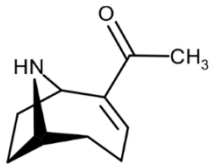
anses



ANATOXIN-A IN SEA FIGS ASSOCIATED WITH HUMAN FOOD POISONINGS IN FRANCE

RONEL BIRÉ

US-EPA WEBINAR – MARCH 22nd 2022

EPA United States Environmental Protection Agency

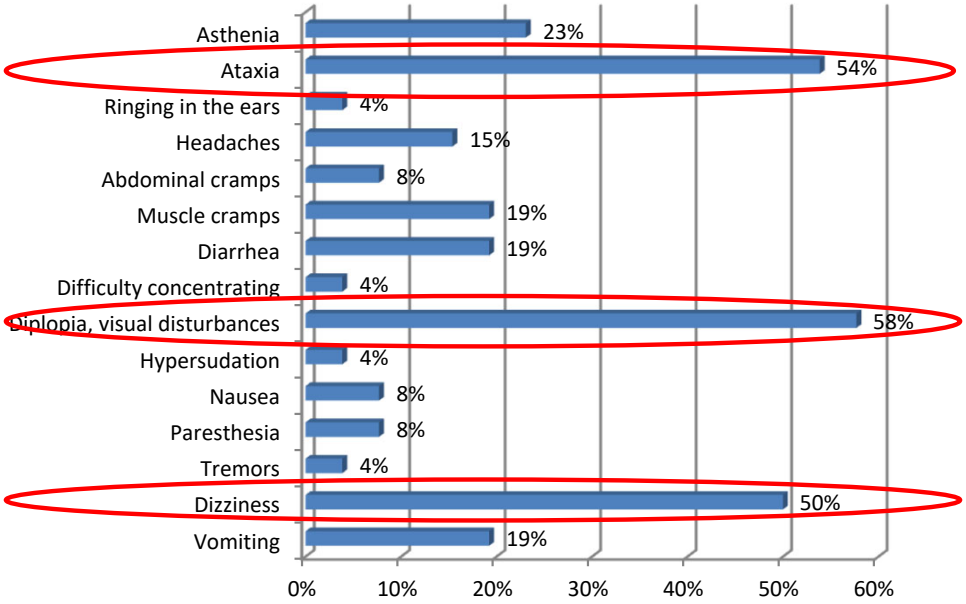
CONNAÎTRE, ÉVALUER, PROTÉGER

Contents

1 — Symptomatology of intoxication related to the consumption of sea figs

Poisoning cases involving sea figs

- Sea figs of the genus Microcosmus, fished in the Mediterranean. Products highly prized for their iodized taste
- 20 poisoning cases between January 2011 and January 2020
- 30 people involved
- 20 women and 10 men
- Aged 17 to 80 years



Schmitt et al. (2019). Cerebellar syndrome associated with ingestion of Mediterranean Microcosmus: a French case series

Sometimes fortuitous nature of the reports!!!

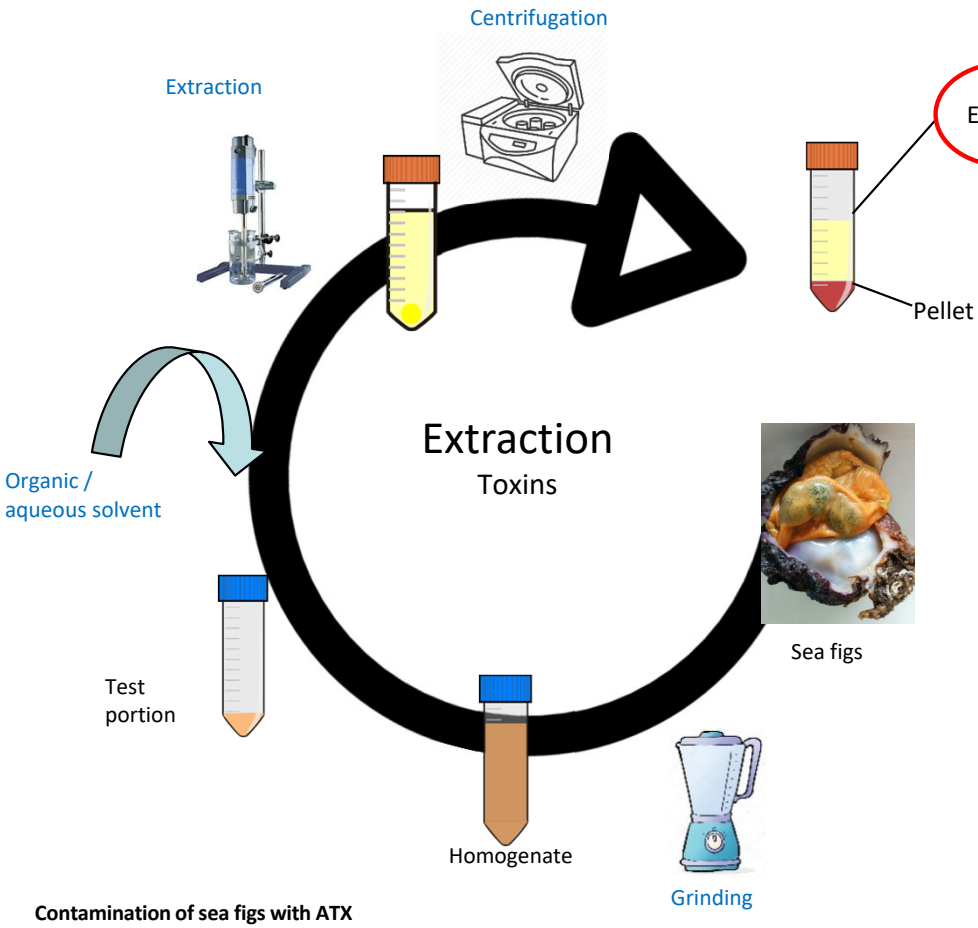
Case of a patient who took an oral medication intended for vaginal administration

