup>a</sup>, Juan Ramon Esquivel Garcia <sup>d,1</sup>, André Andian Padial <sup>b,c</sup>, Denis Moledo de Souza Abessa <sup>a</sup>

<sup>a</sup> Institute of Biosciences, São Paulo State University, São Vicente, Brazil

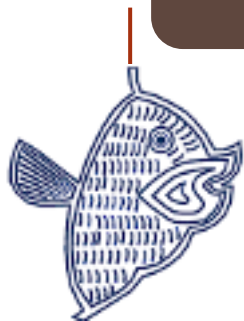
<sup>b</sup> Graduation Program in Ecology and Conservation, Federal University of Paraná, Curitiba, Brazil

<sup>c</sup> Department of Chemistry and Biology, Federal University of Technology-Paraná, Curitiba, Brazil

<sup>d</sup> Panama Fish Farm, Paulo Lopes, Brazil

<sup>\*</sup> Analyses and Synthesis in Biodiversity Lab, Botany Department, Federal University Curitiba, Brazil

# Teste de Toxicidade em embriões de jundiás



PISCICULTURA  
**PANAMÁ**

Reprodução induzida  
dos jundiás  
Verão de 2019

**Solução elutriato (EPA, 2001)**  
1:4 (Sedimento : Água)  
8 pontos do Rio Doce



Desova induzida

Transporte dos ovos







## Teste de Toxicidade com embriões de jundiá

Exposição por 96h  
ao elutriato

Taxa de  
sobrevivência a  
cada 24h

Taxa de  
deformidades a  
cada 24h

Biomarcadores  
bioquímicos  
(96hpf)

Modelo  
matemático

LPO, Dano DNA,  
GST, GSH

Análises Estatísticas Multivariadas (PCA) e  
integração com os dados químicos

# Teste de Toxicidade em embriões de jundiás

- **OECD FET (236)**
- Exposição: 96h
- Controle negativo
- Placa 96 poços – 200  $\mu$ l de elutriato (100% e 50%) ou água reconstituída
- Troca a cada 24h (100 $\mu$ l)
- Anormalidades + Análises bioquímicas ou molecular



- Temperatura 24° C
- Fotoperíodo 12h
- O<sub>2</sub>: 4,4 a 7,8 mg. L<sup>-1</sup>
- pH: 6,6 a 7,2



# Deformidades nos embriões

Controle 48h



GUA 100% com  
Edema cardíaco



Controle 96h



Controle 96h





- Nadadeiras danificadas
- Deformidades faciais
- Deformidades axiais (esqueleto e cauda)

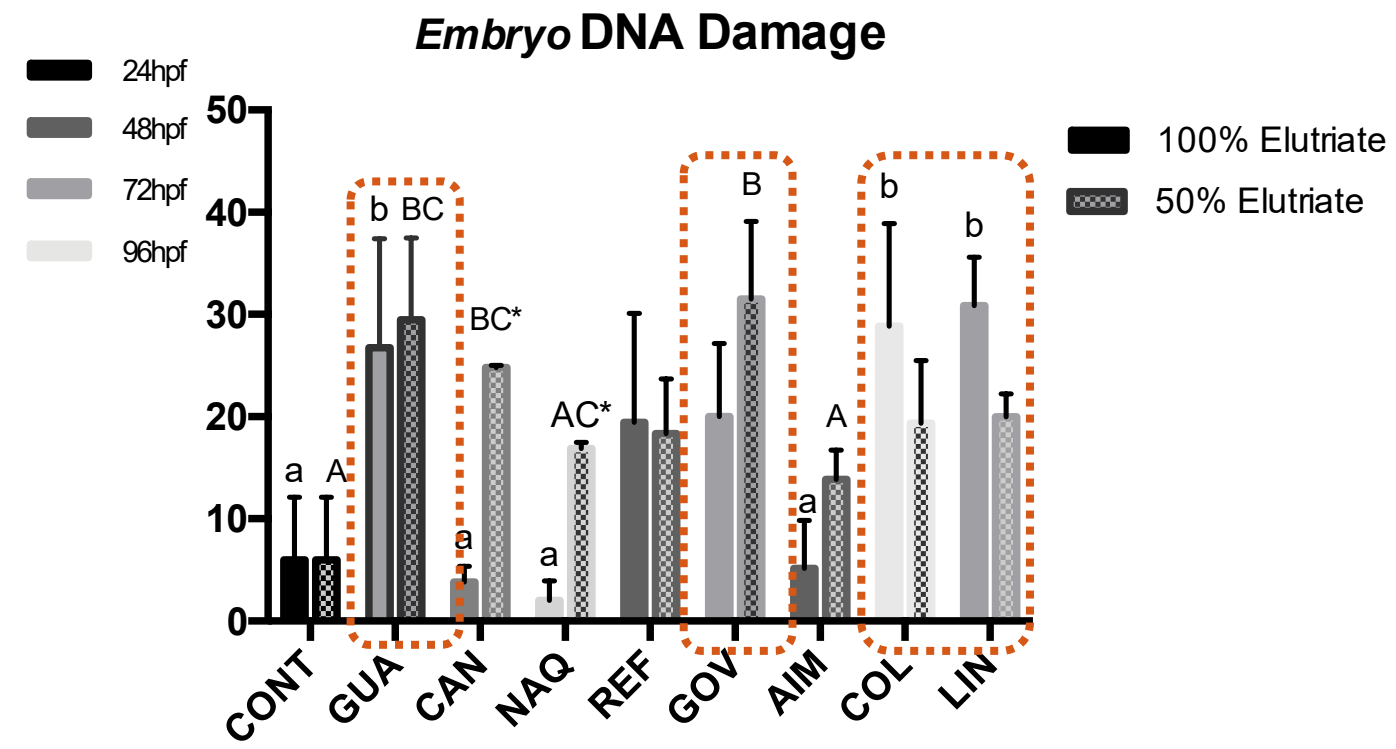
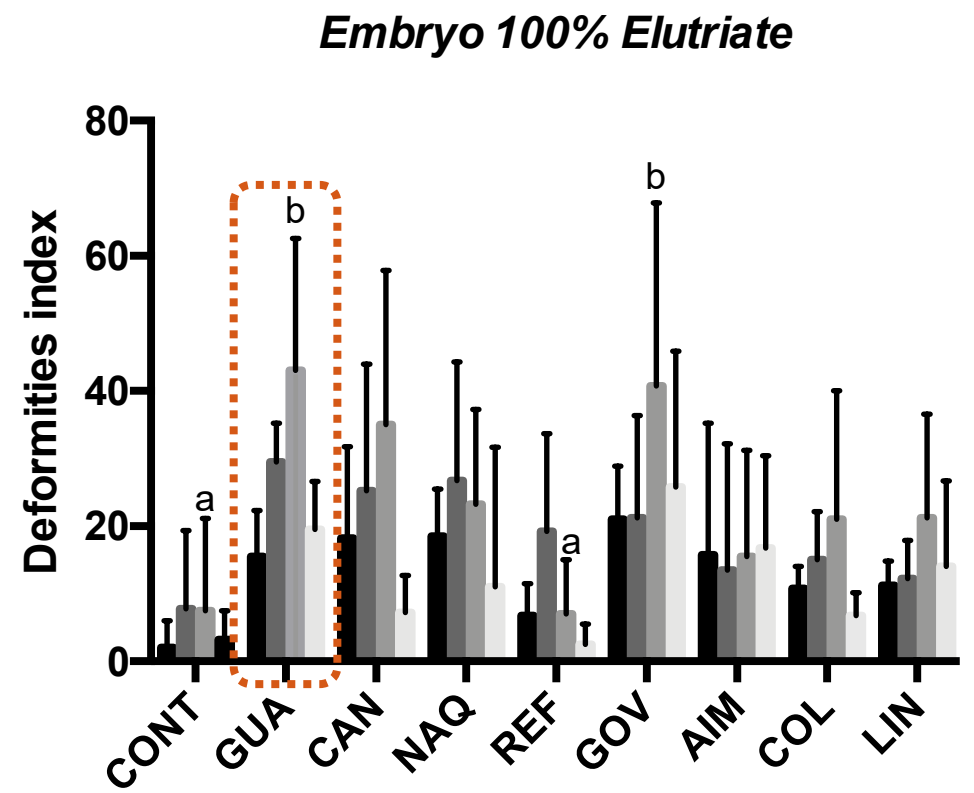


- Ausência de olhos
- Deformidades na estrutura bucal
- Ausência de barbilhões



# RESULTADOS

Apesar de não haver mortalidade significativa para os grupos expostos, verificou-se **maior taxa de deformidades e Dano no DNA** dos embriões expostos ao elutriato do local impactado pela **atividade de mineração (Gualaxo do Norte)**



# MODELAGEM MATEMÁTICA

Efeitos à longo prazo e  
a nível populacional

*Rhamdia quelen* – Jundiá



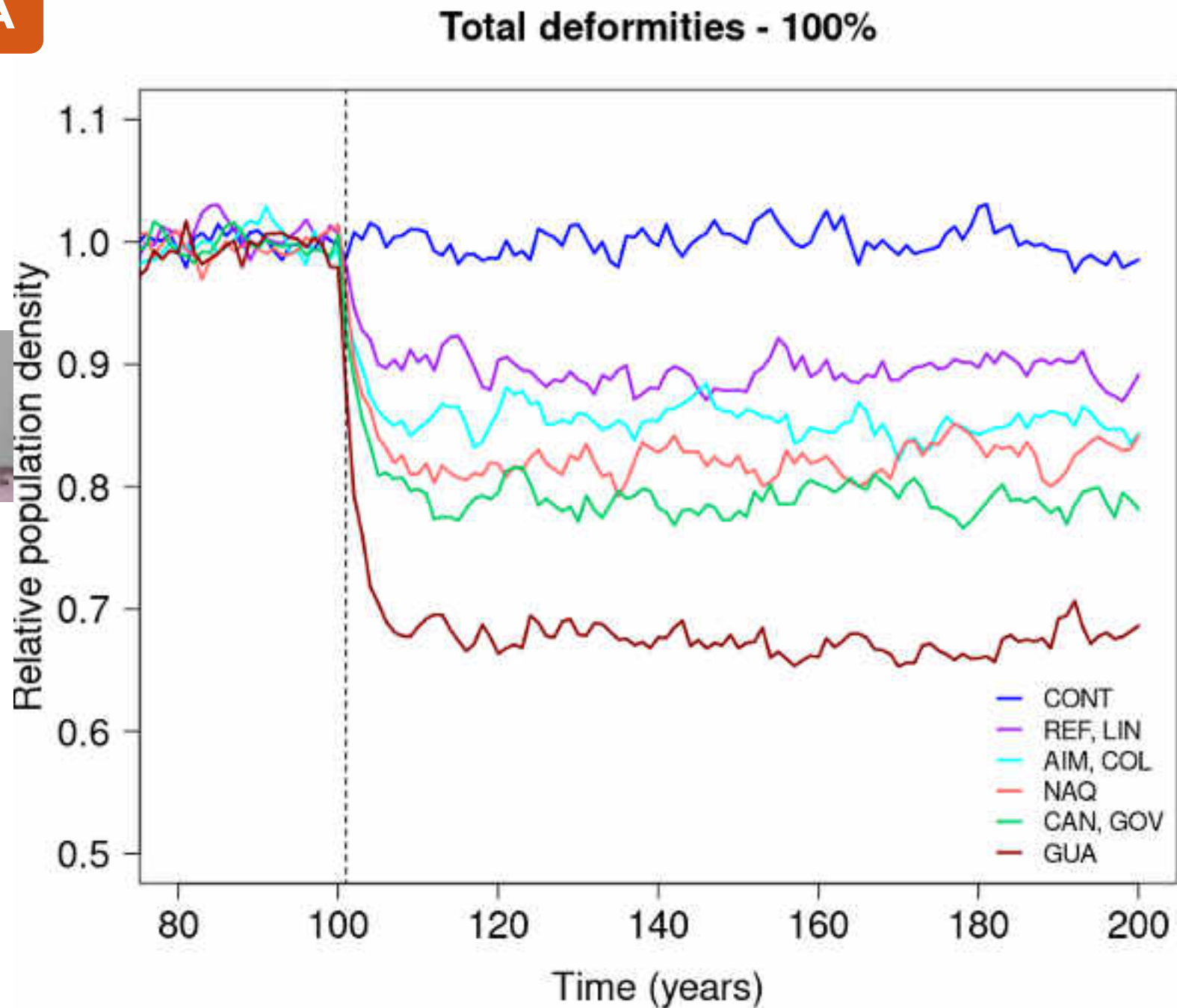
**Impacto Gerado sobre a população  
após 100 a 200 anos, pela taxa de  
indivíduos DEFORMADOS**

Maior impacto nas populações de:

GUALAXO DO NORTE – 66%

CANDONGA – 78%

GOVERNADOR VALADARES – 78%





|                                     | ALTO RD                                                                                                              | MÉDIO RD                                                                                                                                                                                                                 | BAIXO RD                                                                                                                                                                                                                                   |
|-------------------------------------|----------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Químicos</b>                     | <i>Contaminantes inorgânicos<br/>(As; Cd; Fe; Mn)</i>                                                                | <i>Contaminantes orgânicos +<br/>Al, Ti, V</i>                                                                                                                                                                           | <i>Contaminantes orgânicos +<br/>Al, Ti, V</i>                                                                                                                                                                                             |
| <b>Fontes de<br/>poluição</b>       | <i>Actividades de mineração</i><br> | <i>Efluentes industriais e<br/>domésticos</i><br>  | <i>Efluentes industriais e<br/>domésticos<br/>+ Agricultura</i><br>  |
| <b>Biomarcadores<br/>dos peixes</b> | <i>↑ níveis MT, LPO (fígado, cérebro)<br/>↓ GST, GSH<br/>Distúrbios circulatórios (fígado)</i>                       | <i>↑ dano DNA<br/>Respostas do sistema imune<br/>(fígado)</i>                                                                                                                                                            | <i>↑ AChE (brain and muscle),<br/>genotoxicity<br/>Respostas do sistema imune<br/>(fígado)</i>                                                                                                                                             |
| <b>Embriões de<br/>peixes</b>       | <i>Deformidades, Dano DNA</i>                                                                                        | <i>Dano DNA</i>                                                                                                                                                                                                          | <i>↑ GST</i>                                                                                                                                                                                                                               |
| qSQG                                | <b>Maior poluição</b>                                                                                                | <b>Poluição moderada</b>                                                                                                                                                                                                 | <b>Poluição baixa</b>                                                                                                                                                                                                                      |

# Conclusões



Os peixes amostrados no Alto DRB, especialmente nas cabeceiras, apresentaram as maiores respostas de estresse, como LPO e depleção de GSH, provavelmente relacionados aos maiores níveis de metais das atividades de mineração nesta área.

Peixes do médio e baixo DRB, apresentando danos no DNA e respostas de genotoxicidade, indicaram impacto de outras fontes de poluição ao longo do rio, introduzindo produtos químicos inorgânicos e orgânicos

Bagres de inverno apresentaram maior número de correlações significativas de respostas indicando influência da sazonalidade e do nicho frente à exposição aos contaminantes.

# CONSIDERAÇÕES FINAIS

- **Turbidez e metais** reduziram alguns meses após o desastre porém se mantiveram **relativamente altos mesmo 3 anos após o evento (Fe, Mn, Al)**
- Elementos como **As, Cd, Hg são tóxicos** mesmo em baixas concentrações e podem ser potencializados em misturas complexas
- **Efeitos subletais** – crônicos, precisam ser mais investigados, efeitos letais podem subestimar a toxicidade
- Respostas diferentes de acordo com a espécie e sazonalidade
- **Não há um gradiente claro de poluição ao longo do Rio Doce** pois diferentes atividades humanas presentes na BRD interferem na qualidade da água.

Gualaxo do Norte (Mariana, MG)



Plantações de café (Colatina, MG)





# CONSIDERAÇÕES FINAIS

- Apesar de ser esperada uma melhoria na qualidade da água a jusante do rio, a presença de **múltiplas fontes pontuais e difusas** de produtos químicos ao longo do rio, resultando em uma **mistura complexa de contaminantes**, causa **diferentes respostas de estresse e tolerância à ictiofauna** da RDB
- Fatores ambientais, como a **estação chuvosa**, são importantes para determinar a **biodisponibilidade de poluentes**, causando diferentes respostas de estresse em peixes.
- Os **processos contínuos de contaminação** ocorridos ao longo desta bacia hidrográfica, **mesmo antes e depois do rompimento da Barragem e Fundão**, aliados às **falhas** na implementação e **cumprimento** da **legislação ambiental**, dificultam a melhoria da qualidade da água levando a implicações negativas para a saúde humana e ambiental

Upper DRB (Mariana, MG)

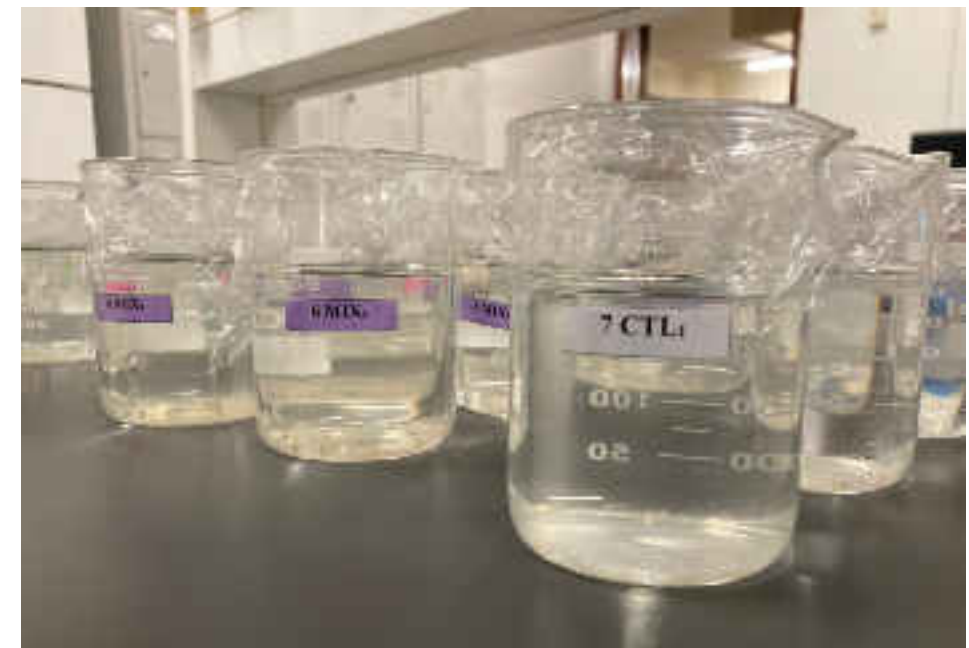
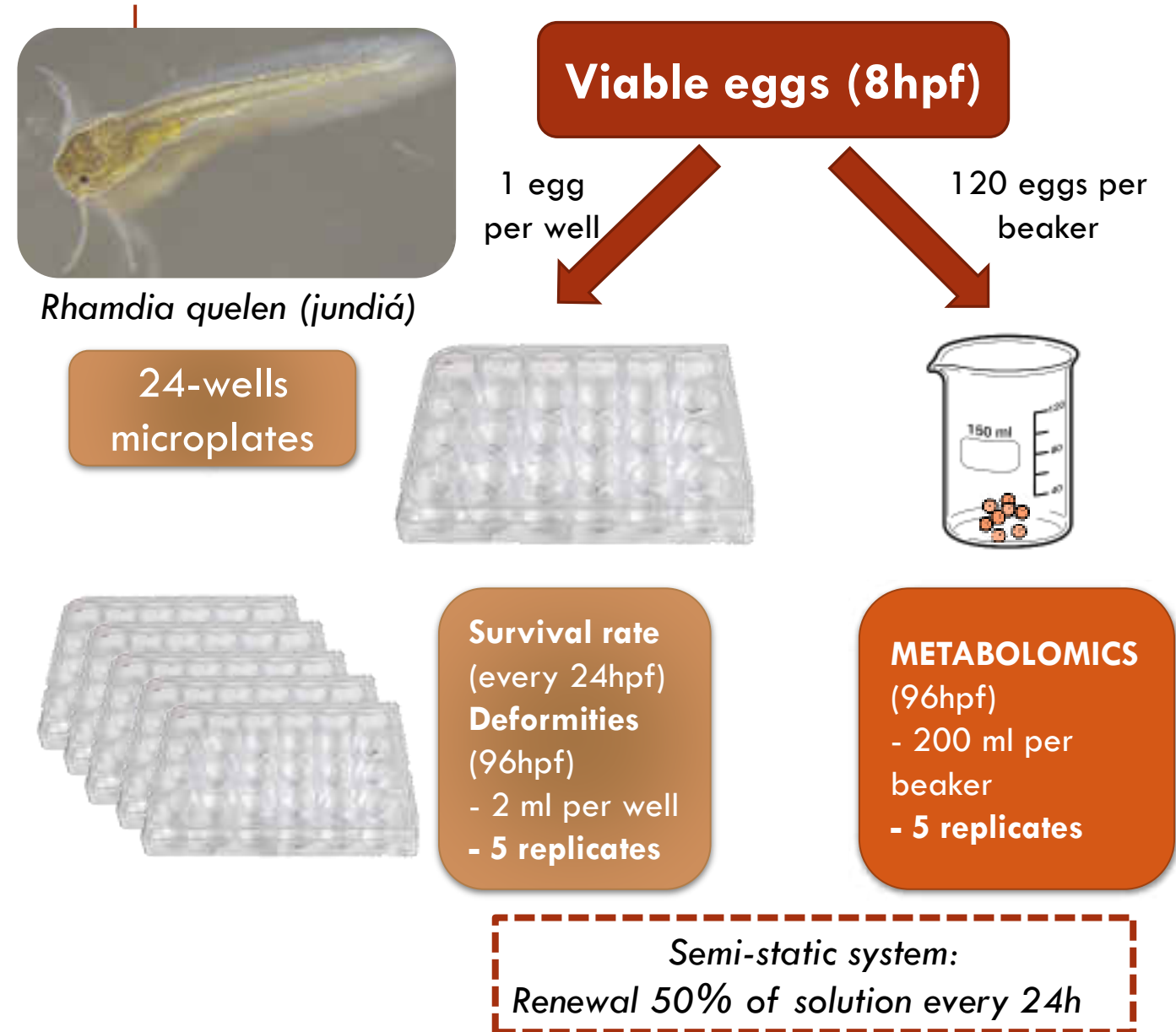


