Effects of dietary mercury on proteome and metabolome in *Dreissena polymorpha*, a sentinel of our aquatic environment

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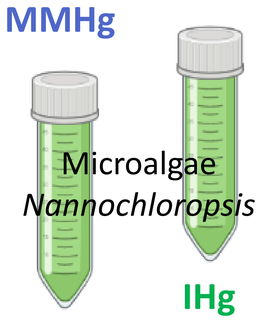
Context

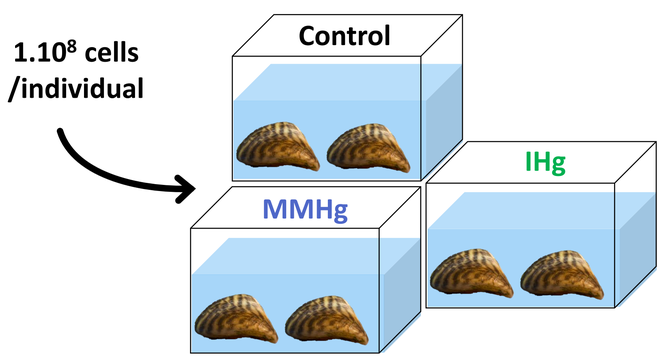
- Bioaccumulation of **methylmercury** (**MMHg**) in the food chain is a recognized health risk but is understudied compared to waterborne **inorganic mercury** (**IHg**)
- Bivalves are at the basis of the food webs
- *D.polymorpha* has great filtration capacities (5 to 400 ml/bivalve/h)
- OMICS gives a global vision of the metabolism and cellular homeostasis

Objectives

- Evaluate the bioaccumulation of dietary **IHg** and **MMHg** in *D.polymorpha*
- Identify and compare molecular toxicity pathways of **IHg** and **MMHg** by targeted approaches at the level of genes, antioxidant proteins and by non-targeted high throughput approaches using metabolomic and proteomic

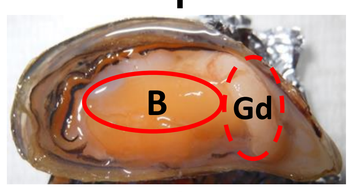
Materials and methods





24h of exposition

Sampling gills (B) and digestive gland (Gd)



Proteomic (nanoLC-MS/MS)

Metabolomic (HPLC-MS)

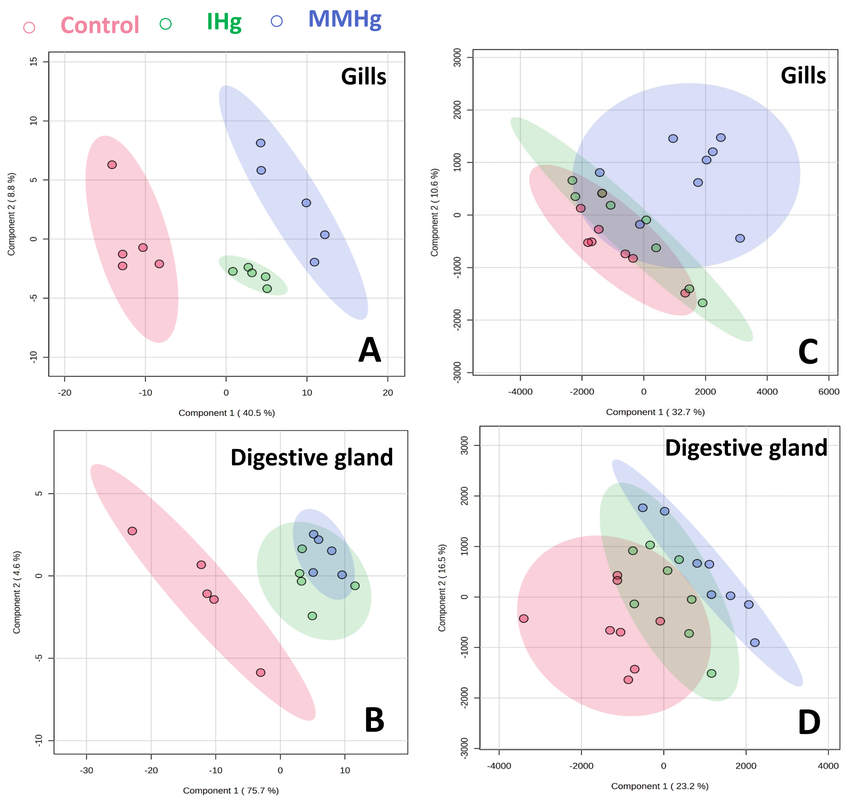
Relative gene expression (RT-qPCR)

Bioaccumulation

Activity of antioxydant proteins and abundance of lipid peroxydation (Spectrophotometry and polarography)