Symptoms actually due to consumption of sea figs!!

Capsules are not only for oral use

2 — Preliminary investigations

Protocol implemented



Analysis of regulated toxins

- Lipophilic toxins
- Domoic acid
- Saxitoxins

Not found

- Hemolytic activity

Complementary investigations

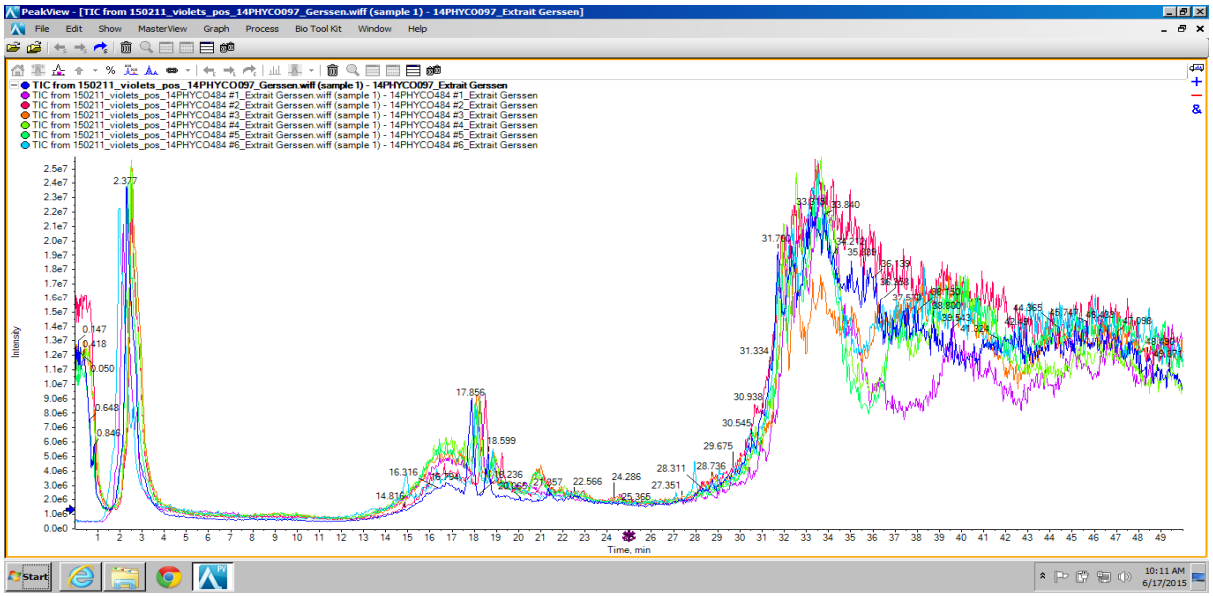
- Non-targeted analysis (high resolution mass spectrometry – LC-HRMS)

Non-targeted analysis - LC-HRMS (I. Dom PhD thesis)

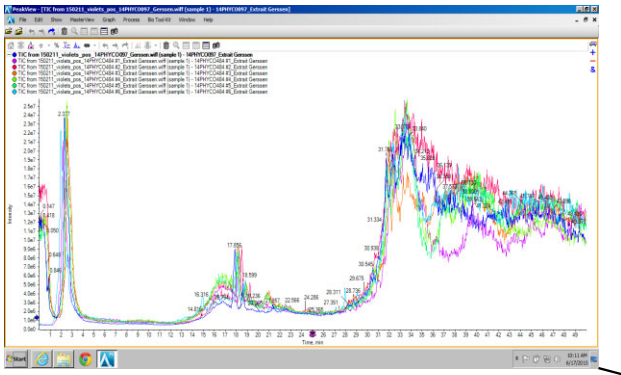
1. **Non discriminating extraction (lipophilic)**
2. **LC-HRMS analysis**
   - **suspect screening (screening of a list of toxins)**
   - **looking for unknown compounds (unbiased analysis)**