Lower DRB (Colatina, MG)



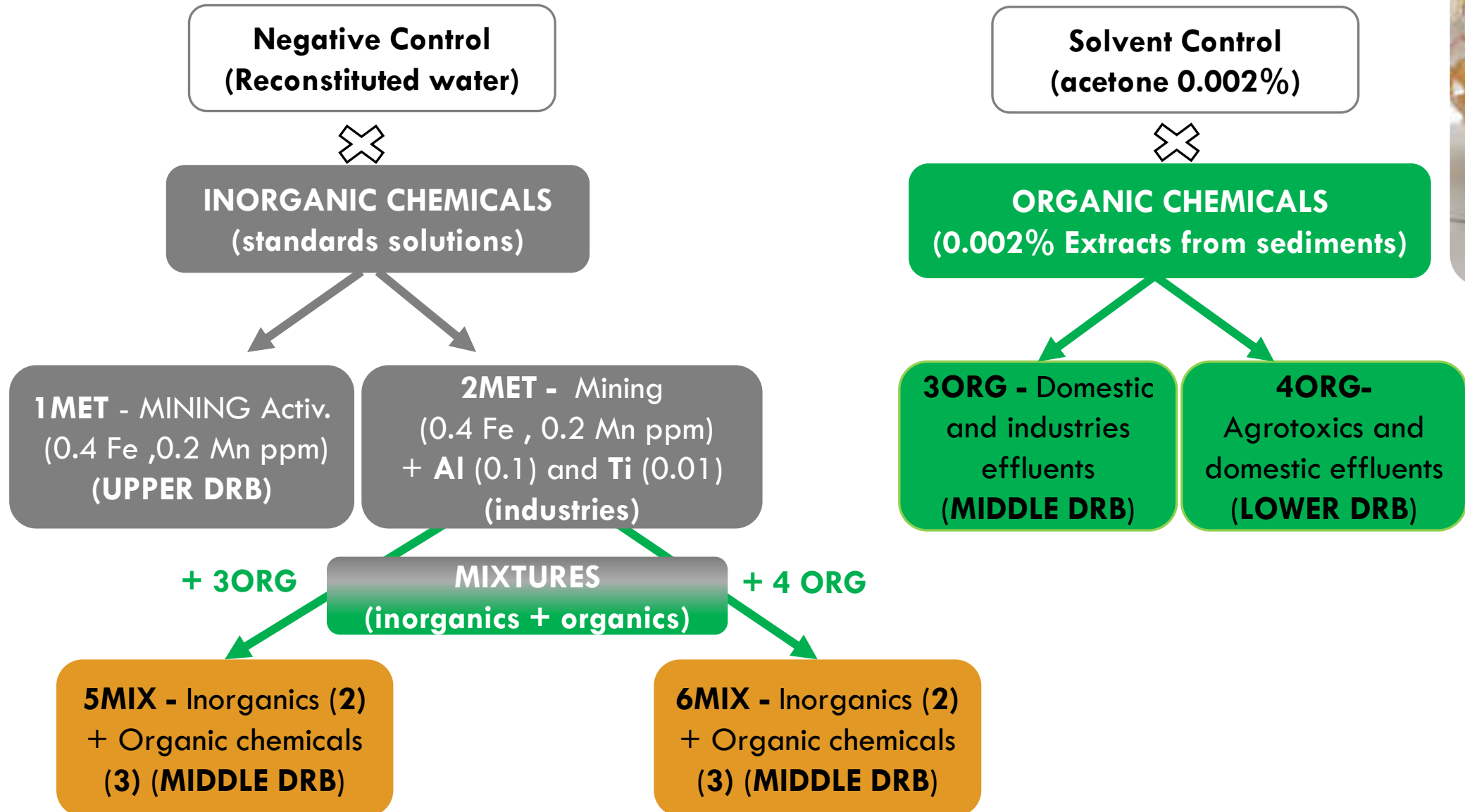
# LC-HRMS-BASED METABOLOMICS AS A POWERFUL APPROACH TO ELUCIDATE MECHANISMS OF ACTION OF ENVIRONMENTAL CHEMICALS FROM DOCE RIVER IN BRAZILIAN FISH EMBRYOS

# 1) FISH EMBRYO TOXICITY TEST





# CONTROL vs. TREATMENT GROUPS

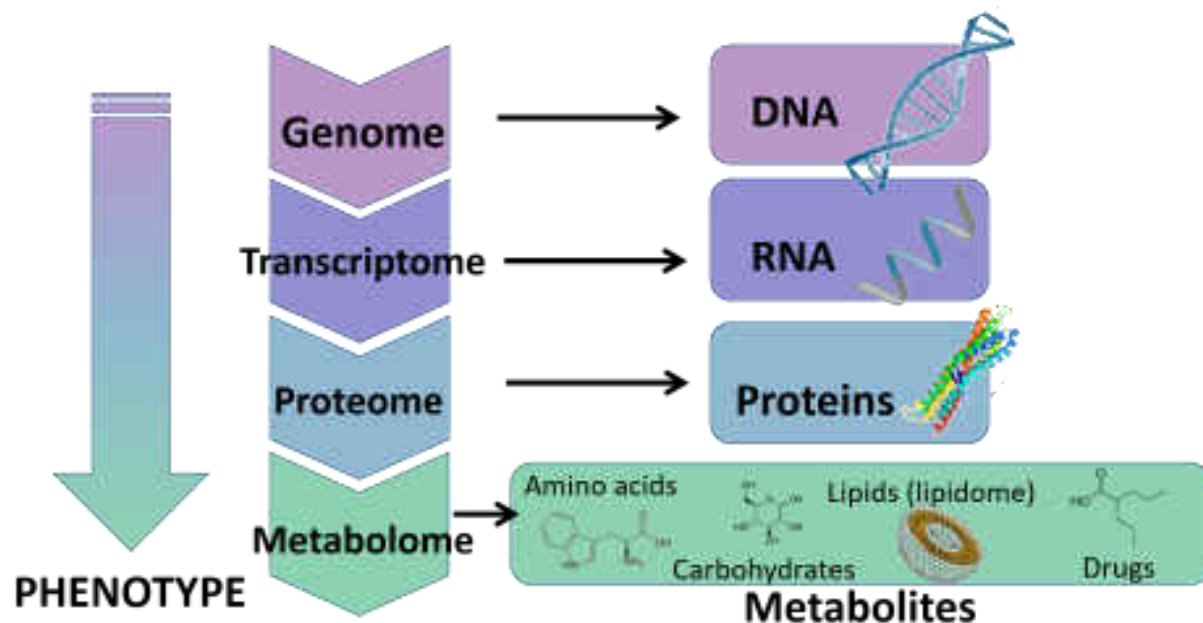


# SURVIVAL RATES

➤ No significant mortality and deformities for the treatment groups and controls

| Survival (%)                     | 1 MET     | 2 MET     | 3 ORG     | 4 ORG     | 5 MIX     | 6 MIX     | 7 CTL     | 8 SOLV    |
|----------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Replicate 1                      | 100       | 79        | 96        | 96        | 100       | 96        | 96        | 83        |
| Replicate 2                      | 100       | 100       | 96        | 100       | 96        | 96        | 100       | 100       |
| Replicate 3                      | 88        | 96        | 92        | 100       | 100       | 100       | 92        | 88        |
| Replicate 4                      | 96        | 92        | 92        | 96        | 75        | 88        | 100       | 100       |
| Replicate 5                      | 92        | 96        | 88        | 100       | 88        | 88        | 96        | 96        |
| <b>Average<br/>(Microplates)</b> | <b>95</b> | <b>93</b> | <b>93</b> | <b>98</b> | <b>92</b> | <b>93</b> | <b>97</b> | <b>93</b> |
| <i>Beakers average</i>           | <i>83</i> | <i>77</i> | <i>80</i> | <i>68</i> | <i>76</i> | <i>60</i> | <i>82</i> | <i>76</i> |

# METABOLOMICS



Elucidate **mechanisms of action** and characterize the **effects** caused by **environmental chemicals** in organisms at the molecular level

*“Directly reflect the underlying biochemical activity and state of cells, being considered as the end point of the “omics” cascade, thus better predicting the molecular phenotype ”*



# ABORDAGENS DA METABOLÔMICA

## Direcionada (Target)

Hipótese pré-formulada

Análise Específica

Correlacionado a padrões  
referências

Identificação pré-  
determinada

Quantificação absoluta

## Não-direcionada (Untarget)

Hipótese gerada

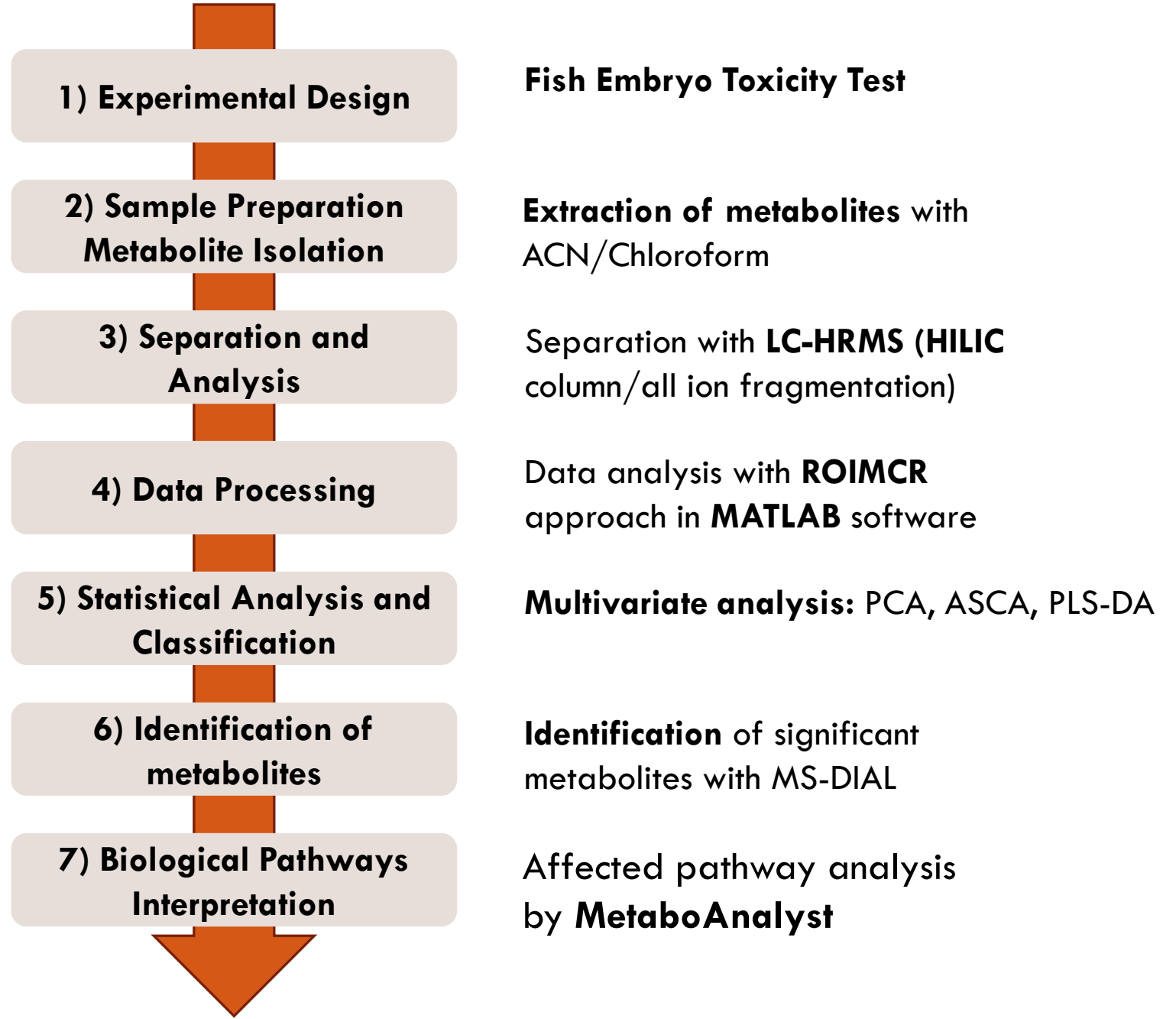
Análise  
Global/Compreensiva

Correlacionado a bancos  
de dados/livrarias

Identificação qualitativa

Quantificação relativa

# *UNTARGET METABOLOMICS WORKFLOW*



## 2) METABOLITES EXTRACTION

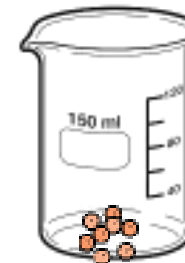
### FISH EMBRYO TOXICITY TEST

Jundiá (*Rhamdia quelen*)



Treatment groups  
and controls

200 ml **Treatment  
groups/ controls**



120 eggs per  
beaker

**Samples**  
Pools of ~50 embryos  
(50 mg)

- 5 replicates
- Stored at -80° C
- Add surrogates



3. ADD 900 µL of  
ice-cold  
**METHANOL** and  
**HOMOGENIZE**

5. ADD 500 µL of  
water and 300 µL  
of **CHLOROFORM**

**UPPER**



**LOWER**



MeOH  
(METABOLITES)

ACN



Ressuspension

MeOH



Chloroform  
LIPIDS



# MIXTURES OF METABOLITES' STANDARDS FOR TARGET ANALYSIS

**BIG MIX**  
(95 compounds)

Surrogates

Methionine sulfone

Neurotransmitters

LIPIDOMIX

PIPES\*

Metabolites  
STD - 2 ppm

**Mix 1  
aminoacids**  
(17 compounds)

**Mix 2  
nucleosides**  
(12 compounds)

**Mix 3 Organic  
acids**  
(17 compounds)

**Mix 4 Individual**  
(29 compounds)

**Mix 5 Hormones  
+ Lipids MeOH**  
(17 compounds)

**INJECTION into QTOF**

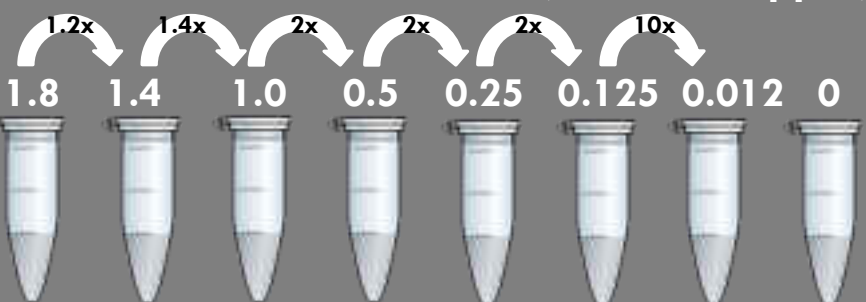


Only Surrogates  
Each MIX of STDs (1, 2, 3, 4, 5)  
BIG MIX (5 replicates at 1 ppm, std curve)



Fish embryo samples (-/+)

**BIG MIX Standard Curve (1.8 – 0.01 ppm)**



and Blank  
of extrac.

\*PIPES after extraction

# 3)LC- HRMS analysis

## Chromatographic Separation (LC)

| UPLC system (BRUKER)                         | Column HILIC (ACQUITY UPLC BEH) |            |
|----------------------------------------------|---------------------------------|------------|
| A1 – Aqueous phase                           | 50:50 (Acetonitrile/Water)      |            |
| B1 – Organic phase                           | 90:10 (Acetonitrile/Water)      |            |
| 10mM ammonium formate (NH4COOH) + 0.2% HCOOH |                                 |            |
| Injection volume: 2 µL                       | Flux: 0.4 ml/min                | RT: 10 min |



## HRMS parameters

 All-ion fragmentation (MS1/MS2)  
**UNTARGETED ANALYSIS**

| BRUKER IMPACT II QTOF                             | <u>Full scan broad band acquisition (MS1/MS2)</u> |
|---------------------------------------------------|---------------------------------------------------|
| Mass range (m/z)                                  | 60 – 1000                                         |
| Normalized collision energy                       | VIP-HESI Ion source                               |
| Positive and Negative mode (separate acquisition) |                                                   |
| <b>Positive</b>                                   | 20-30 eV (scan: 3 x 3.0 Hz)                       |
| <b>Negative</b>                                   | 24-36 eV (scan: 3 x 1.0 Hz)                       |





# 4) Data analysis (one sample for each standard mixture)

## Aminoacid Standard mixture

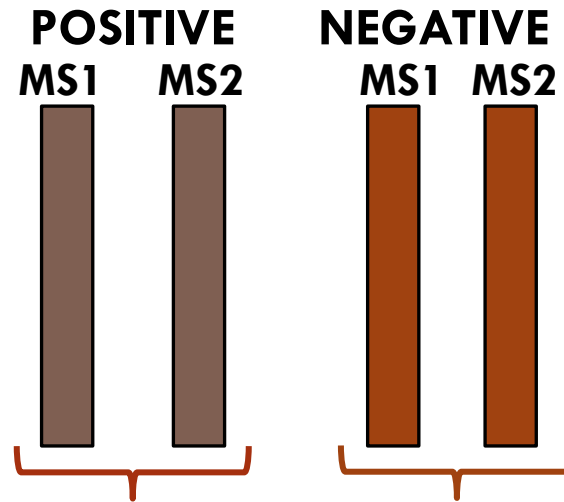
- Analytical standard
- Sigma Aldrich
- 17 compounds
- Diluted to ~ 2ppm

## Regions of Interest (ROI) parameters

Intensity threshold: 0.5% of max.  
Intensity

Minimum occurrence: 5

Mass error tolerance: 0.005



ROI  
Augmented  
MS12 +



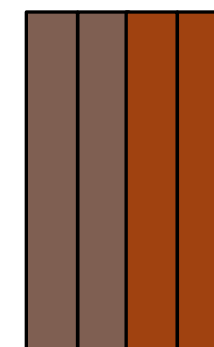
+

ROI  
Augmented  
MS12 -



=

Augmented  
MS12 +/-



MCR-ALS



25 components  
Quantification

Elution  
profiles

Identification

Spectra  
profiles

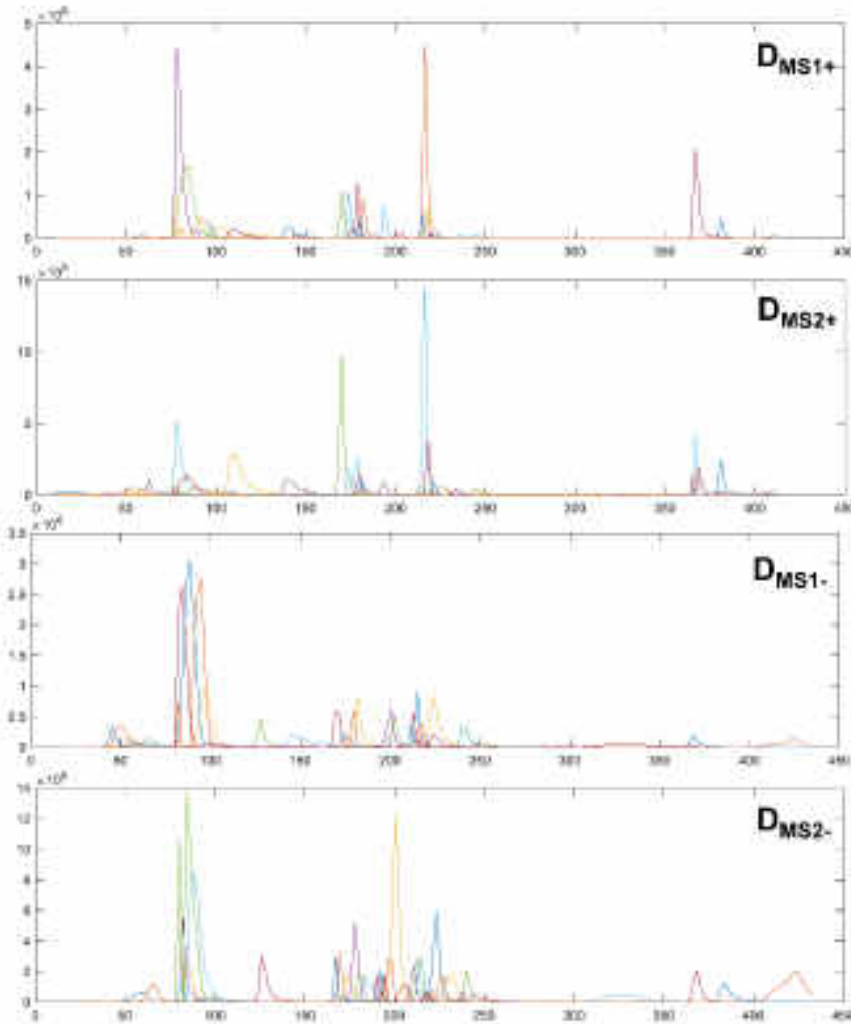
## MCR-ALS

$$D = CS^T$$

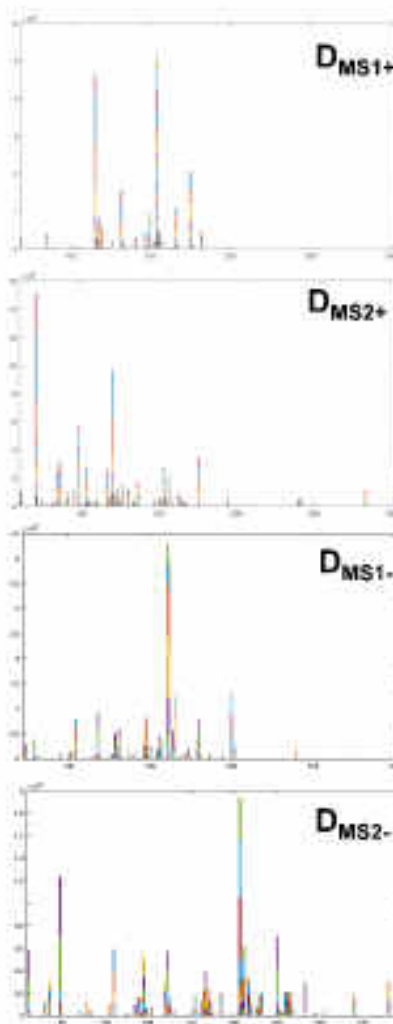
**D** é a matriz de resposta instrumental, **C** é a matriz de concentração relativa e **S** é uma matriz de espectros puros