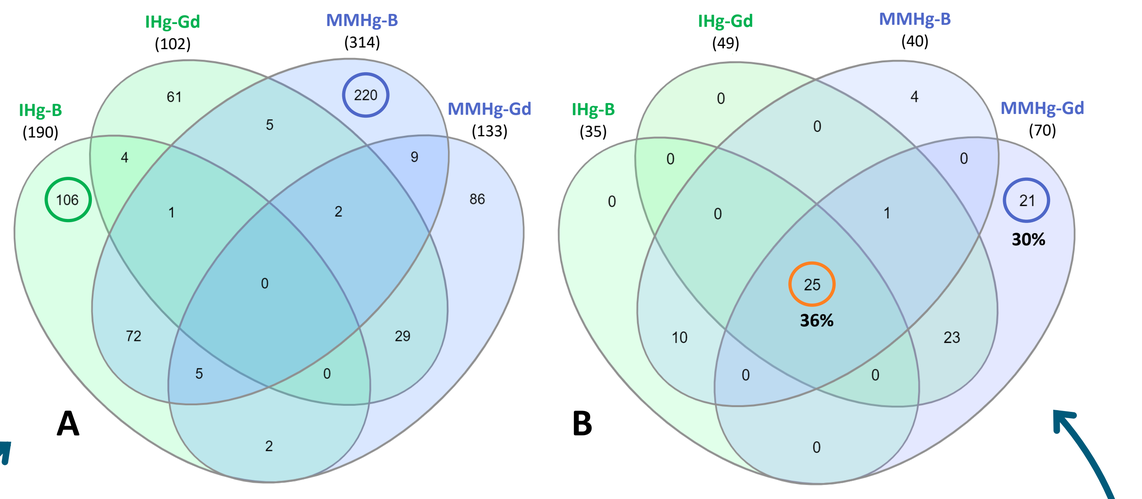
Fg = femtogram (10^{-15} g)

Proteomic & metabolomic



PLS DA of spectral count for proteins (A,B) and peptide mass (C,D) modulated by at least one exposure vs control in gills and the digestive gland (n=5 to 8)

→ Specific protein modulations are observed for **IHg** and **MMHg** vs control (A,C)
→ The response of metabolites is less marked than at the proteome level, and is more discriminating for **MMHg**



*Venn diagram of modulated proteins (A) and metabolites (B) by **IHg** et **MMHg** (p<0,05 et FC>1,5)*

→ **MMHg** modulates twice as much protein as **IHg** in the gills
→ **MMHg** modulate **30%** of the metabolites in the digestive gland and **36%** are modulated both by **IHg** and **MMHg**

Metabolic pathways affected by dietary Hg analysed by Webgestalt (KEGG)

	Gills	Digestive gland
IHg	- Molecular signaling pathways - Energy metabolism	- Molecular signaling pathways - Metabolism of sugars - Metabolism of amino acids
MMHg	- Metabolism of glycerolipids	- DNA repair mechanism - RNA transport

→ Molecular toxicity pathways affected by **IHg** and **MMHg** are different and differ in organs

Bioaccumulation

THg bioaccumulation in D.polymorpha in µg THg/g dw, percentage of MMHg and bioaccumulation factor (BAF) (n=8)

	Gills	*BAF*	*Digestive gland*	*BAF*
Control	0.04 ±0.0 (1,9%)		0.04 ±0.0 (0%)	
IHg	**0.73 ±0.4** (3,8%)	*0.36*	1.03 ±0.7 (0%)	*0.51*
MMHg	0.30 ±0.1 (1,1%)	*0.13*	**7.9 ±3.1** (0%)	*3.68*

→ **IHg** is more accumulated in gills (*vs **MMHg***) while **MMHg** is more accumulated in the digestive gland (*vs **IHg***)

Antioxydant responses

Relative gene expression

Relative expression level of antioxydant genes. Significant modulations are in bold (p<0.05 vs control, n=8)

		cat	*gst*	*sod*	*mt*
Gills	**Control**	1.1 ±0.4	1.3 ±0.3	1.1 ±0.5	1.1 ±1.1
	IHg	1.2 ±1.2	**0.3 ±0.3**	1.4 ±1.2	1.5 ±1.2
	MMHg	1.0 ±1.1	1.0 ±0.9	1.1 ±0.7	1.4 ±0.9
Digestive gland	**Control**	1.2 ±0.4	0.9 ±0.5	1.1 ±1.0	1.0 ±0.9
	IHg	1.0 ±0.8	**0.1 ±0.7**	1.0 ±0.7	0.6 ±0.4
	MMHg	1.1 ±0.8	0.4 ±0.4	1.1 ±1.0	0.7 ±0.6

Enzymatic activities and lipid peroxydation

Enzymatic activities of antioxydant proteins and lipid peroxydation. Significant modulations are in bold (p<0.05 vs control, n=8)

		CAT	*GST*	*SOD*	*LOOH*
Gills	**Control**	24.4 ±13.9	82.9 ±29.4	3.1 ±1.0	16.7 ±10.2
	IHg	27.6 ±4.6	**135.2 ±28.4**	3.5 ±1.4	25.9 ±9.0
	MMHg	27.7 ±8.9	**157.6 ±39.8**	4.4 ±2.0	20.3 ±12.7
Digestive gland	**Control**	108.3 ±29.2	235 ±95.8	4.9 ±1.5	59.3 ±26.4
	IHg	84.2 ±15.6	207 ±60.8	4.3 ±1.8	77.2 ±14.9
	MMHg	**38.2 ±10.5**	210.2 ±70.6	4.2 ±1.7	56.2 ±27.0

→ Significant changes in relative **gst** gene expression and enzyme activity is observed, suggesting an increase of ROS production without lipid peroxydation

Conclusion

- **MMHg** caused more specific responses than **IHg** in the proteome and metabolome of *D.polymorpha*
- Bioaccumulation and molecular toxicity pathways of **IHg** and **MMHg** were distinct
- **MMHg** resulted in a higher alteration of metabolome in digestive gland in congruence with bioaccumulation
- OMICS were more sensitive than antioxydant responses

Perspectives

- RNA-seq
- Kinetik up to 96h
- Subcellular distribution and speciation of Hg
- Isotopic Hg exposure to follow bioconversion

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