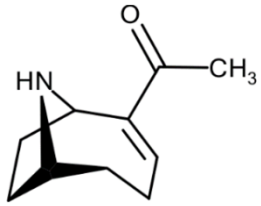
LC-MS/MS high resolution

API 5600 QTof



Non-targeted analysis - LC-HRMS (I. Dom PhD thesis)



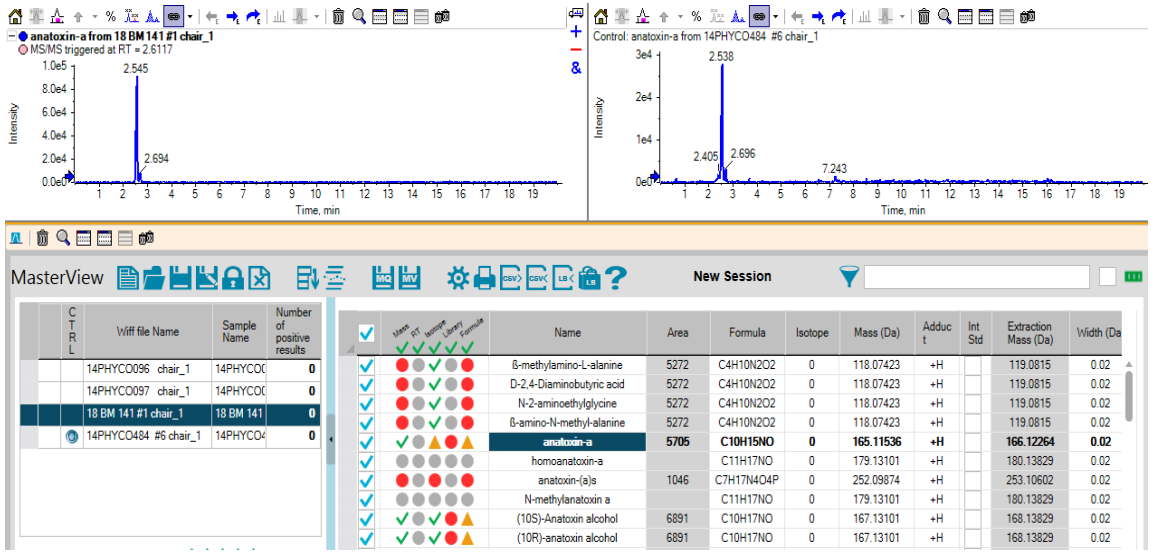


ATX structure

List of 820 toxins (marine and freshwater toxins)

⇨ Suspicion of anatoxin-a (ATX) but

- *Chromatographic method not adapted (co-elution of ATX-a and Phe, isobaric compounds)*
- *ATX found in 1 sample but for 1 replicate only (out of 3)*
- *unexpected result (freshwater cyanotoxin in a marine organism)*