# Multivariate curve Resolution (Resolução multivariada de curvas com mínimos quadrados alternantes)

Elution profiles



Mass spectra

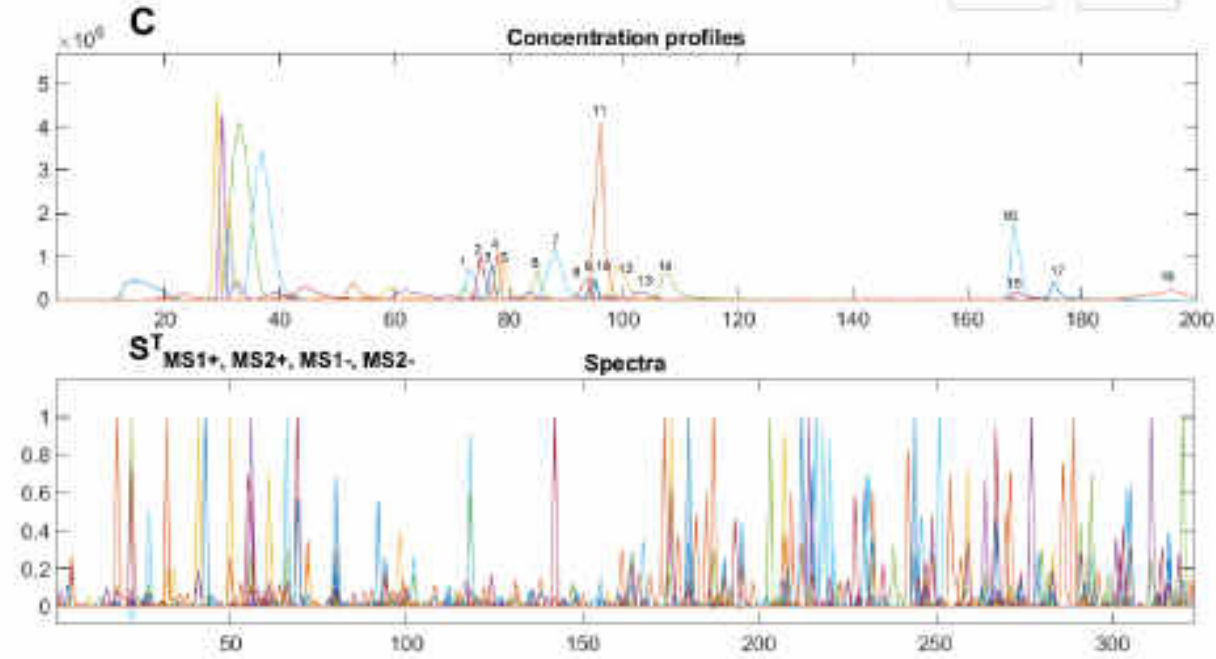


$$D_{MS1+, MS2+, MS1-, MS2-} = C S^T_{MS1+, MS2+, MS1-, MS2-}$$

ALS optimization

Copy

Print



Aromatic side-chains

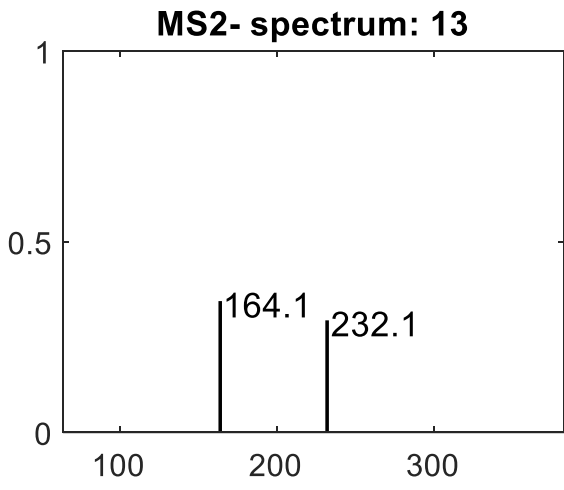
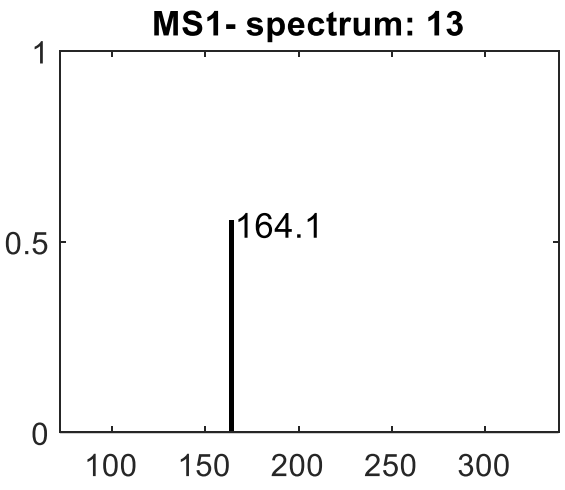
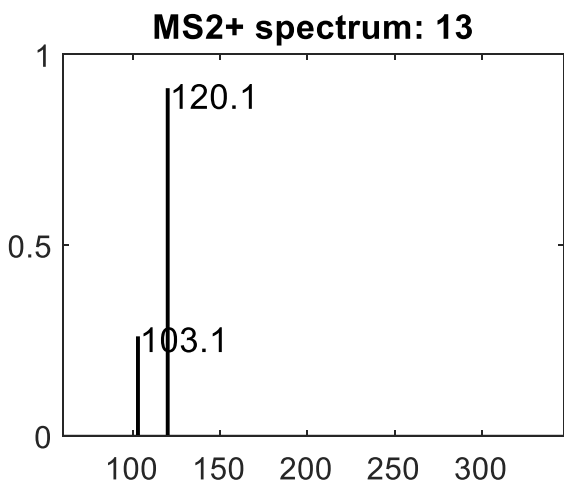
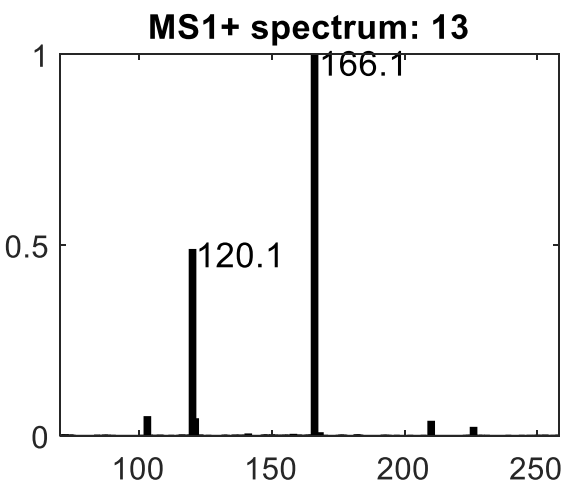
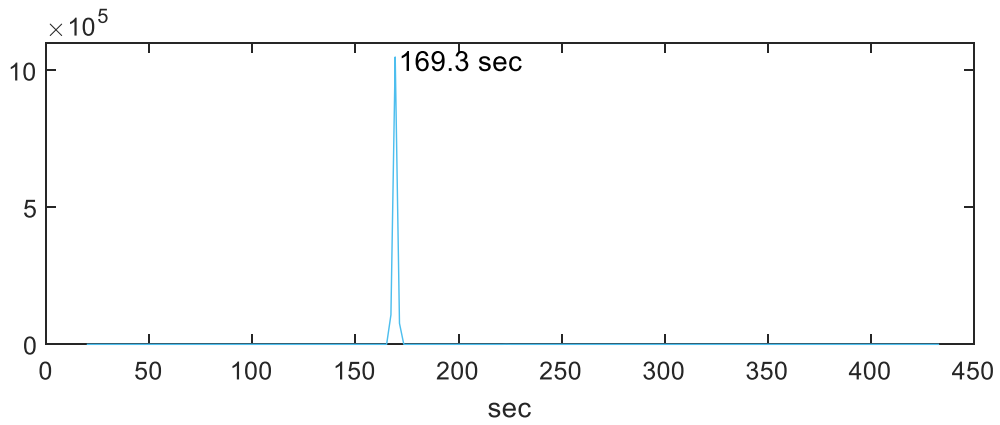
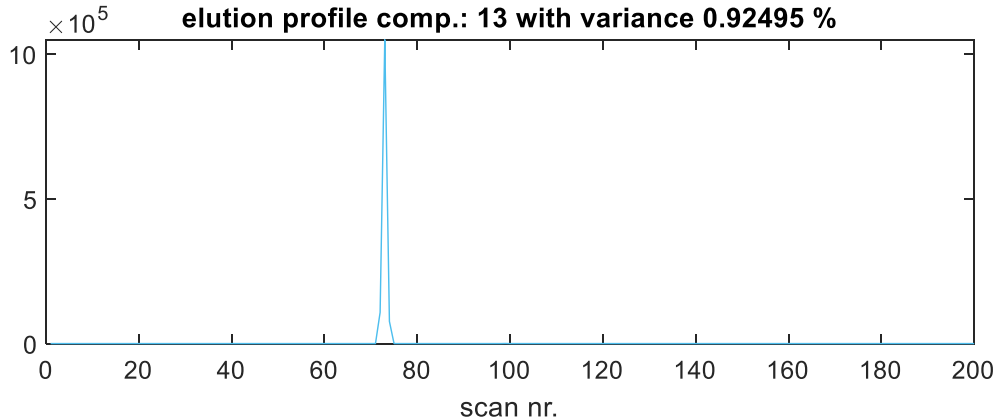
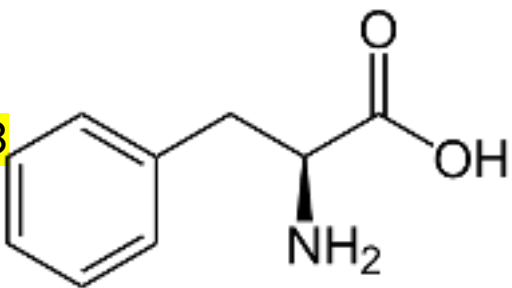
# L-PHENYLALANINE \*

[H+] 166.086805

MS2 + 120.0796/103.0533

[H-] 164.0671

MS2 - 147.041/



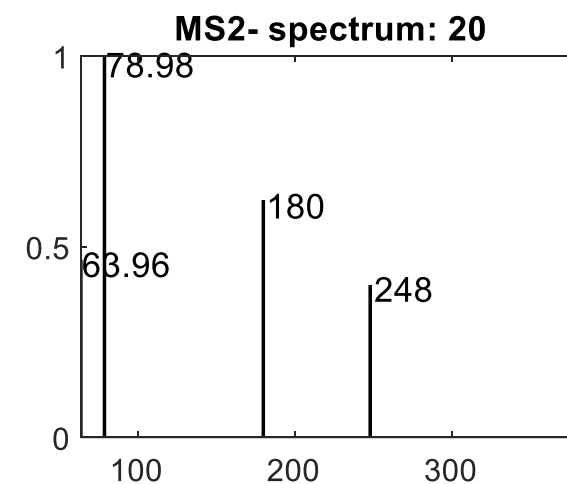
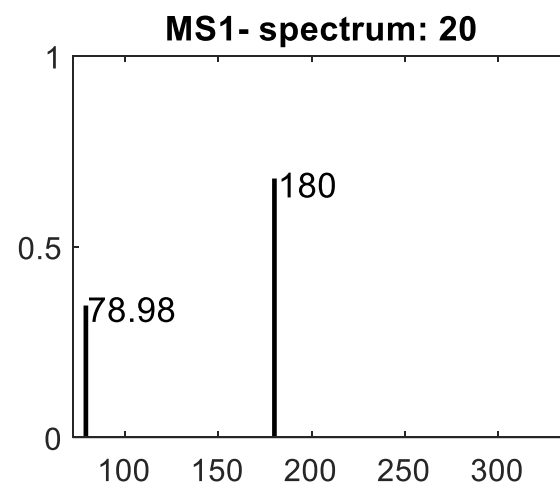
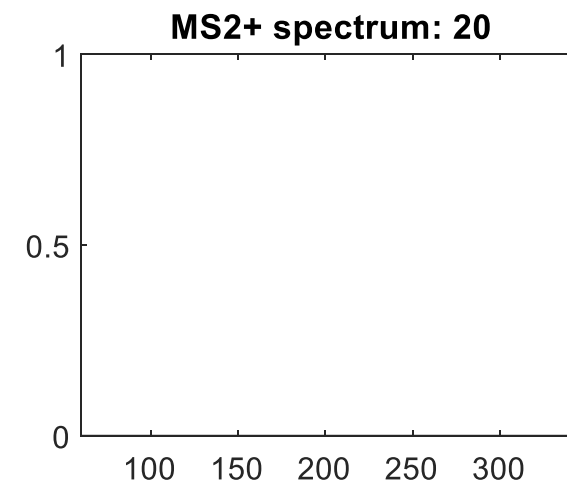
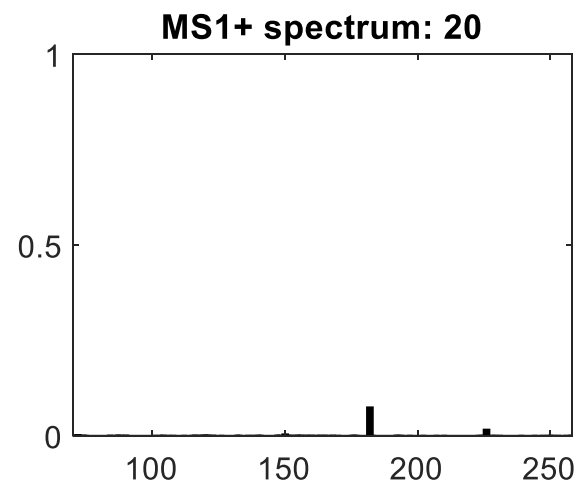
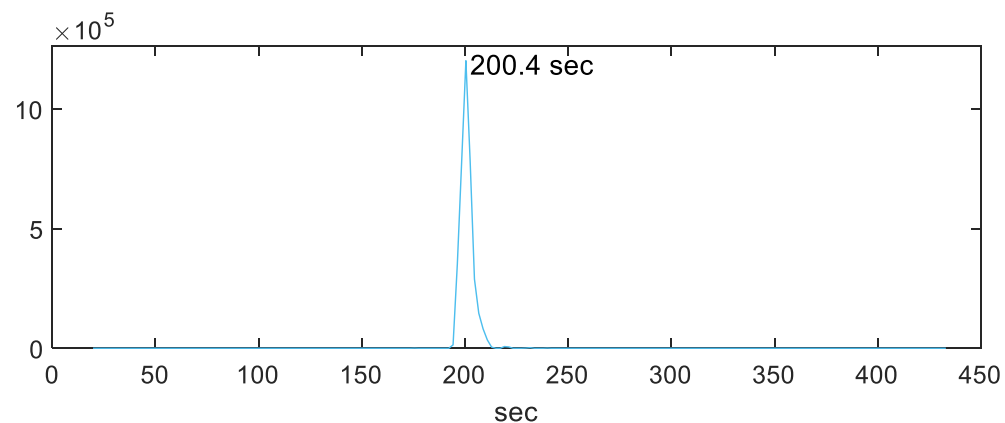
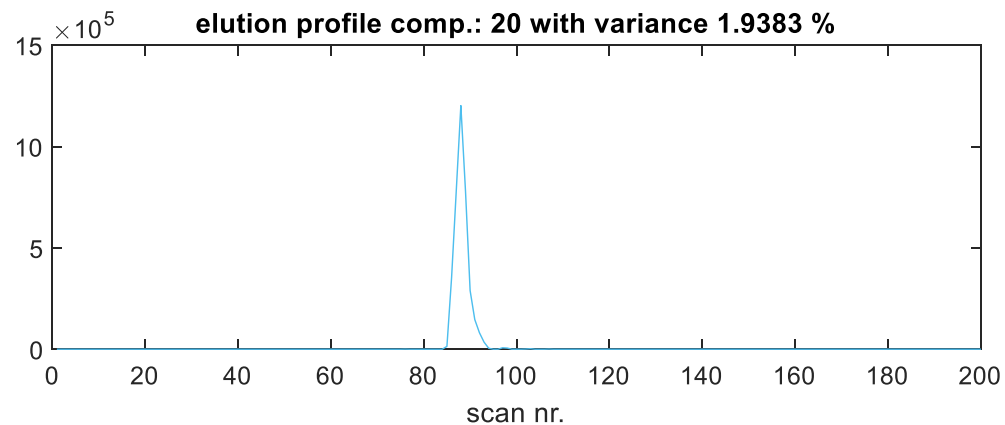
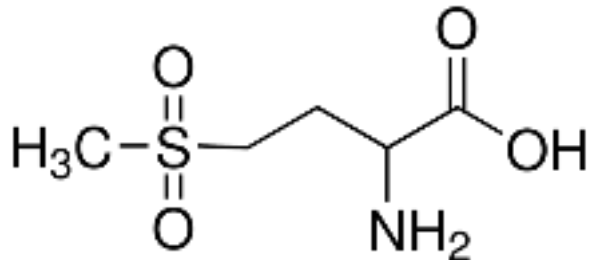
# METHIONINE SULFONE (SURROGATE)