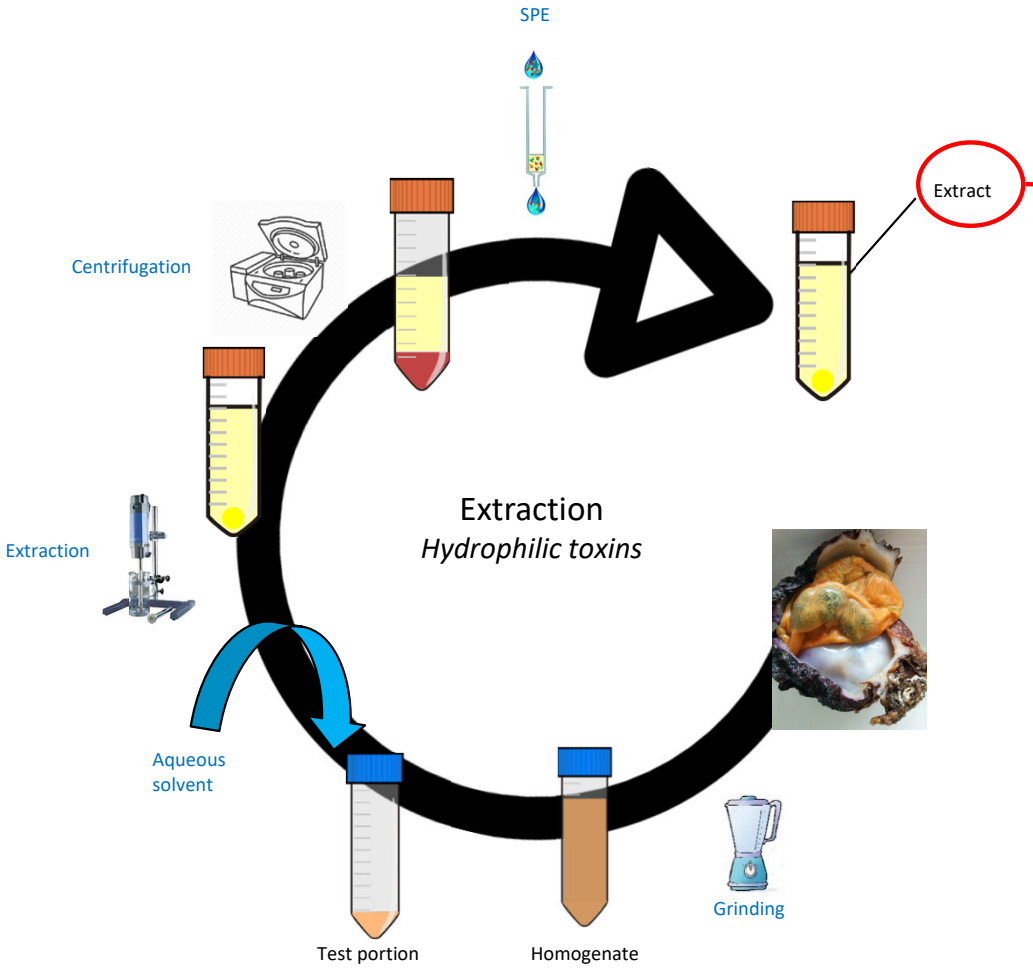


Need for additional analyses

↳ Method adapted for hydrophilic toxins (cyanotoxins) → HILIC

3 — Complementary investigations

Protocol implemented



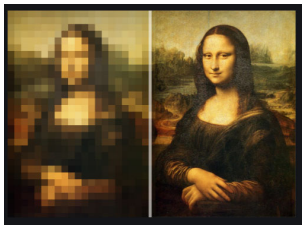
LCMS analyses in low and high resolution (HILIC-MS)

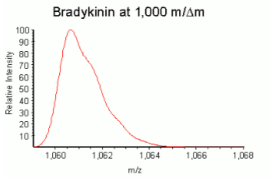
TSQ Vantage
(low resolution)

API 5600 Qtof
(high resolution)

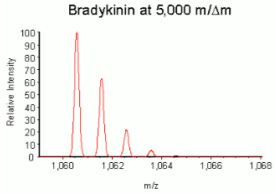
Low (LR) versus high resolution (HR)

LR HR





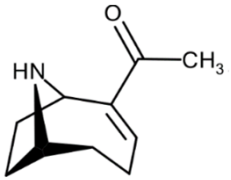
LR



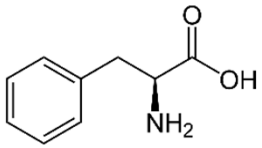
HR

Targeted analyses: HILIC-MS/MS (low resolution)

Analytical method developped by the national reference laboratory for marine biotoxins as part of the monitoring of emerging compounds in France (EmergTox)



ATX: 165.11536 Da

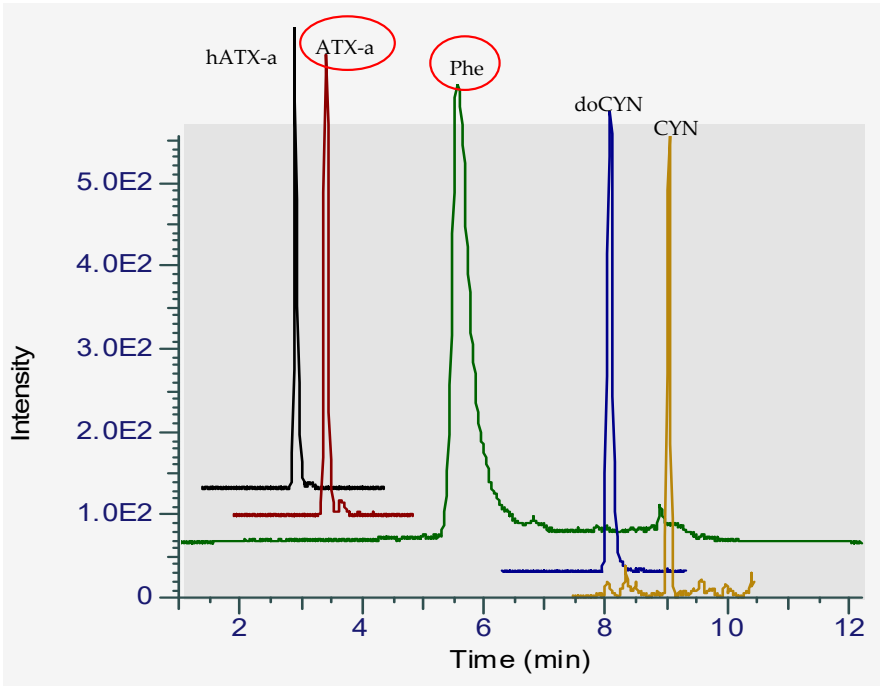


Phenylalanine (Phe) : 165.07898 Da

In low resolution, no mass distinction (165.1 Da)!

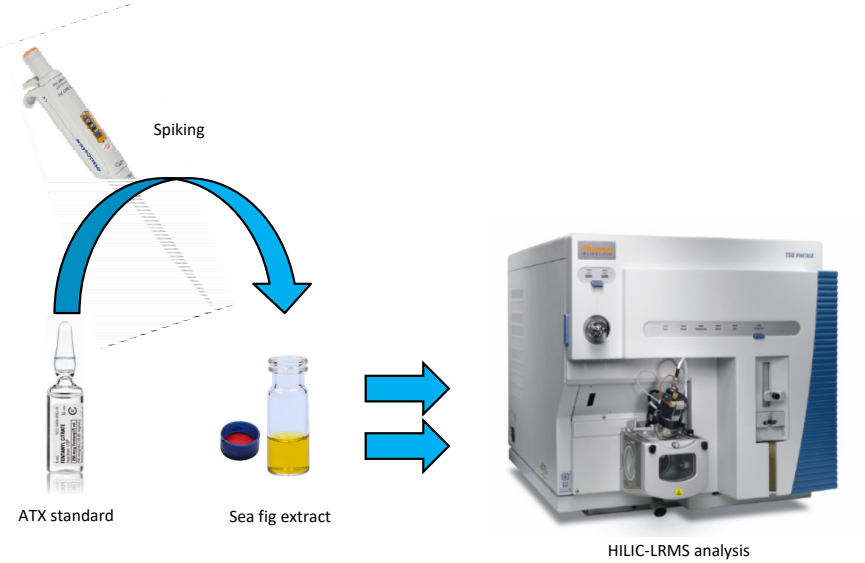
→ *<u>Prerequisite</u>: need for good chromatographic separation ATX / Phe*

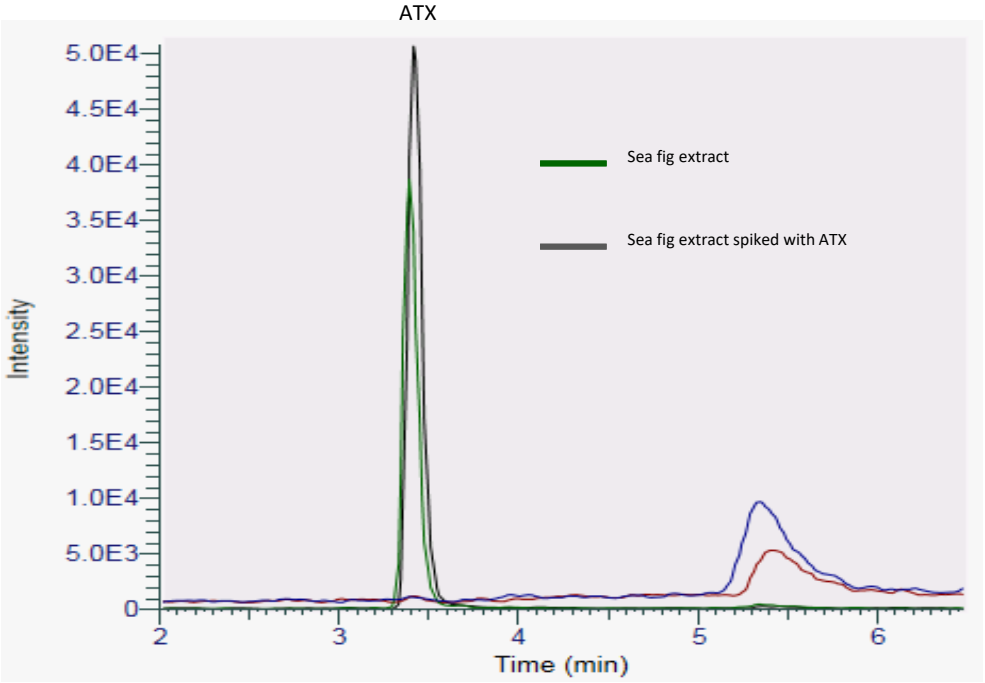
Chromatogram of a mixed solution of toxins after HILIC-LRMS analysis



Targeted analysis - HILIC-MS/MS (low resolution)