[H<sup>+</sup>] 182.0487

MS2 + 136.0466

[H<sup>-</sup>] 180.0330

MS2 - 78.9835





Cationic side-chains

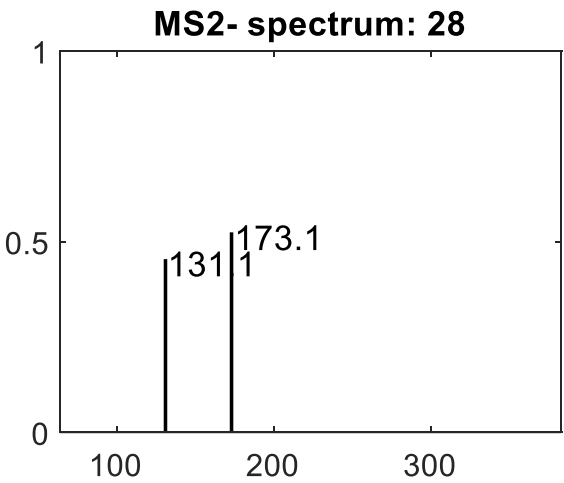
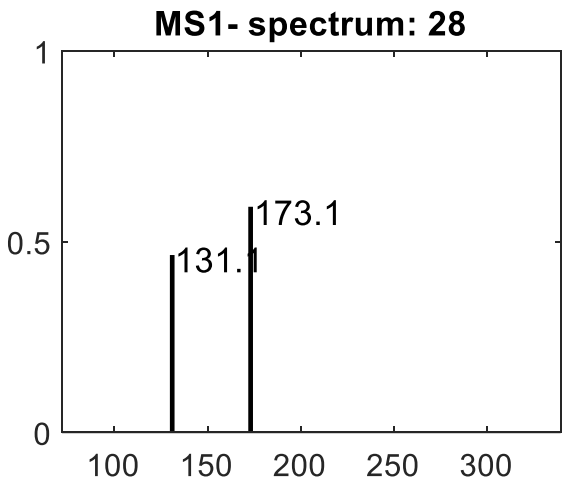
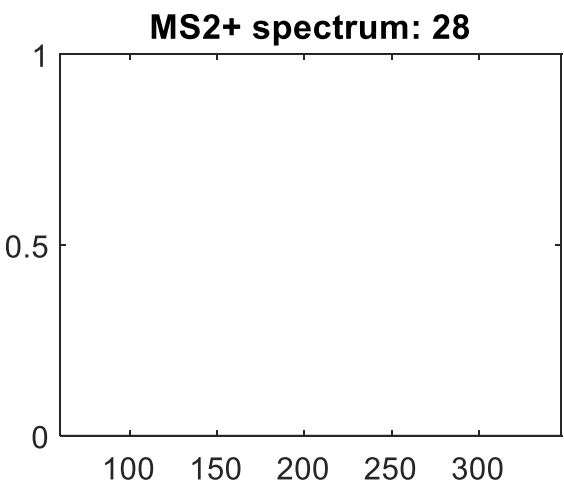
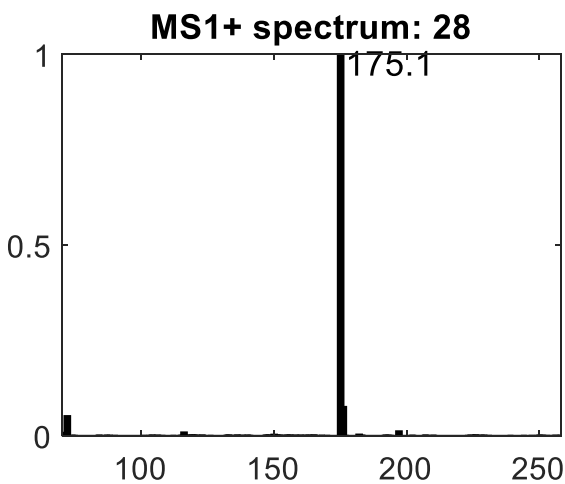
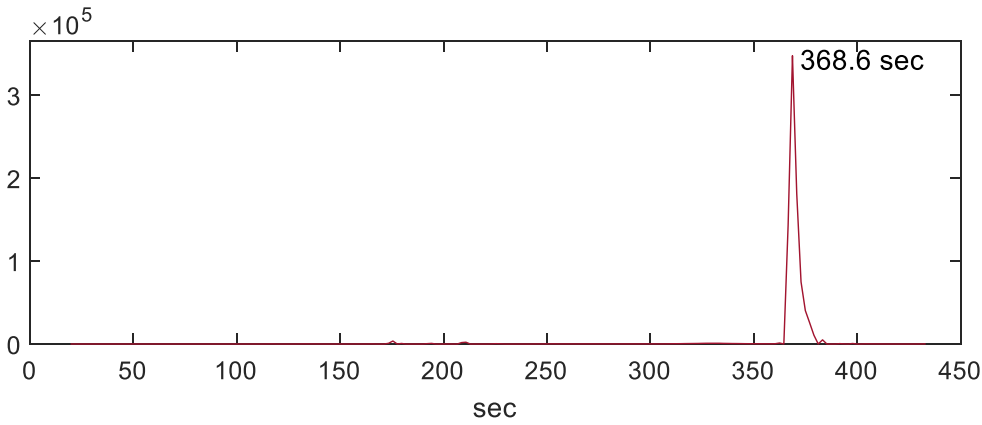
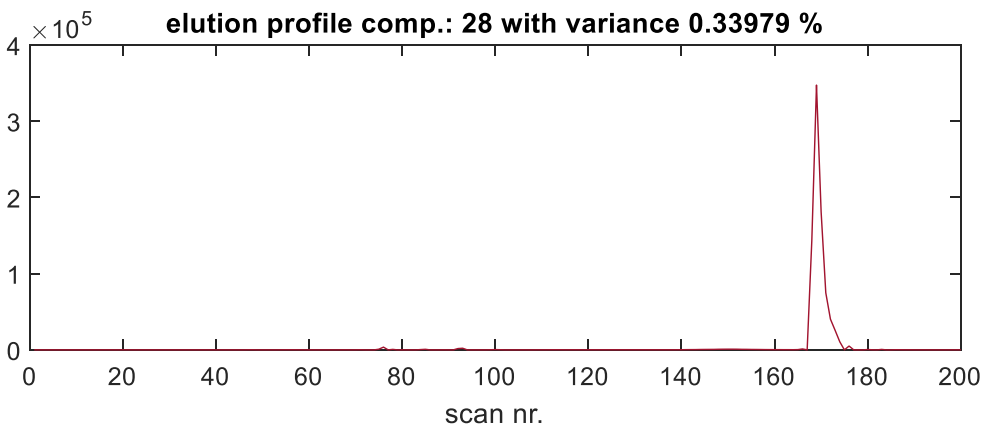
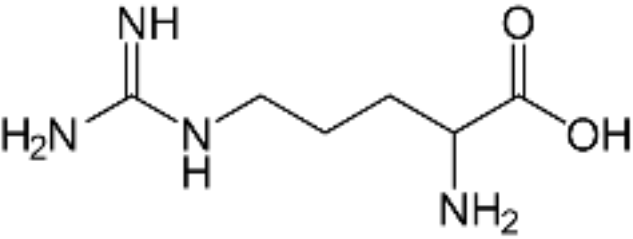
L-ARGININE

[H+] 175.11950

MS2 +130.0965

[H-] 173.1039

MS2 - 131.0787



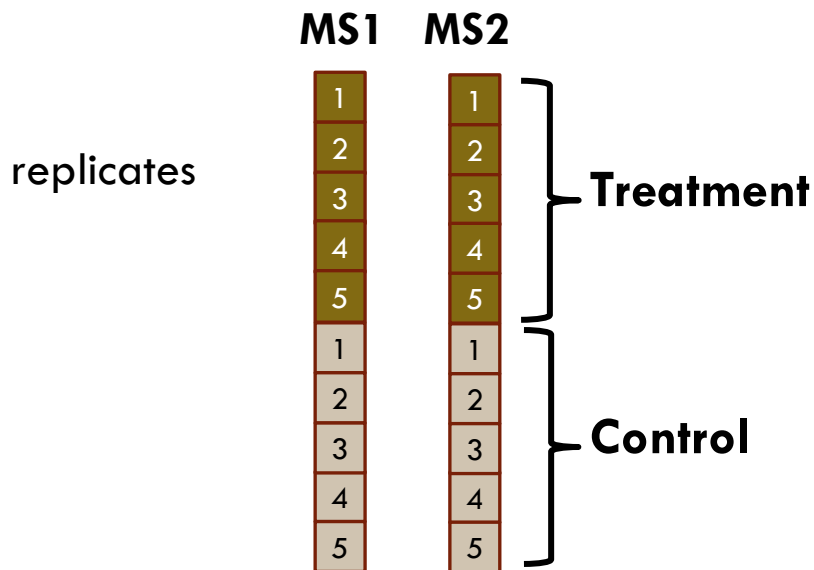
# IDENTIFICATION OF METABOLITES – STD. MIXTURES

|                                | POSITIVE Acq. |            |            | NEGATIVE Acq. |            |            |                   |
|--------------------------------|---------------|------------|------------|---------------|------------|------------|-------------------|
|                                | Data Analysis | MS-DIAL    | ROIMCR*    | Data Analysis | MS-DIAL    | ROIMCR*    | TOTAL (+/-)       |
| MIX 1 (17)<br>Aminoacids       | 15            | 15         | 15         | 13            | 13         | 15         | 17 (100%)         |
| MIX 2 (12)<br>Nucleosides      | 8             | 9          | 8          | 9             | 10         | 5          | 11 (91.6%)        |
| MIX 3 (17)<br>Organic acids    | 3             | 4          | 4          | 9             | 8          | 7          | 11 (64.7%)        |
| MIX 4 (29)<br>Individual comp. | 13            | 11         | 15         | 16            | 12         | 6          | 22 (75.8%)        |
| MIX 5 (17)<br>Hormones         | 5             | 7          | 6          | 8             | 7          | 4          | 11 (64.7%)        |
| MIX 6 (6)<br>Surrogates        | 2             | 3          | 3          | 2             | 2          | 2          | 3 (50.0%)         |
| <b>TOTAL (95)</b>              | 46 (48.4%)    | 49 (51.5%) | 48 (50.5%) | 57 (60%)      | 52 (54.7%) | 35 (36.8%) | <b>75 (78.9%)</b> |

\*need to re-check to confirm results

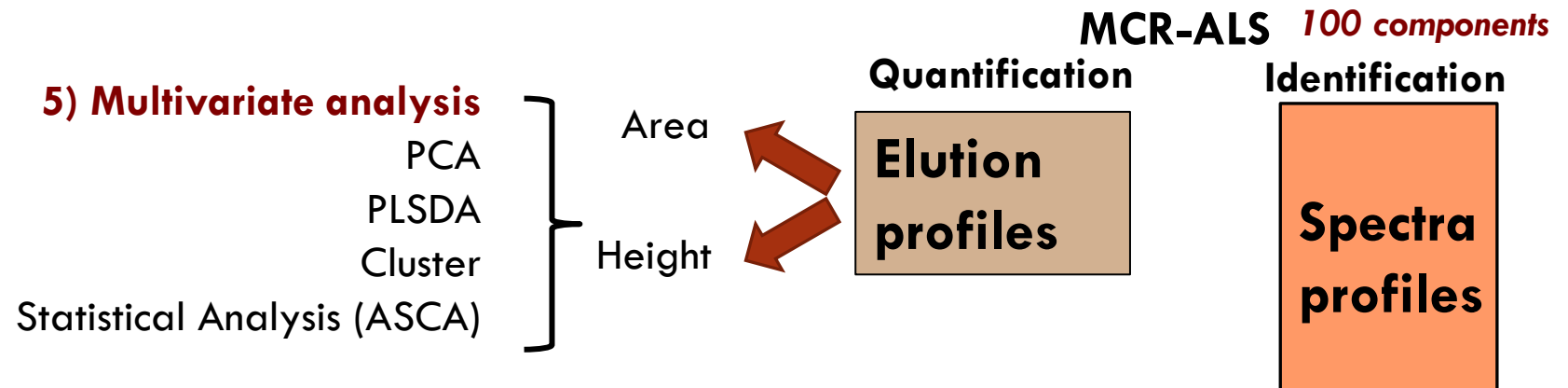
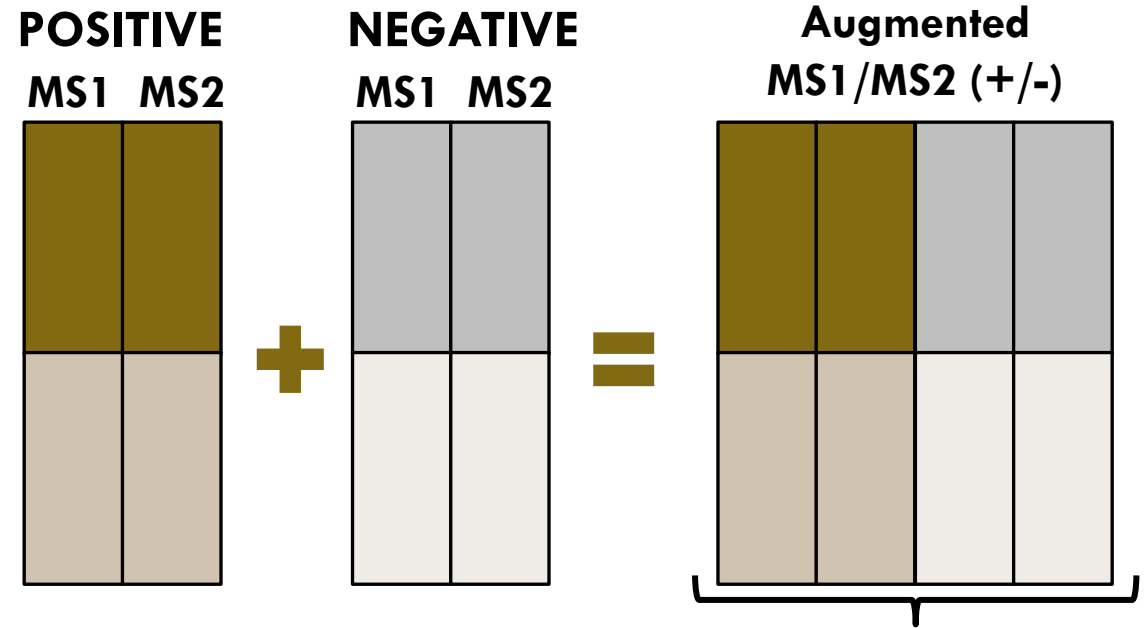
# ROI-MCR

Simultaneous analysis of:  
Treatment group (5 replicates)  
X  
Control group (5 replicates)

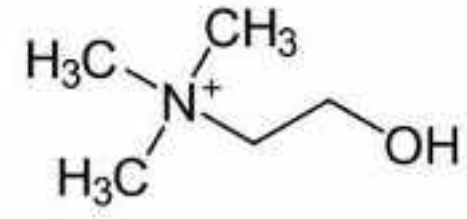


## FISH EMBRYOS (Multi-sample analysis)

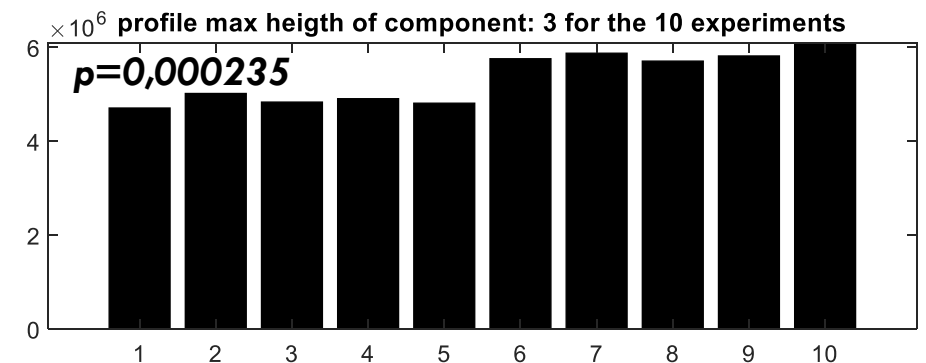
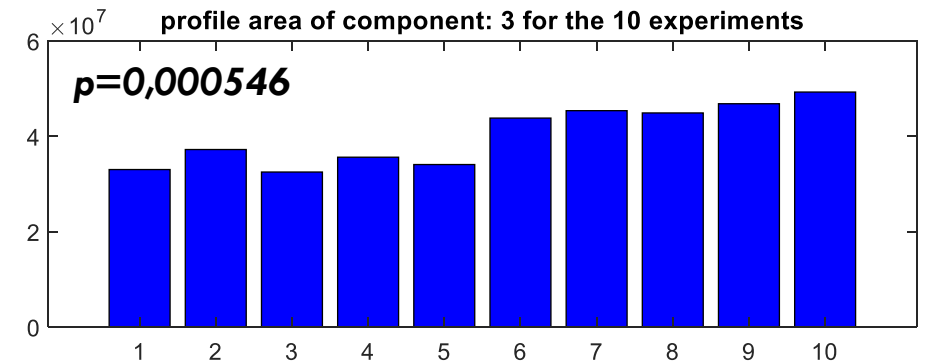
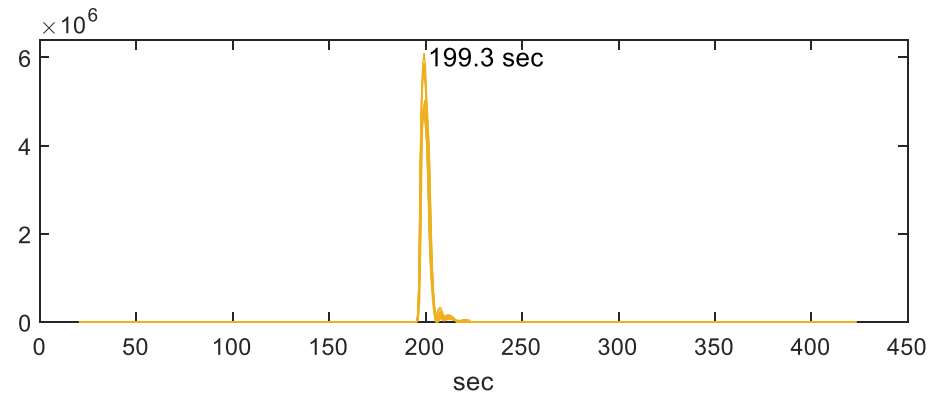
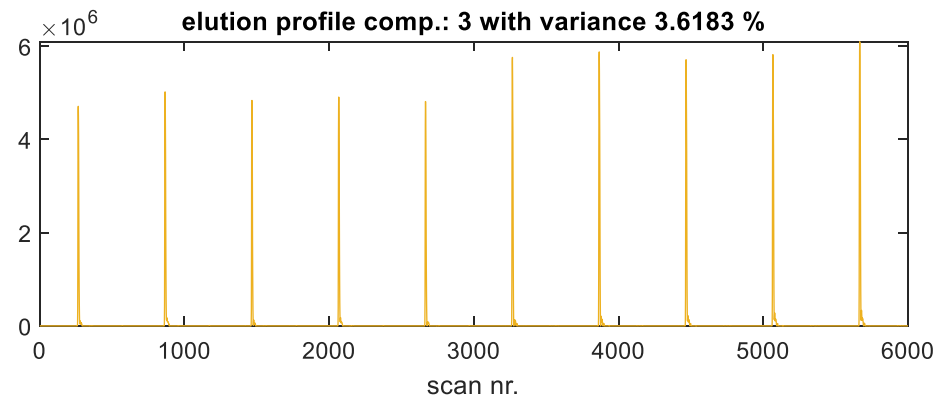
Analysis of  
augmented MS1 2  
performed both  
separated +/- and  
jointly



# CHOLINE



Vitamin-like essential nutrient -  
Neurotransmitter  
RT - 199.252 (MS-DIAL)  
Control > Treatment