Verification of the presence of ATX in the sea fig extracts





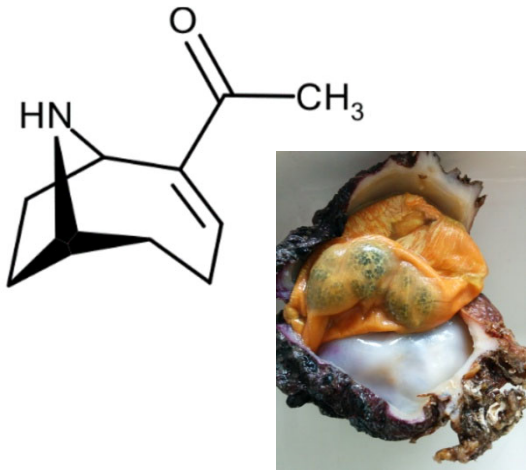
- Presence of a single peak at the retention time of ATX
- Increase in ATX-a peak intensity correlated with the amount of ATX added to the sea fig extract

Targeted analysis - HILIC-MS/MS (low resolution)

ATX concentrations found in the sea fig samples

Samples	ATX concentration (µg/kg)
FP-1-2011	194
FP-6-2012	1240
FP-17-2018	1133
Sea fig Control	22
Mussel control	< LOD*

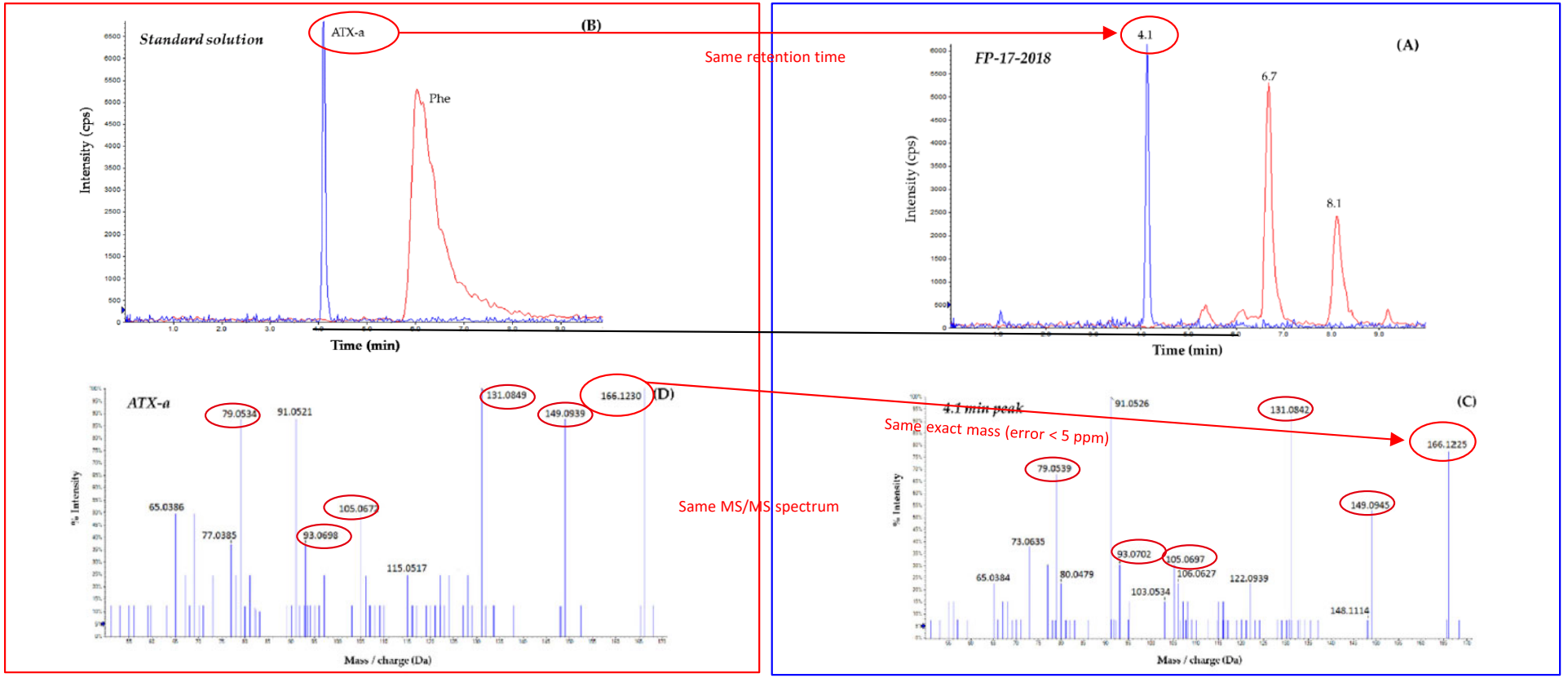
(*) LOD = limit of detection (8 µg/kg)



Confirmatory analyses: HILIC-MS/MS (high resolution)

ATX standard

Sea fig sample



Confirmatory analyses: HILIC-MS/MS (high resolution)