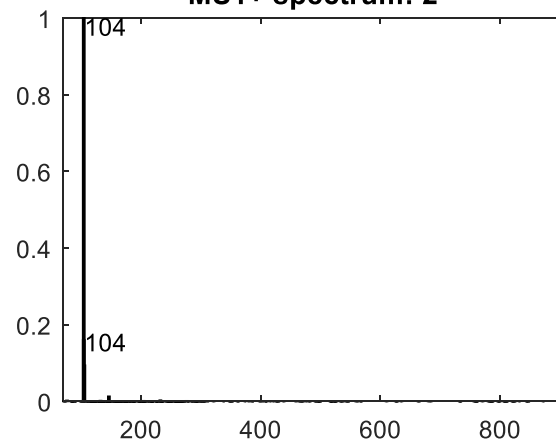
# CHOLINE

## POSITIVE

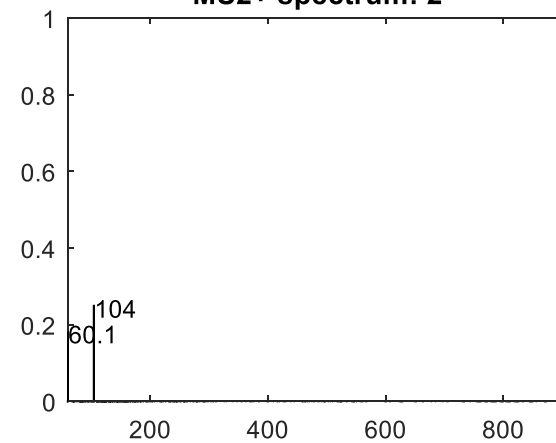
[H+] 104.1056  
MS2 60.0802

Analysis of augmented  
MS1 2 +/-

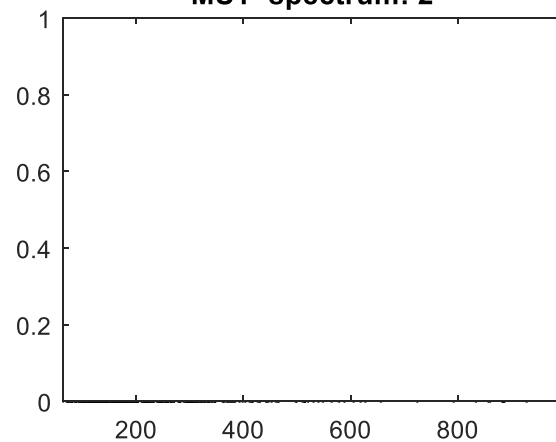
MS1+ spectrum: 2



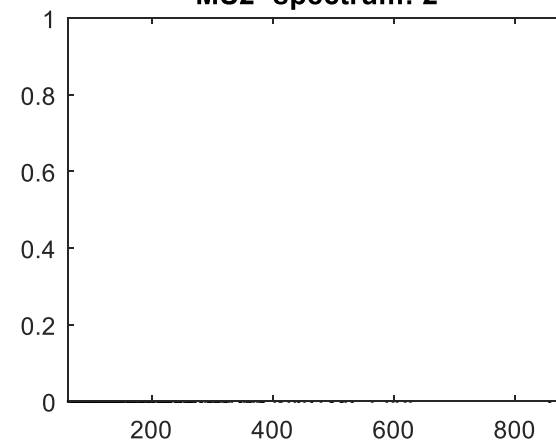
MS2+ spectrum: 2



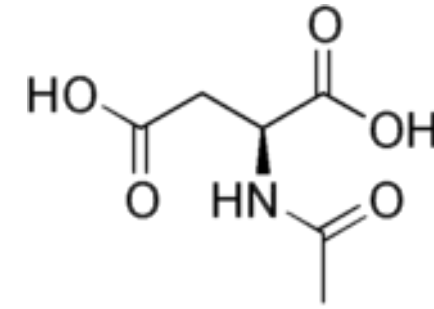
MS1- spectrum: 2



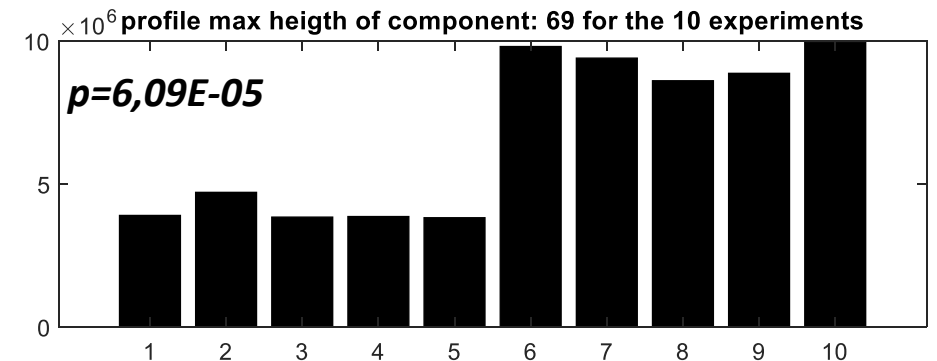
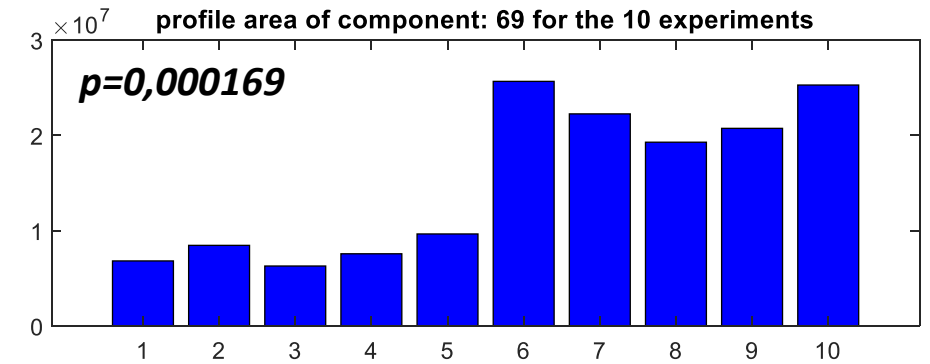
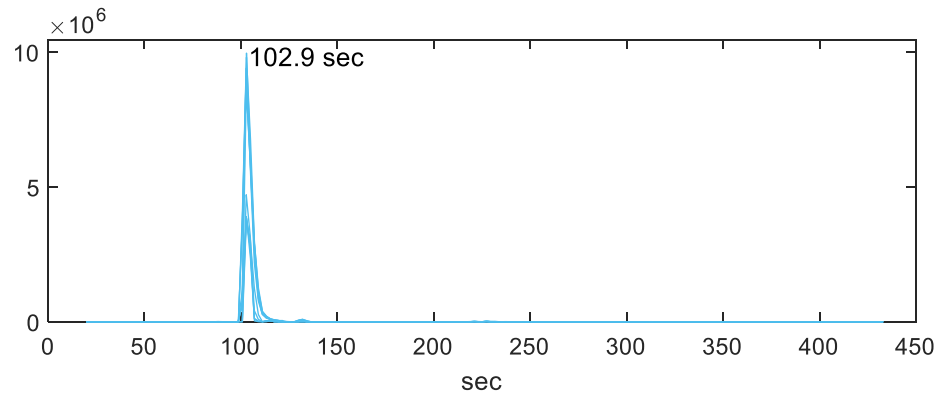
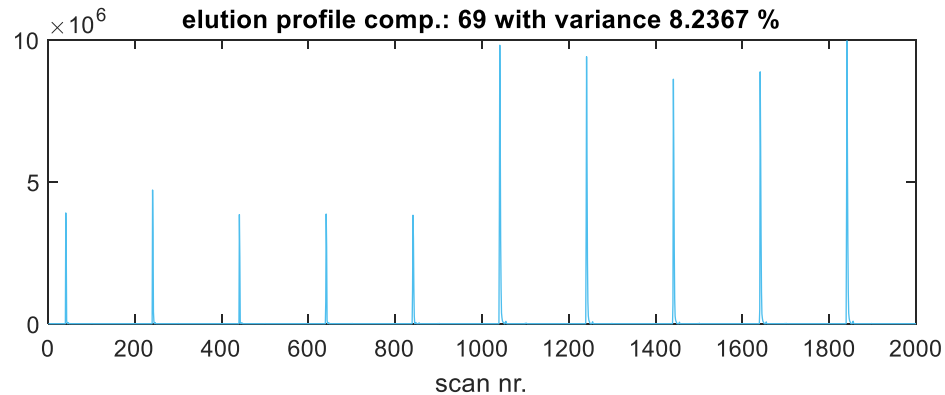
MS2- spectrum: 2



# N-ACETYL-L-ASPARTIC ACID



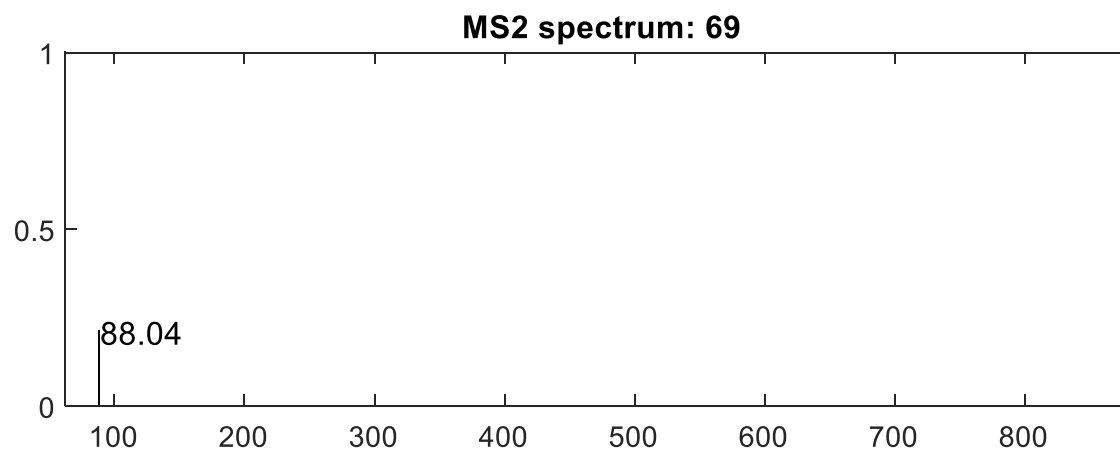
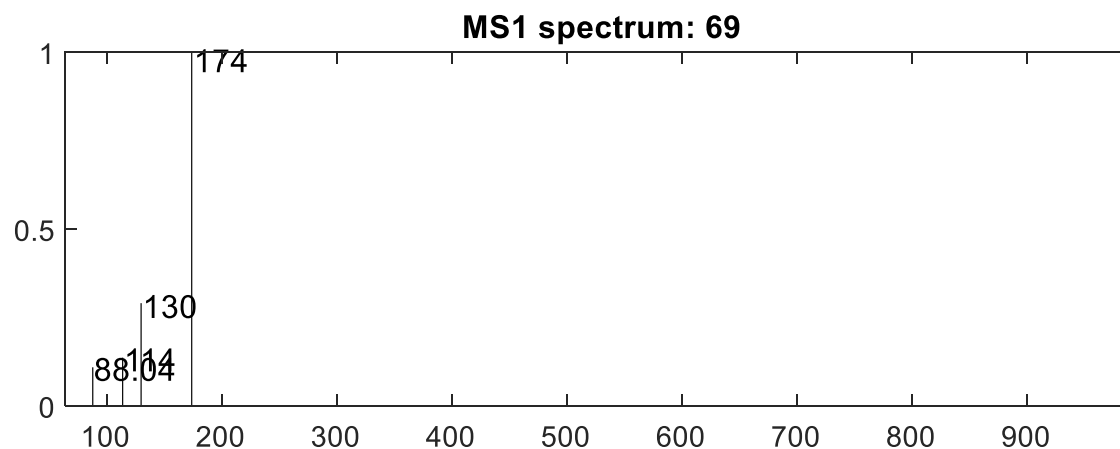
Derivative of aspartic acid – Neuro-functions  
RT – 103.8 (MS-DIAL)  
Control > Treatment



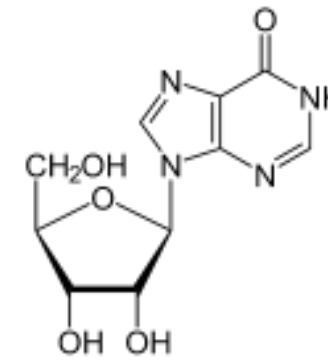
# N-ACETYL-L-ASPARTIC ACID

**NEGATIVE**

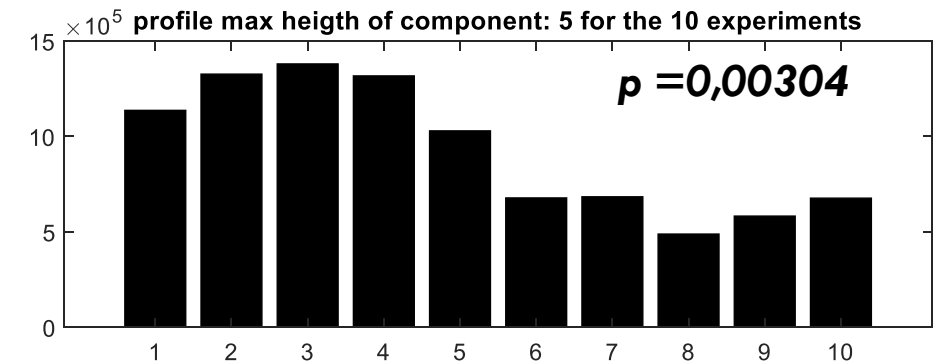
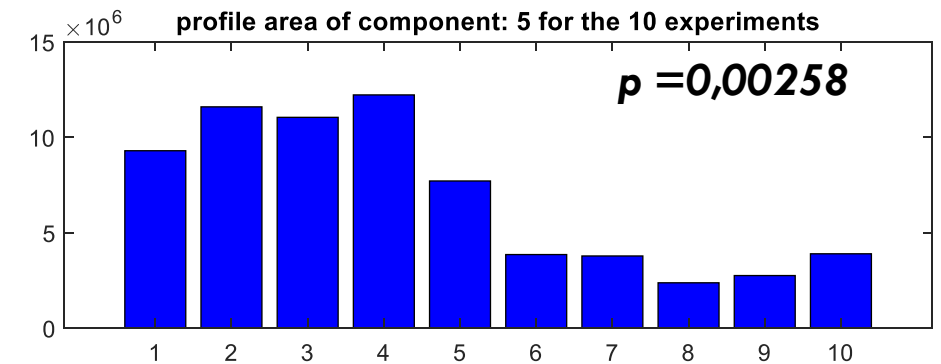
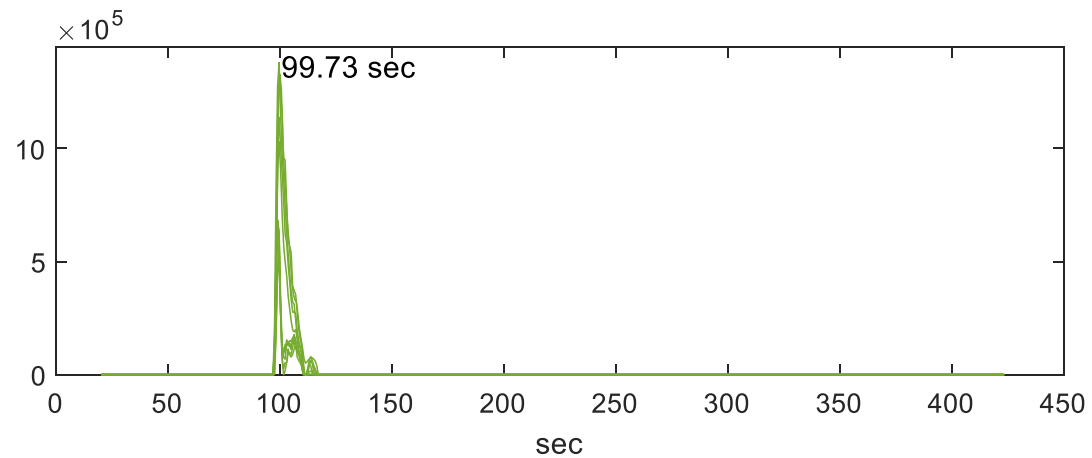
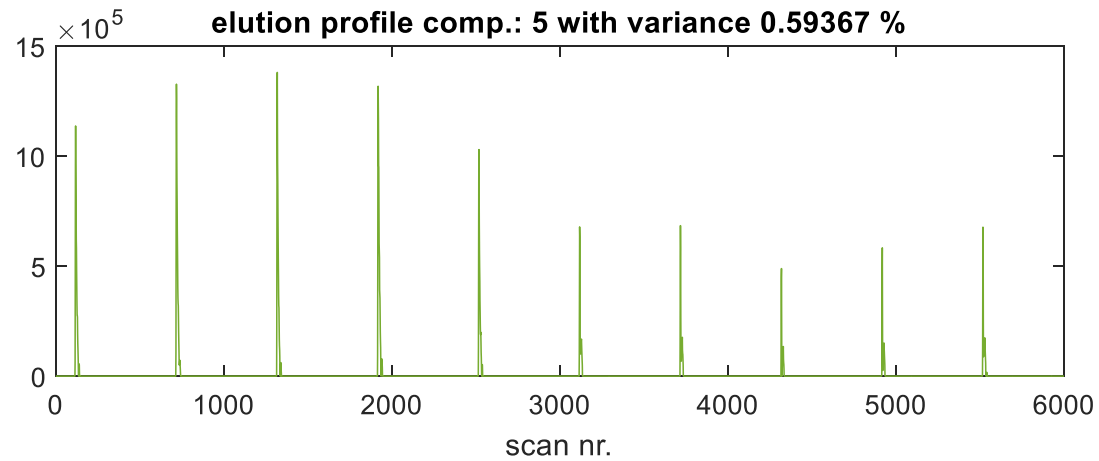
[H-] 174,0345  
MS2 88,0375;  
130,05



# INOSINE



Nucleoside – Nucleic acids  
RT 100.2 (MS-DIAL)  
Treatment > Control

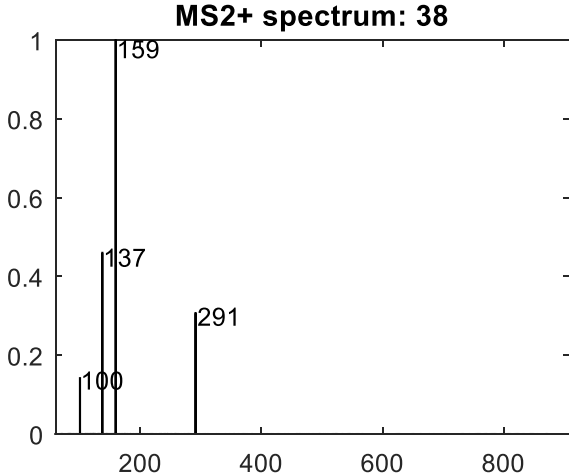
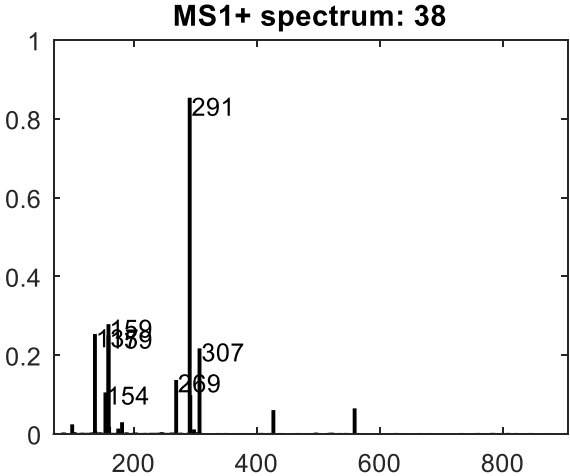




# INOSINE

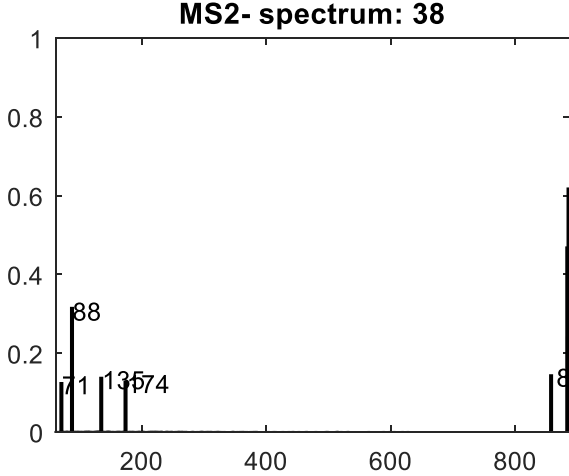
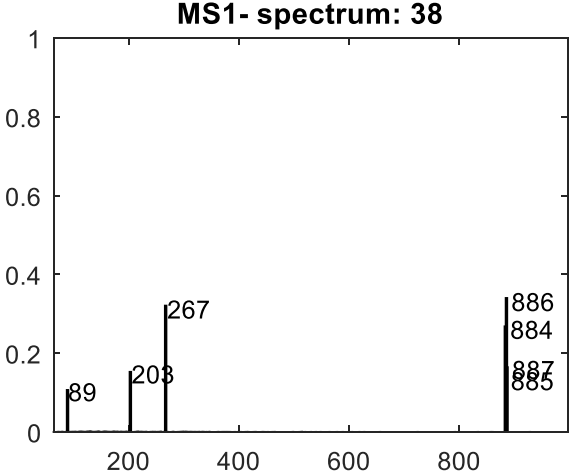
**POSITIVE**

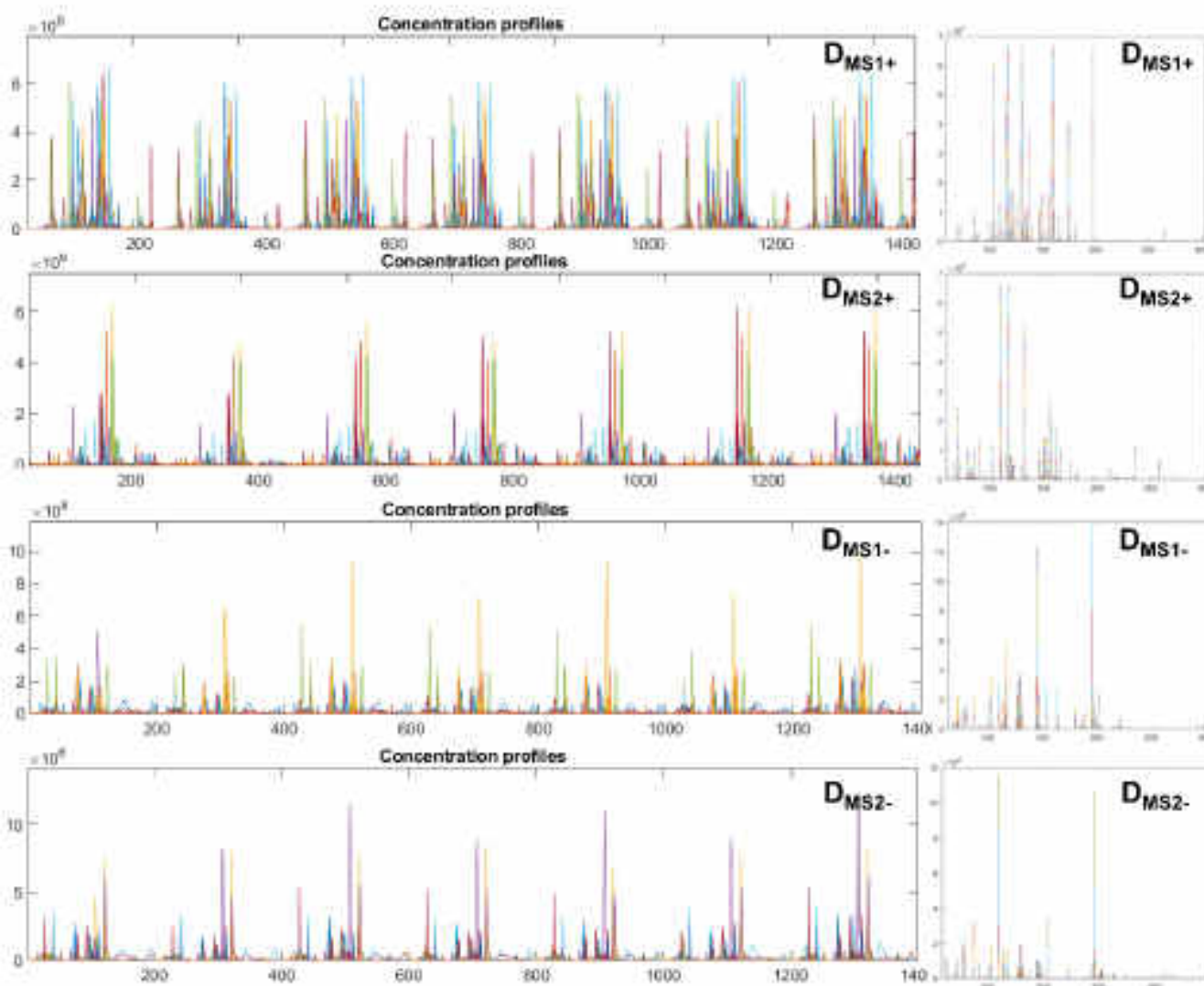
[H+] 269.0886  
MS2 137.0443;  
291.0662;159.0259



**NEGATIVE**

[H-] 267.07295





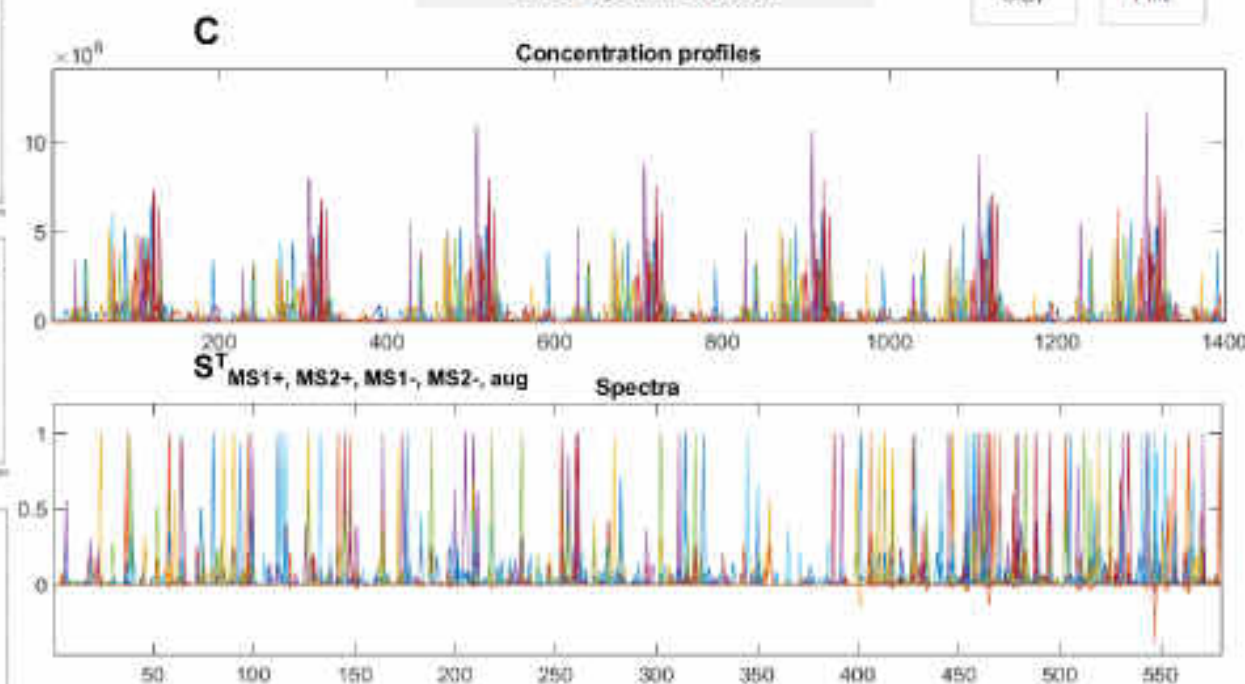
**MCR Bilinear model**

$$D_{MS1+, MS2+, MS1-, MS2-, aug} = C S^T_{MS1+, MS2+, MS1-, MS2-, aug}$$

ALS optimization

Copy

Print



Plots are optimum at the iteration for 27

CONVERGENCE IS ACHIEVED!!!

Std. dev of residuals vs. exp. data = 4040.9402

Fitting error (lack of fit) in % (PCA) = 2.5721

Fitting error (lack of fit) in % (exp) = 2.2388

Percent of variance explained (R<sup>2</sup>) at the optimum is = 89.6024

Information

MCR Bands

Back 2 const Back 2 param

# 6) IDENTIFICATION OF SIGNIFICANT METABOLITES

## MS-DIAL

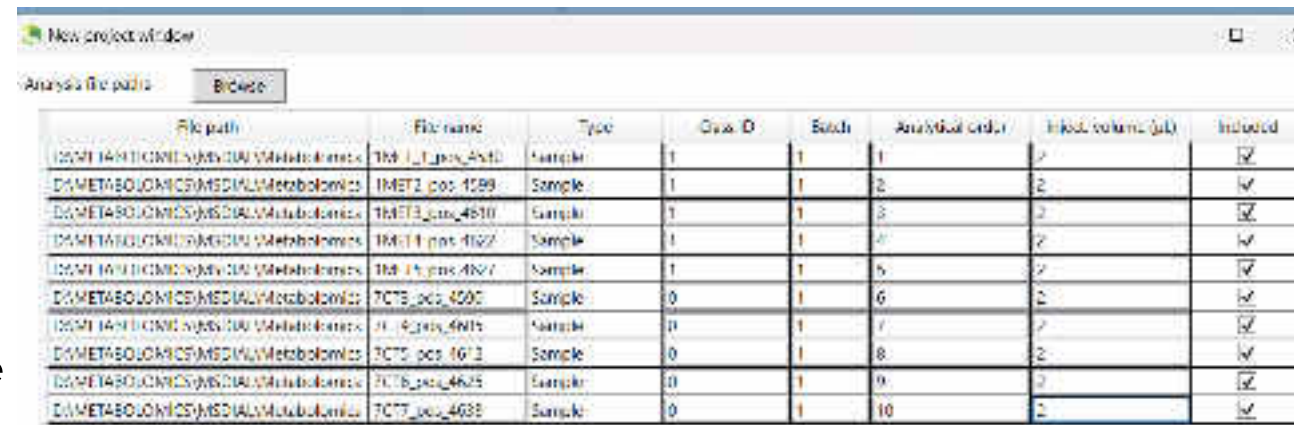
- ✓ Raw data (cdf files → **abf files**)
- ✓ Treatment x control groups
- ✓ Selection of Reference Matched metabolites
- ✓ MS2 spectra match with available database
- ✓ Mass error < 0.005 (MS1, MS2)
- ✓ Total score > 850.00

## ROIMCR

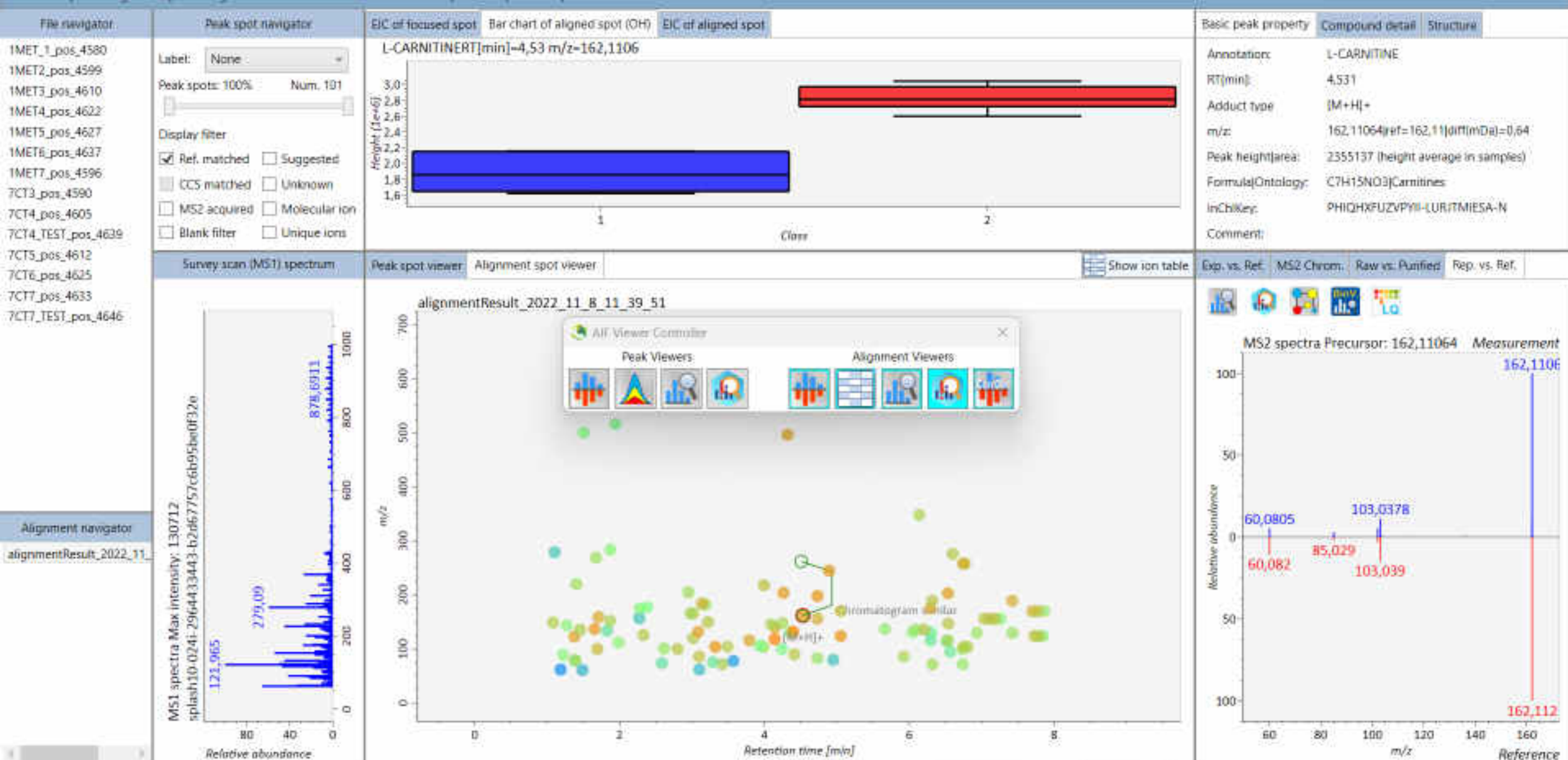
### Identification of significant components (Student's T test)

- ✓ MS2 spectra match with list obtained from MS-DIAL
- ✓ Mass error < 0.005 (MS1, MS2)

✓ Separated analysis of Positive and Negative acquisition files



| File path                           | File name      | Type   | Class ID | Batch | Analytical order | Inject volume (µl) | Included |
|-------------------------------------|----------------|--------|----------|-------|------------------|--------------------|----------|
| D:\METABOLOMICS\MSDIAL\Metabolomics | IMET1_pos_4510 | Sample | 1        | 1     | 1                | 2                  | ✓        |
| D:\METABOLOMICS\MSDIAL\Metabolomics | IMET2_pos_4589 | Sample | 1        | 1     | 2                | 2                  | ✓        |
| D:\METABOLOMICS\MSDIAL\Metabolomics | IMET3_pos_4610 | Sample | 1        | 1     | 3                | 2                  | ✓        |
| D:\METABOLOMICS\MSDIAL\Metabolomics | IMET4_pos_4622 | Sample | 1        | 1     | 4                | 2                  | ✓        |
| D:\METABOLOMICS\MSDIAL\Metabolomics | IMET5_pos_4627 | Sample | 1        | 1     | 5                | 2                  | ✓        |
| D:\METABOLOMICS\MSDIAL\Metabolomics | TC7S_pos_4600  | Sample | 0        | 1     | 6                | 2                  | ✓        |
| D:\METABOLOMICS\MSDIAL\Metabolomics | TC7S_pos_4615  | Sample | 0        | 1     | 7                | 2                  | ✓        |
| D:\METABOLOMICS\MSDIAL\Metabolomics | TC7S_pos_4612  | Sample | 0        | 1     | 8                | 2                  | ✓        |
| D:\METABOLOMICS\MSDIAL\Metabolomics | TC7S_pos_4625  | Sample | 0        | 1     | 9                | 2                  | ✓        |
| D:\METABOLOMICS\MSDIAL\Metabolomics | TC7T_pos_4638  | Sample | 0        | 1     | 10               | 2                  | ✓        |





Alignment Table

Num of rows: 101 Metabolite Name Filter Comment Filter 60.06 Mz Range 999.63 0.5 RT Range 8.0

| ID  | RT(min) | m/z      | Type                | Fill % | Metabolite name               | Comment | Correlation | S/N     | ANOVA P-value | Fold change (Max/Min) | BarChart |
|-----|---------|----------|---------------------|--------|-------------------------------|---------|-------------|---------|---------------|-----------------------|----------|
| 634 | 1.71    | 159.1114 | [M+Na] <sup>+</sup> | 0.57   | myrcene                       |         | 0.96        | 18624.0 | 1.05E-08      | 2.86                  |          |
| 696 | 4.53    | 162.1106 | [M+H] <sup>+</sup>  | 1.00   | L-CARNITINE                   |         | 1.00        | 47065.8 | 1.04E-06      | 1.51                  |          |
| 691 | 2.98    | 166.0840 | [M+H] <sup>+</sup>  | 0.14   | L-Phenylalanine; CE0; COLNVLD |         | 0.51        | 4165.5  | 2.96E-01      | 1.21                  |          |
| 692 | 3.02    | 166.0843 | [M+H] <sup>+</sup>  | 0.86   | L-Phenylalanine; CE0; COLNVLD |         | 0.62        | 5384.4  | 6.79E-02      | 1.22                  |          |
| 725 | 5.05    | 170.0901 | [M+H] <sup>+</sup>  | 1.00   | N(PAI)-METHYL-L-HISTIDINE     |         | 0.74        | 8548.5  | 2.48E-03      | 1.90                  |          |
| 726 | 7.86    | 170.0902 | [M+H] <sup>+</sup>  | 0.07   | N(PAI)-METHYL-L-HISTIDINE     |         | 0.47        | 3178.4  | 6.20E-02      | 2.49                  |          |
| 727 | 7.70    | 170.0903 | [M+H] <sup>+</sup>  | 0.43   | N(PAI)-METHYL-L-HISTIDINE     |         | 0.71        | 11860.7 | 4.42E-03      | 5.14                  |          |

Compound search

A:Confidence B:Unsettled C:Unknown Cancel Selected library ID: 5030

RT tol. [min]: 0.5 MS1 tol. [Da]: 0.01 MS2 tol. [Da]: 0.02 Search

File information

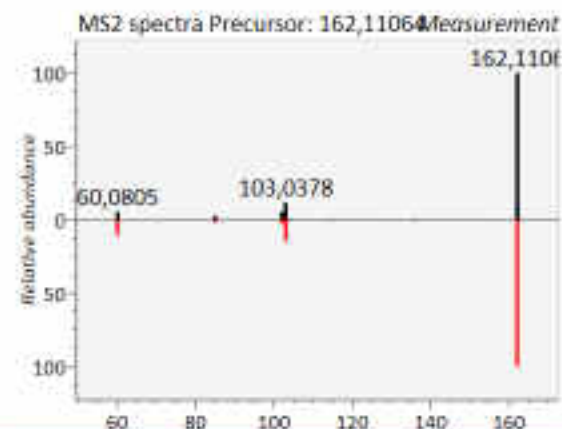
| File ID | File name          | RT[min] | Accurate mass | Molecular species  | Identified  |
|---------|--------------------|---------|---------------|--------------------|-------------|
| 13      | 7CT7_TEST_pos_4646 | 4.53235 | 162.1106      | [M+H] <sup>+</sup> | L-CARNITINE |

Library information

| Library ID | Name                     | Adduct             | RT[min] | Pre. m/z | Dot prod. | Rev. prod. | Presence | Instrument | Comment               |
|------------|--------------------------|--------------------|---------|----------|-----------|------------|----------|------------|-----------------------|
| 5030       | L-CARNITINE              | [M+H] <sup>+</sup> | -1      | 162.11   | 0.93      | 0.93       | 1.00     |            | DB#=CCMSLIB00000577   |
| 827        | L-CARNITINE              | [M+H] <sup>+</sup> | 3.9069  | 162.1121 | 0.93      | 0.93       | 1.00     | LC-ESI-QFT | DB#=EMBL-MCF_spec11   |
| 825        | L-CARNITINE              | [M+H] <sup>+</sup> | 3.7293  | 162.1121 | 0.93      | 0.93       | 1.00     | LC-ESI-QFT | DB#=EMBL-MCF_spec11   |
| 1405       | Carnitine                | [M+H] <sup>+</sup> | 7.6705  | 162.1122 | 0.92      | 0.92       | 1.00     | LC-ESI-QFT | DB#=FiehnHILIC000242  |
| 17821      | L-CARNITINE              | [M+H] <sup>+</sup> | -1      | 162.112  | 0.91      | 0.91       | 1.00     |            | DB#=CCMSLIB00005464   |
| 34473      | L-Carnitine              | [M+H] <sup>+</sup> | -1      | 162.113  | 0.90      | 0.90       | 1.00     | LC-ESI-QTO | DB#=PR100159; origin= |
| 64022      | L-Carnitine hydrochlorid | [M+H] <sup>+</sup> | -1      | 162.113  | 0.90      | 0.90       | 1.00     | LC-Q-TOF/  | DB#=PT102690; origin= |
| 34629      | L-Carnitine              | [M+H] <sup>+</sup> | -1      | 162.113  | 0.89      | 0.89       | 1.00     | LC-ESI-QTO | DB#=PR100320; origin= |
| 64179      | L-Carnitine              | [M+H] <sup>+</sup> | -1      | 162.113  | 0.89      | 0.89       | 1.00     | LC-Q-TOF/  | DB#=PT107170; origin= |

Search results:

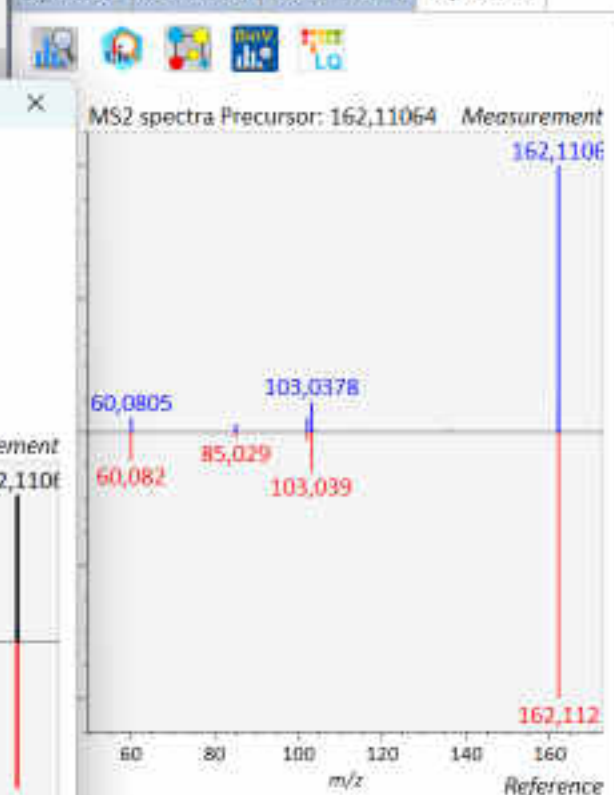
Name: L-CARNITINE  
 RT similarity score: -1000  
 Accurate mass similarity score: 997,9485  
 Dot product similarity score: 933,564  
 Reverse dot product similarity score: 933,5639  
 Total score: 962,4072



Basic peak property Compound detail Structure

Annotation: L-CARNITINE  
 RT(min): 4.531  
 Adduct type: [M+H]<sup>+</sup>  
 m/z: 162.11064(ref=162.111|diff(mDa)=0.64)  
 Peak height(area): 2355137 (height average in samples)  
 Formula(Ontology): C7H15NO3|Carnitines  
 InChIKey: PHIQHXFZVPYU-LURITMIESA-N  
 Comment:

Exp. vs. Ref. MS2 Chrom. Raw vs. Purified Rep. vs. Ref.



# 7) BIOLOGICAL PATHWAYS



**MetaboAnalyst 5.0** - user-friendly, streamlined metabolomics data analysis

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## Module Overview

| Input Data Type                                | Available Modules ( <b>click on</b> a module to proceed, or scroll down for more details) |                                          |                          |                           |                  |                 |
|------------------------------------------------|-------------------------------------------------------------------------------------------|------------------------------------------|--------------------------|---------------------------|------------------|-----------------|
| Raw Spectra<br>(mzML, mzXML or mzData)         |                                                                                           |                                          | LC-MS Spectra Processing |                           |                  |                 |
| MS Peaks<br>(peak list or intensity table)     |                                                                                           |                                          | Functional Analysis      | Functional Meta-analysis  |                  |                 |
| Annotated Features<br>(compound list or table) |                                                                                           | Enrichment Analysis                      | Pathway Analysis         | Joint-Pathway Analysis    | Network Analysis |                 |
| Generic Format<br>(.csv or .txt table files)   | Statistical Analysis<br>[one factor]                                                      | Statistical Analysis<br>[metadata table] | Biomarker Analysis       | Statistical Meta-analysis | Power Analysis   | Other Utilities |

# METABOANALYST

Please upload your data use one of the options below

[Compound List](#)

[Concentration Table](#)

[Metabolomics Workbench Data](#)

Please enter a one-column compound list:

Choline  
Hypoxanthine  
Inosine  
Dopamine Hydrochloride  
L-CARNITINE  
N-Acetylhistidine  
L-Methionine  
Proline  
Nicotinamide  
Isoleucine  
Valine  
Homoproline  
Butyrylcarnitine  
Betaine  
Acetylcarnitine  
L-Phenylalanine  
Acetylcarnitine  
Targinine  
N,N-Dimethylarginine  
Nicotinamide

Input Type:

Compound Name

☐

Use our example data

Submit

Select a pathway library

X *Danio rerio* (zebrafish) (KEGG)

Click the corresponding **Pathway Name** to view its graphical presentation; click **Match Status** to view the pathway compounds (with matched ones highlighted).