List of ATX analogues searched in the sea fig samples

Toxin	Formula	Mass (Da)	Extraction Mass $[M + H]^+$ (Da)
ATX-a	C10H15NO	165.11536	166.12264
hATX-a	C11H17NO	179.13101	180.13829
Carboxy ATX-a	C11H15NO3	209.10519	210.11247
Carboxy hATX-a	C12H17NO3	223.12084	224.12812
Carboxy dihydroATX-a	C11H17NO3	211.12084	212.12812
N-methyl ATX a	C11H17NO	179.13101	180.13829
(10*S*)-ATX alcohol	C10H17NO	167.13101	168.13829
(10*R*)-ATX alcohol	C10H17NO	167.13101	168.13829
nor ATX-a	C9H13NO	151.09971	152.10699
Dihydro ATX-a	C10H17NO	167.13101	168.13829
Dihydro hATX-a	C11H19NO	181.14666	182.15394
Epoxy ATX-a	C10H15NO2	181.11028	182.11756
Epoxy hATX-a	C11H17NO2	195.12593	196.13321
ATX-(a)s	C7H17N4O4P	252.09874	253.10602
Phe	C9H11NO2	165.07898	166.08626

None of the ATX analogues were found in the sea fig samples (non-targeted analyses in "suspect screening" mode)

4 — Study of the variability of contamination levels in sea figs

Samples analyzed

- *Microcosmus sulcatus*
- Area: FAO 37.2.1, Adriatic Sea (Croatia)
- Sample related to a food poisoning in the Gard department (jan. 2020) → 3 peoples (2×W 54 y/o, W 17 y/o). Important quantities of sea figs eaten by the two 54-y/o women

- *Microcosmus sulcatus*
- Area: FAO 37.2, Central Mediterranean Sea
- Sample coming from a store in the Var department (Géant Casino of Hyères), not related to any food poisoning

- *Microcosmus sp.*
- Thau lagoon
- Sample bought from a fisherman, not related to any food poisoning

Samples analyzed

Sample preparation

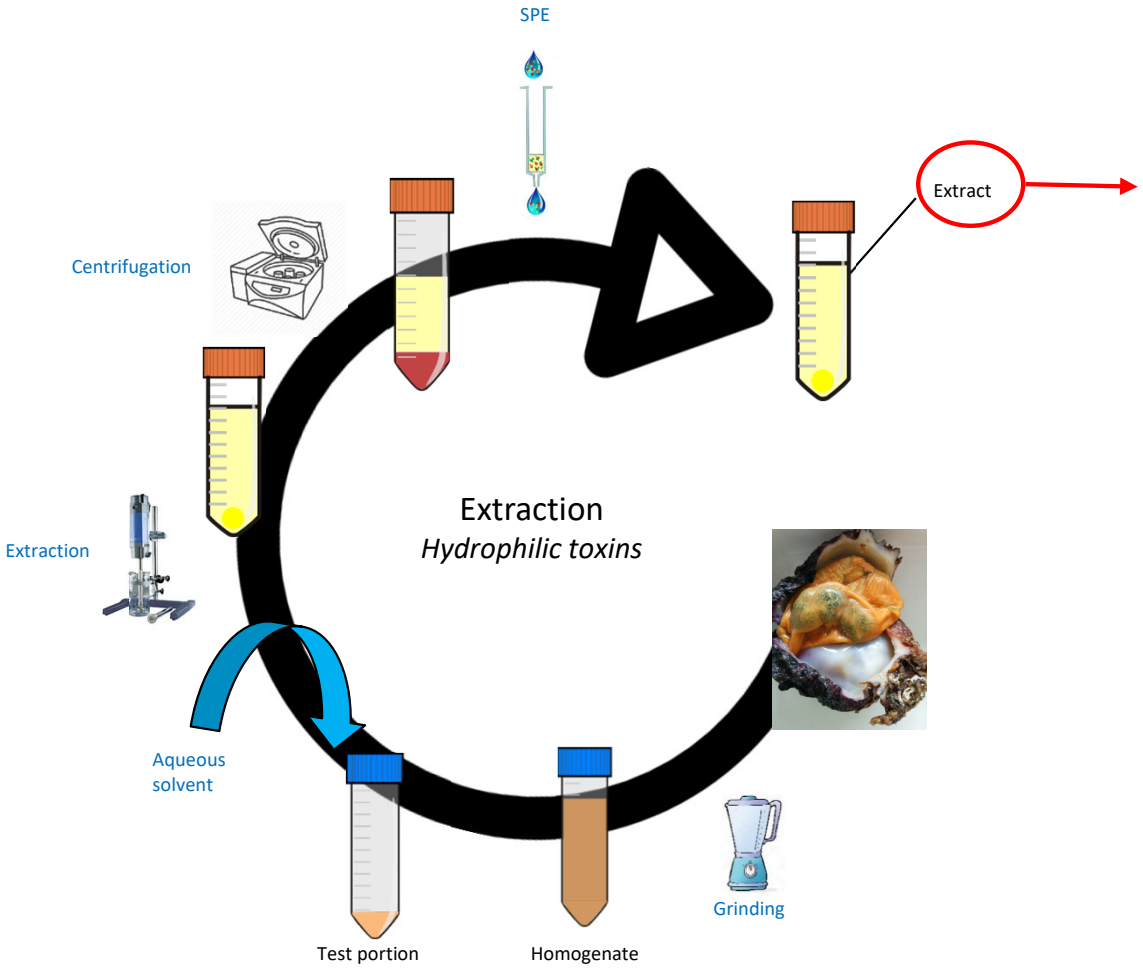
- Cleaning
- Shucking
- Weighing of each individual (flesh versus liquid/exudate)