Pathway Name

[Aminoadyl-tRNA biosynthesis](#)

[Valine, leucine and isoleucine biosynthesis](#)

[Lysine degradation](#)

[Phenylalanine, tyrosine and tryptophan biosynthesis](#)

[Glycine, serine and threonine metabolism](#)

[Arginine and proline metabolism](#)

[Valine, leucine and isoleucine degradation](#)

[Taurine and hypotaurine metabolism](#)

[Phenylalanine metabolism](#)

[Arginine biosynthesis](#)

[Nicotinate and nicotinamide metabolism](#)

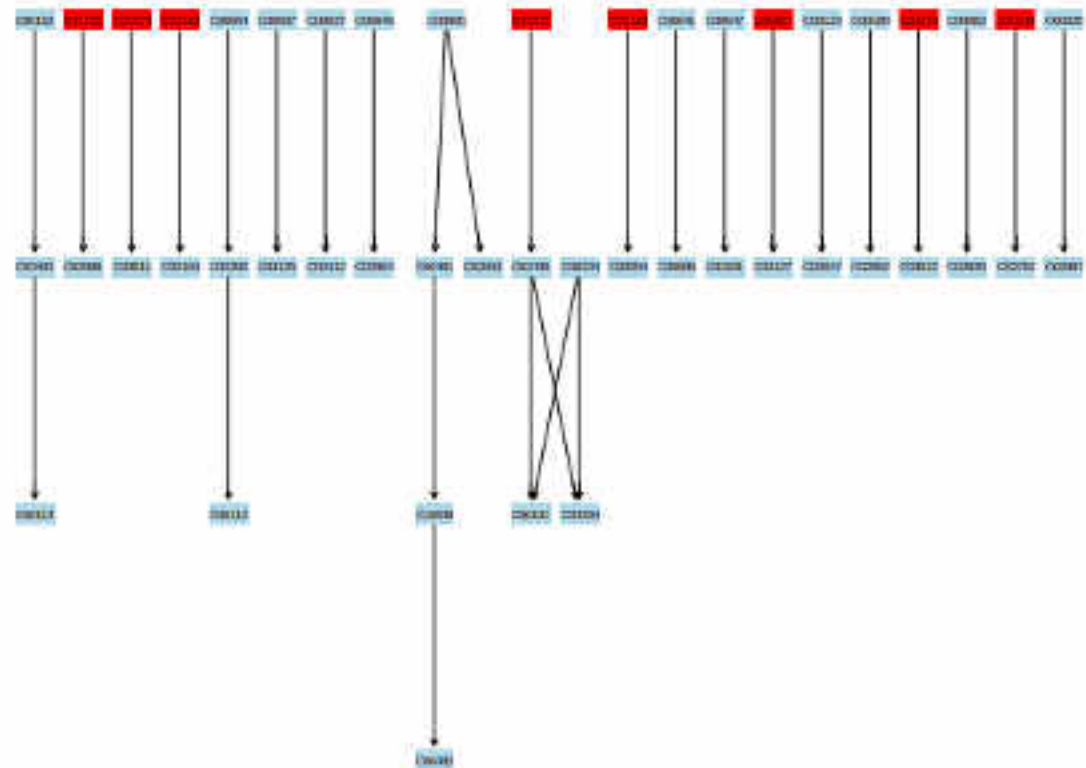
[Histidine metabolism](#)

[Purine metabolism](#)

[beta-Alanine metabolism](#)

[Pantothenate and CoA biosynthesis](#)

[Cysteine and methionine metabolism](#)



# SIGNIFICANT IDENTIFIED METABOLITES

## MS-DIAL analysis

| MSDIAL results         | 1 MET      |      | 2 MET      |      | 3 ORG      |      | 4 ORG      |      | 5 MIX      |      | 6 MIX      |      |
|------------------------|------------|------|------------|------|------------|------|------------|------|------------|------|------------|------|
|                        | Pos        | Neg  | Pos        | Neg  | Pos        | Neg  | Pos        | Neg  | Pos        | Neg  | Pos        | Neg  |
| Significant DOWN       | 54         | 20   | 33         | 8    | 46         | 22   | 57         | 19   | 43         | 20   | 58         | 18   |
| Significant UP         | 5          | 0    | 1          | 1    | 2          | 0    | 4          | 2    | 3          | 0    | 2          | 0    |
| TOTAL Matched          | 101        | 50   | 98         | 49   | 99         | 55   | 106        | 43   | 118        | 49   | 104        | 49   |
| Rate                   | 0.58       | 0.40 | 0.35       | 0.18 | 0.48       | 0.40 | 0.58       | 0.49 | 0.39       | 0.41 | 0.58       | 0.37 |
| Total POS and NEG      | 79 (52%)   |      | 43 (29%)   |      | 70 (45%)   |      | 82 (55%)   |      | 66 (40%)   |      | 78 (51%)   |      |
| Affected pathways (23) | 18 (78.2%) |      | 12 (52.1%) |      | 15 (65.2%) |      | 14 (60.8%) |      | 14 (69.8%) |      | 16 (69.5%) |      |

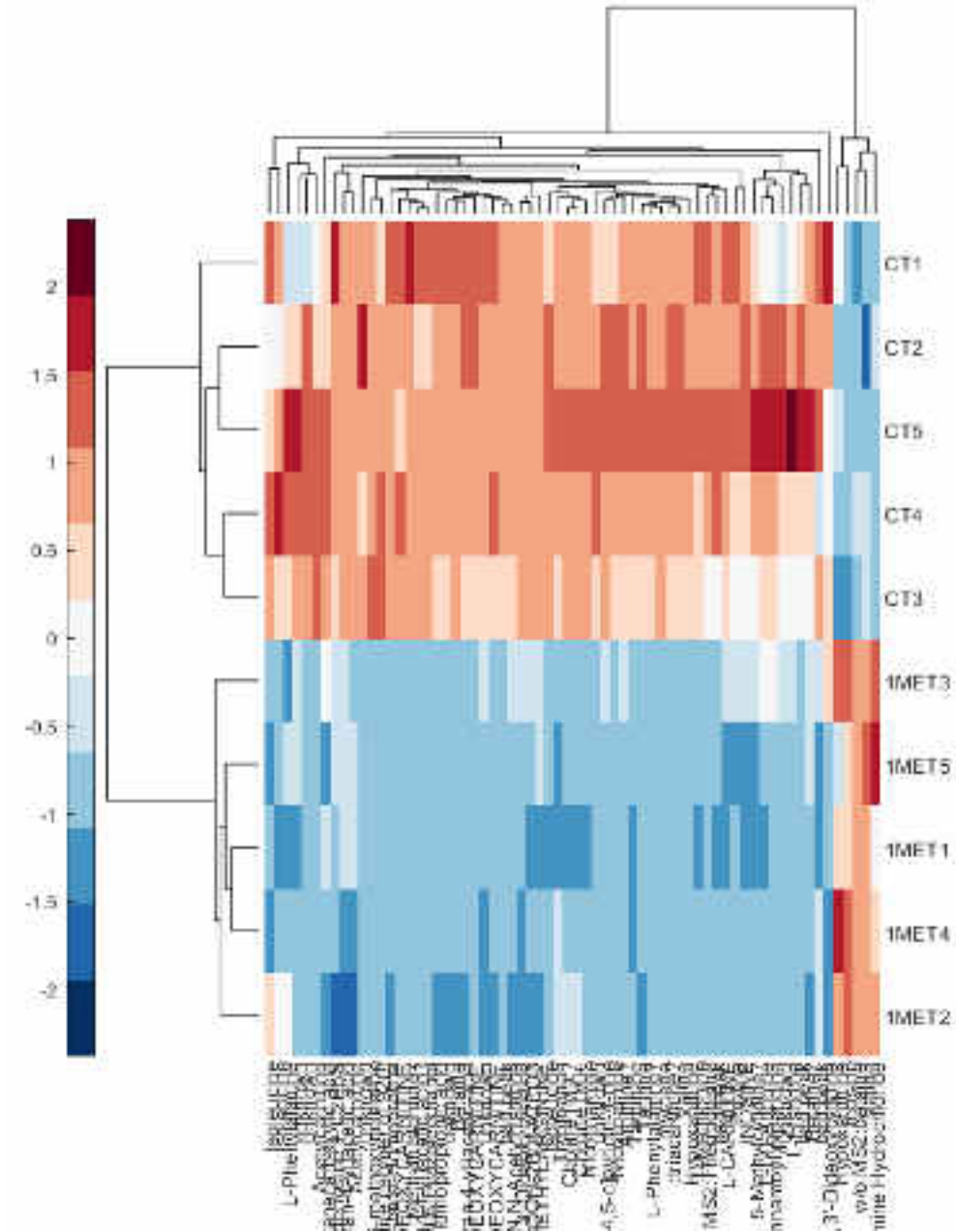
## ROIMCR analysis

| ROIMCR results         | 1 MET       |      | 2 MET      |      | 3 ORG      |      | 4 ORG      |      | 5 MIX      |      | 6 MIX      |      |
|------------------------|-------------|------|------------|------|------------|------|------------|------|------------|------|------------|------|
|                        | Pos         | Neg  | Pos        | Neg  | Pos        | Neg  | Pos        | Neg  | Pos        | Neg  | Pos        | Neg  |
| Significant DOWN       | 63          | 61   | 68         | 48   | 50         | 26   | 50         | 31   | 36         | 24   | 40         | 61   |
| Significant UP         | 3           | 6    | 2          | 4    | 2          | 1    | 3          | 4    | 1          | 2    | 3          | 4    |
| TOTAL                  | 0.66        | 0.67 | 0.7        | 0.52 | 0.52       | 0.27 | 0.53       | 0.35 | 0.37       | 0.26 | 0.43       | 0.65 |
| Total POS and NEG      | 133 (66.6%) |      | 122 (61%)  |      | 79 (39.5%) |      | 88 (44%)   |      | 63 (31.5%) |      | 108 (54%)  |      |
| Affected pathways (23) | 13 (56.5%)  |      | 12 (52.1%) |      | 7 (30.4%)  |      | 15 (65.2%) |      | 11 (47.8%) |      | 18 (78.2%) |      |

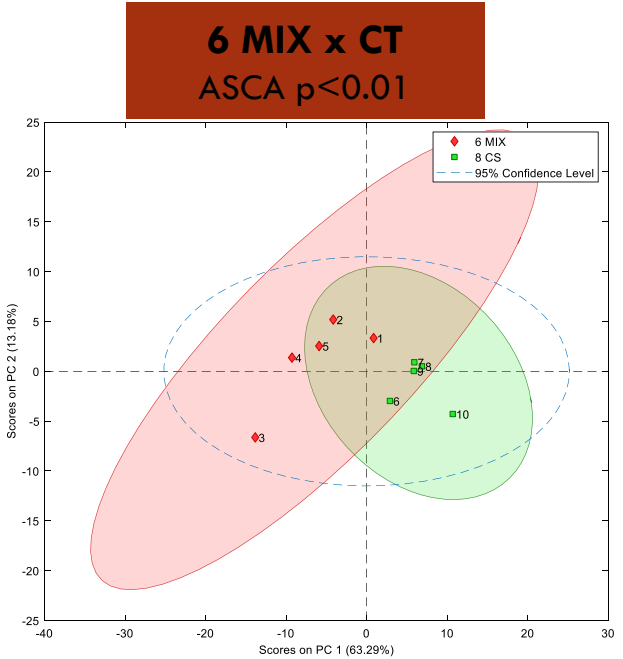
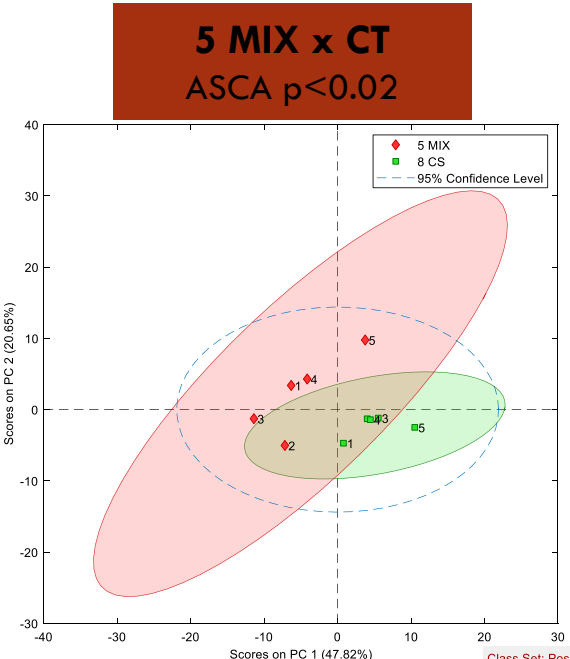
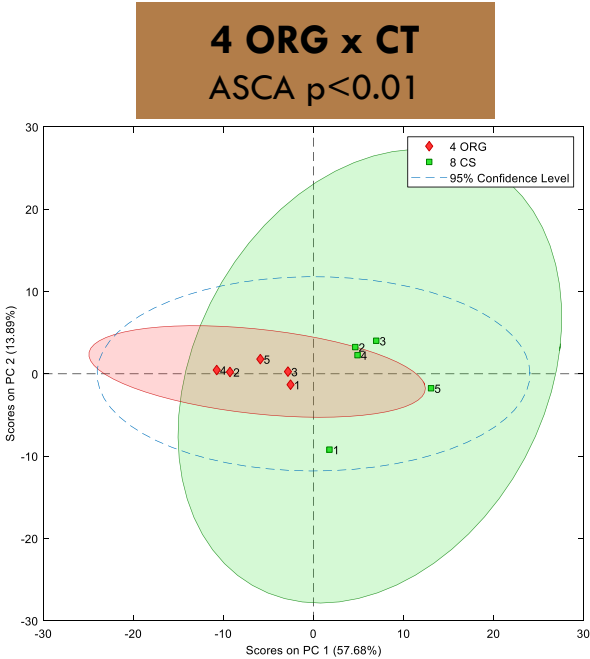
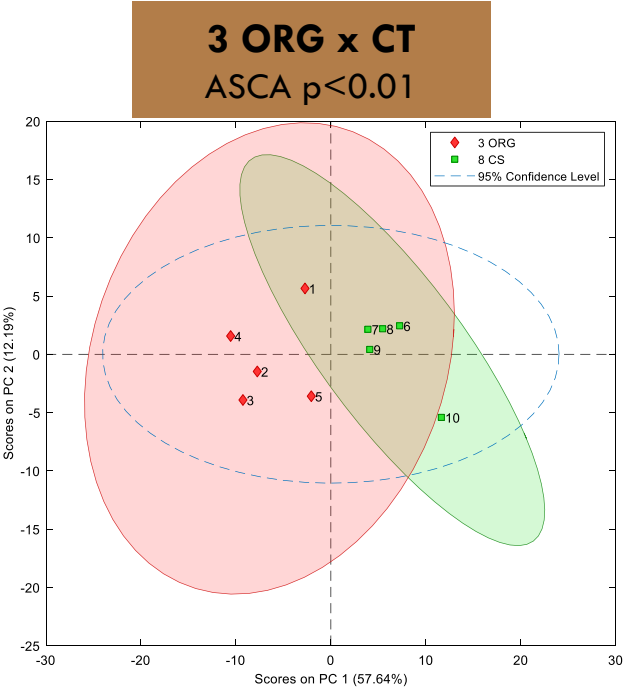
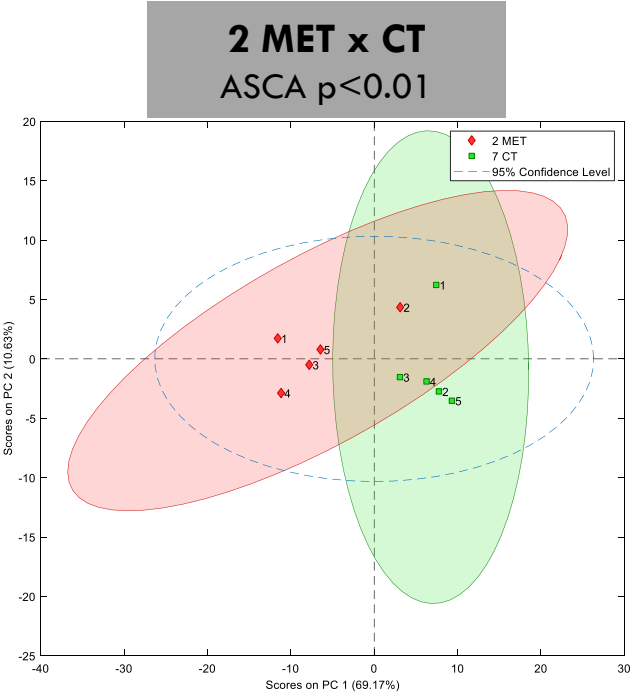
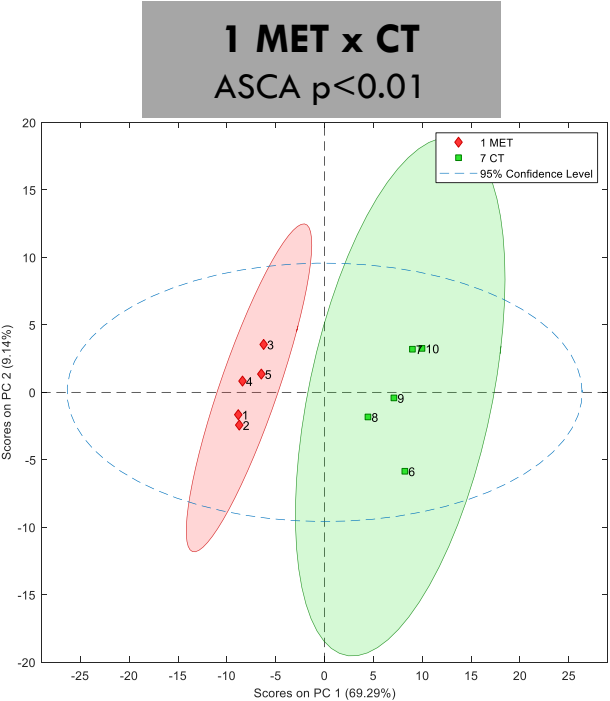


# 1 MET X CT

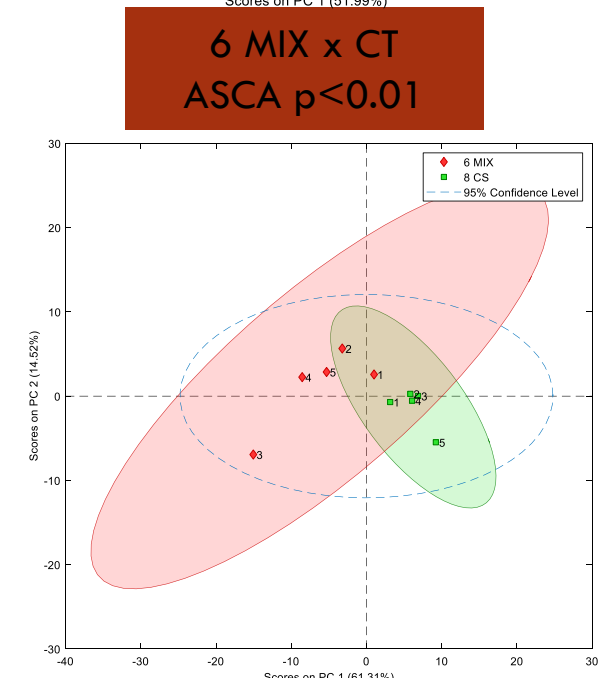
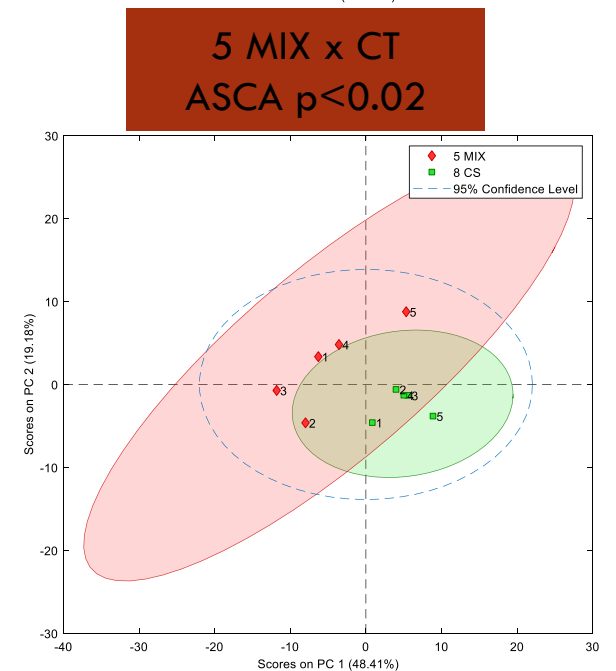
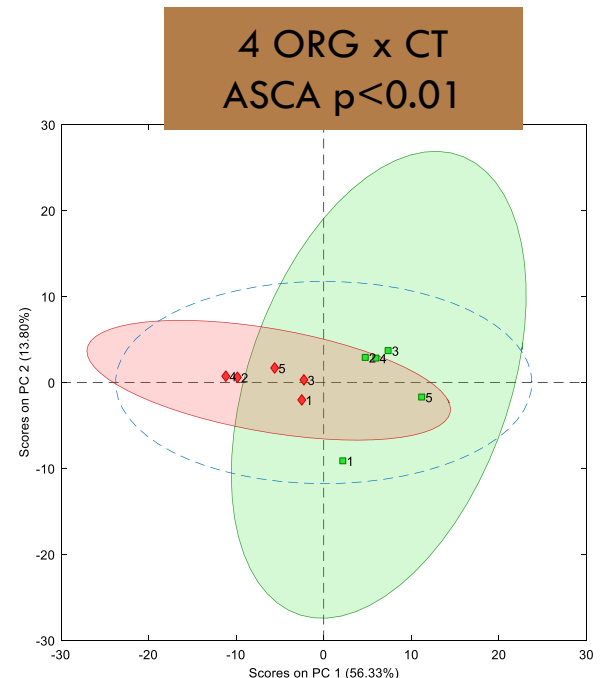
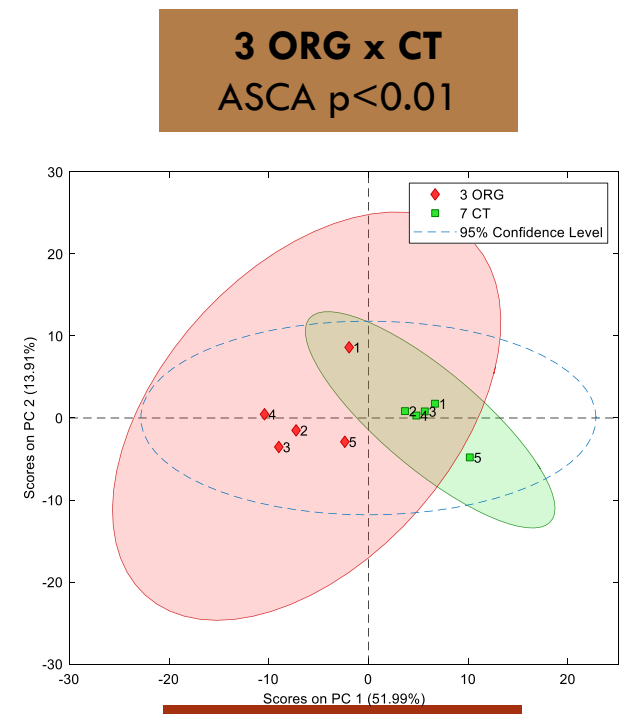
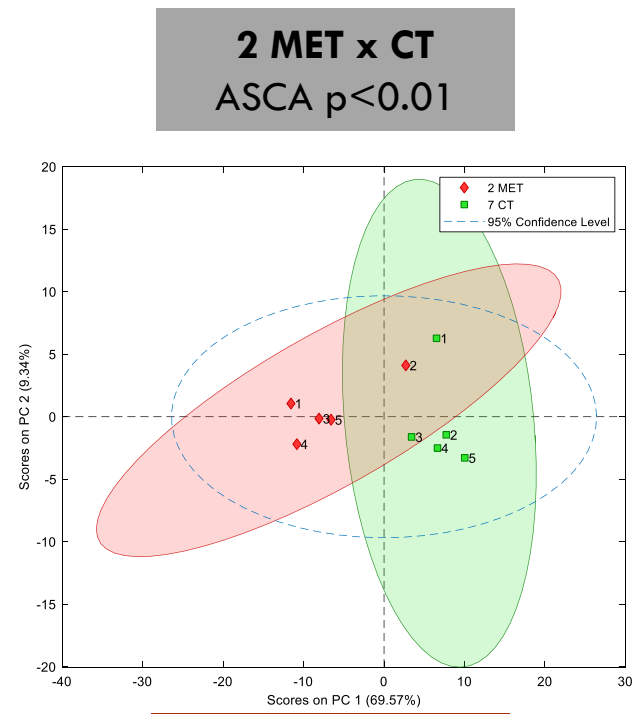
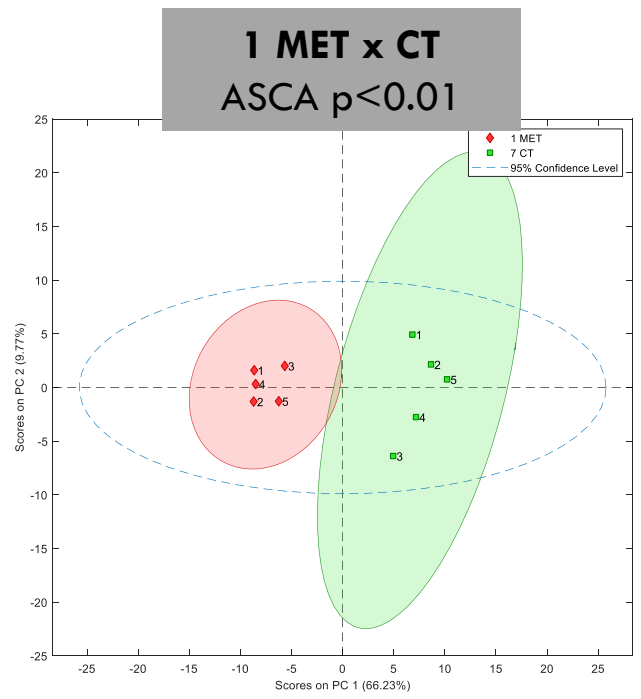
| Metabolites                                                                                                                                | Pathways                                                  | p value  | FDR      |
|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|----------|----------|
| <b>Aminoacids</b> ( <i>Histidine; Phenylalanine; Arginine; Methionine; Valine; Isoleucine; Tryptophan; Proline; Glutamine; Glutamate</i> ) | <u><b>Aminoacyl-tRNA biosynthesis</b></u>                 | 1.06E-11 | 8.97E-10 |
| (N-Acetyl-L-aspartate; L-Aspartate; L-Glutamate; L-Glutamine; Succinate)                                                                   | <u><b>Alanine, aspartate and glutamate metabolism</b></u> | 8.43E-6  | 3.54E-4  |
| Taurine (antioxidant); Hypotaurine                                                                                                         | <u><b>Taurine and hypotaurine metabolism</b></u>          | 0.0026   | 0.0320   |



# Positive AREA Scores

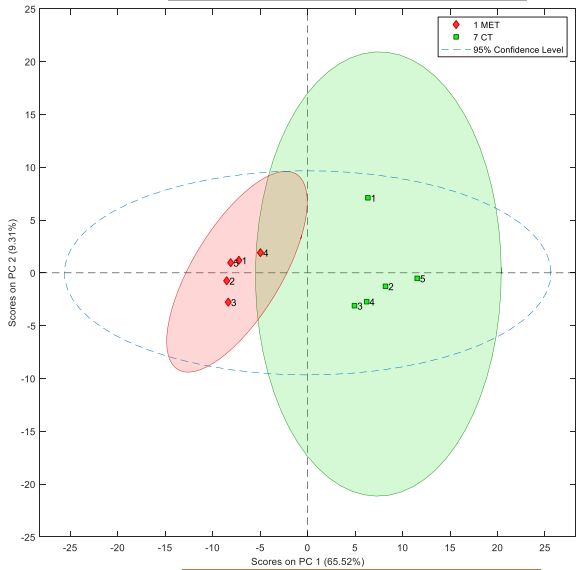


Positive HEIGHT  
scores

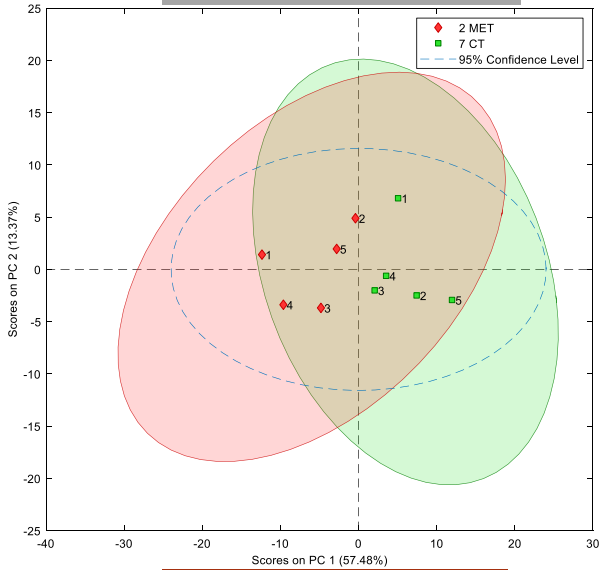


# Negative HEIGHT scores

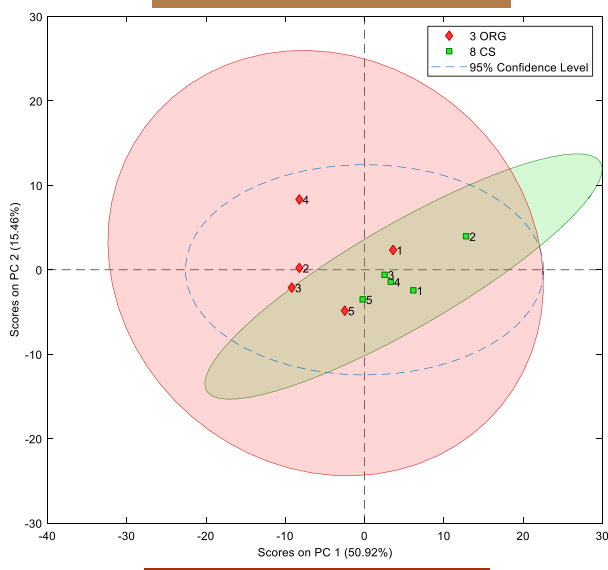
**1 MET x CT**  
ASCA p<0.01



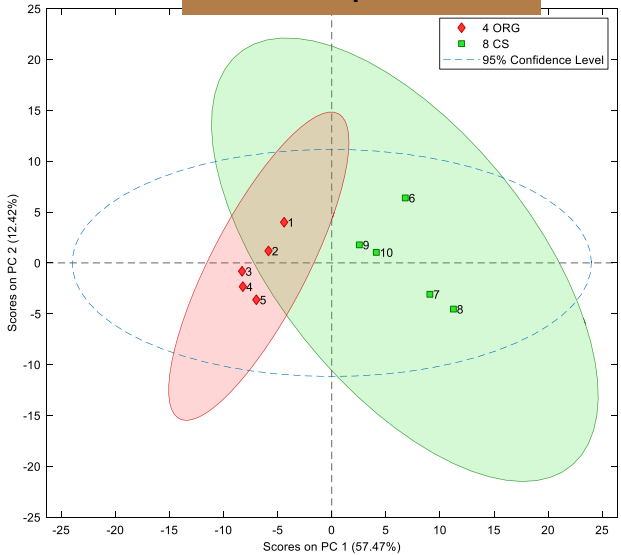
**2 MET x CT**  
ASCA p<0.01



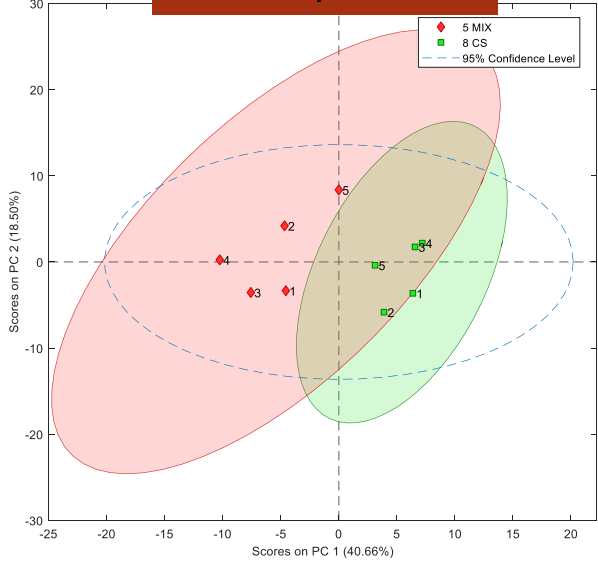
**3 ORG x CT**  
ASCA p<0.02



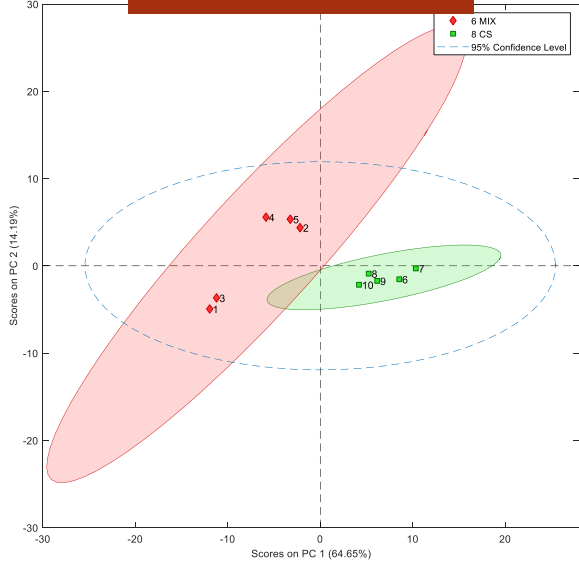
**4 ORG x CT**  
ASCA p<0.01



**5 MIX x CT**  
ASCA p<0.01



**6 MIX x CT**  
ASCA p<0.01

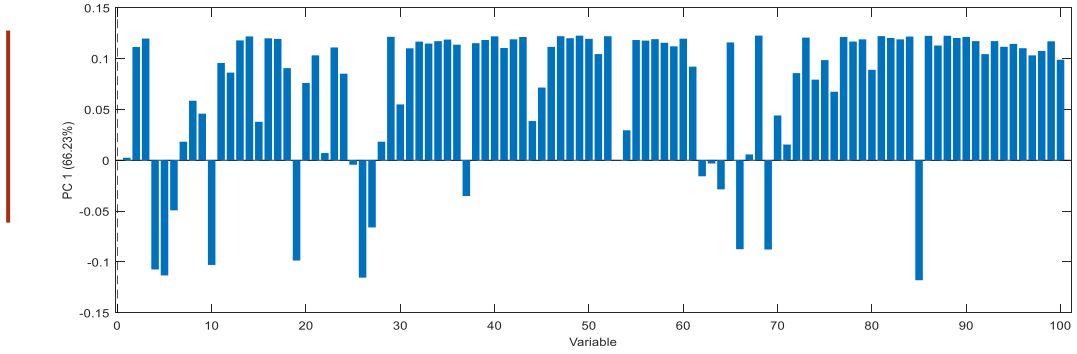




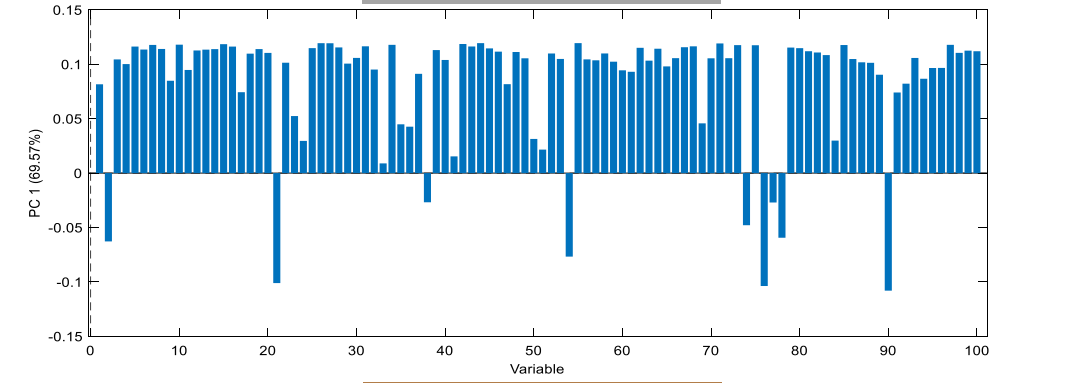
# Positive height Loadings

↑Positive loadings – ↑ levels in Control groups

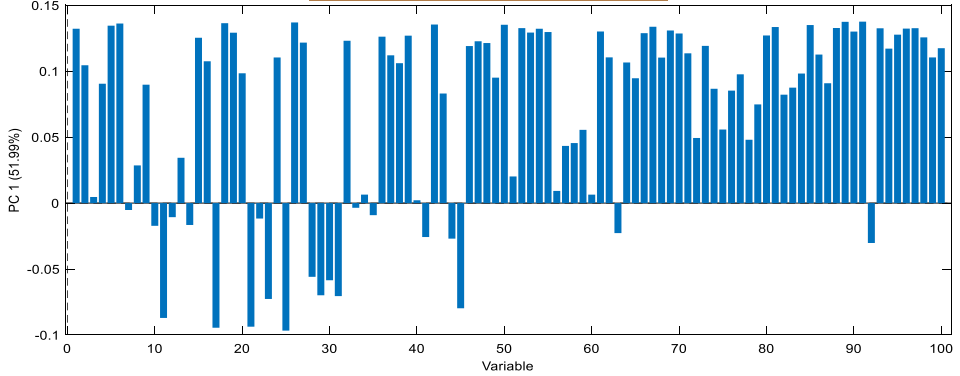
1 MET x CT



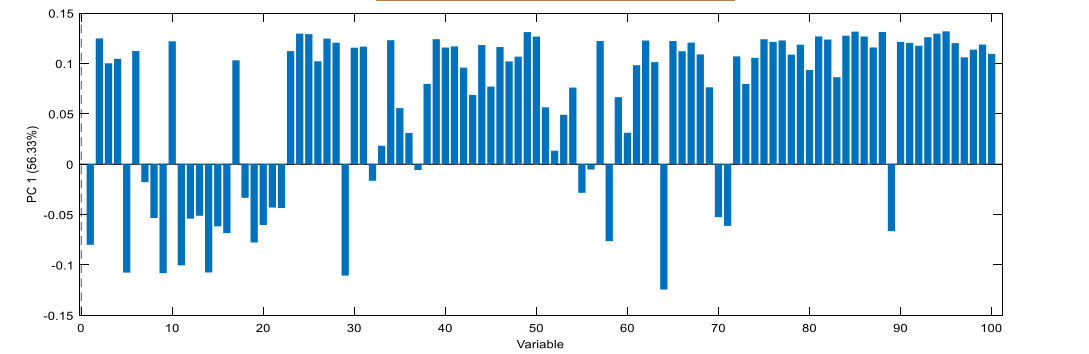
2 MET x CT



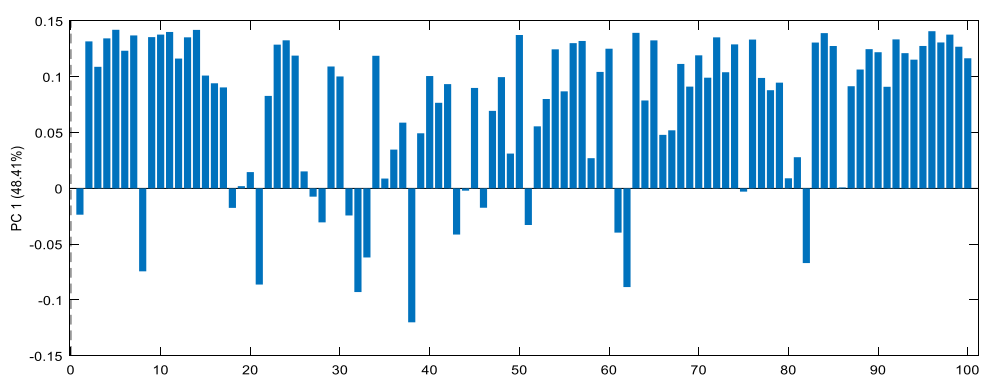
3 ORG x CT



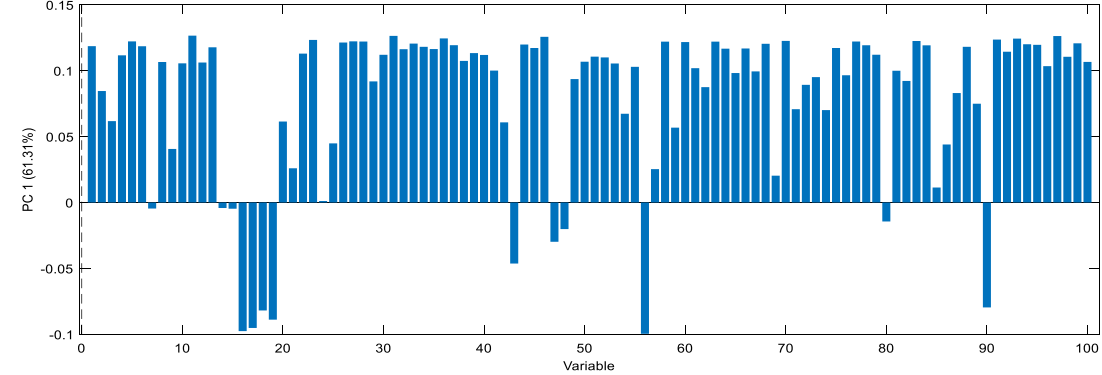
4 ORG x CT



5 MIX x CT



6 MIX x CT



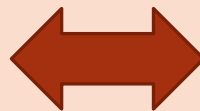
# CONCLUSIONS

- **All treatments showed to significantly reduce many essential metabolites (aminoacids, nucleosides) when compared to controls**
- **Metal exposure** (1 MET) triggered the most significant effects when compared to the control group.
- **Organic mixtures** also induced significant effects in fish embryos.
- The **mixture of additional contaminants** to the metals from 1 MET, may have reduced their bioavailability, and thus causing lower toxicity.
- **Complex mixtures of chemicals induced distinct patterns of responses** that should be further investigated.

# NEXT STEPS

- Look for **potential biomarkers** for each exposure condition
- Interpret the **relationships** of the affected **pathways** with the **chemicals** exposure
- **Concluding** the analysis of the **standards mixtures**
- **Target analysis** of the metabolites in the samples
- Perform **Lipidomics** analysis
- **Integrate metabolomics + lipidomics + chemical datasets** to provide a better interpretation of the potential effects of the pollutants from the Doce River

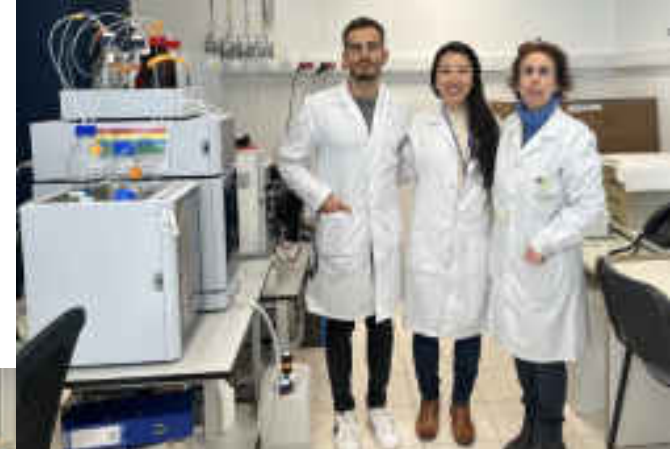
Biological  
data



Chemical  
data



# ACKNOWLEDGEMENTS



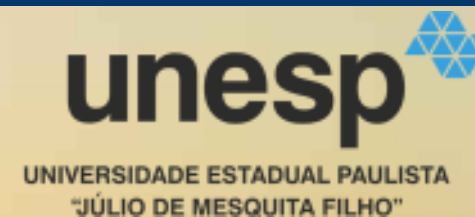


## ACKNOWLEDGEMENT





# OBRIGADA!



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