- 9 individuals
- Total weight of the animals 12.4 – 38.4 g
- Mean = 24.7 g, SD = 9.0 g
- Flesh/liquid ratio: 0.5 – 3.3

- 12 individuals
- Total weight of the animals 20.7 – 86.6 g
- Mean = 49.0 g, SD = 21.9 g
- Flesh/liquid ratio: 0.4 – 1.2

- 5 individuals
- Total weight of the animals 15.3 – 59.0 g
- Mean = 32.9 g, SD = 17.7 g
- Flesh/liquid ratio: 0.5 – 0.8

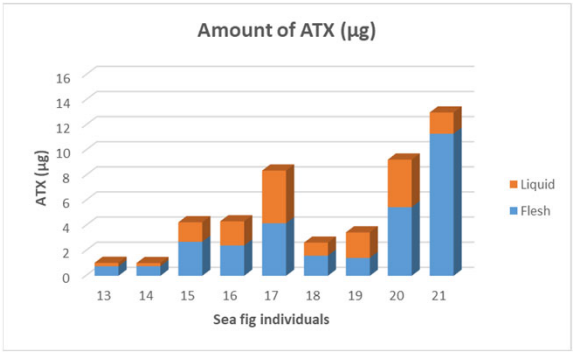
Protocol implemented

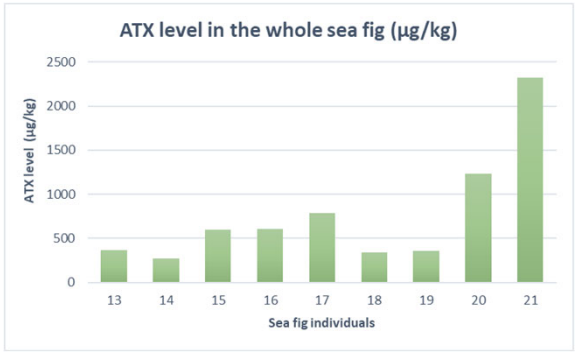


Analysis in low resolution (HILIC-MS)

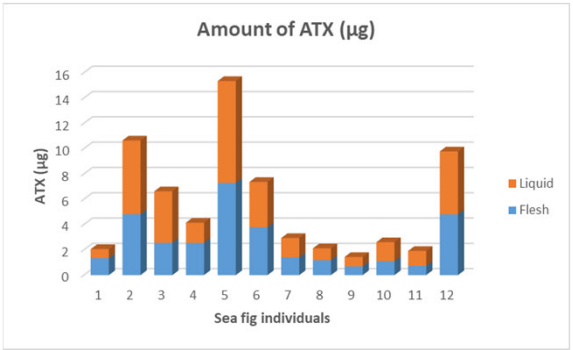
TSQ Vantage
(low resolution)

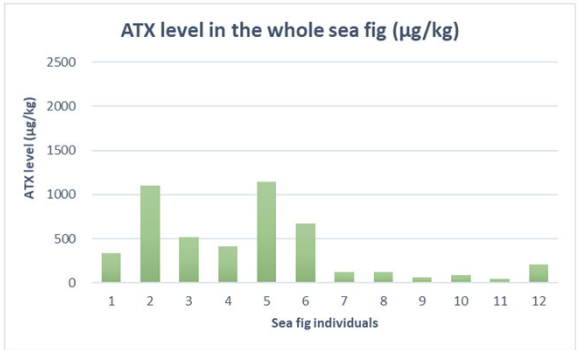

Gard
GARD


Amount of ATX (µg)
ATX (µg)
Sea fig individuals
Liquid
Flesh

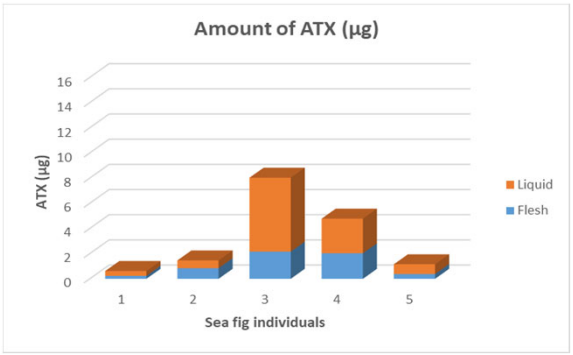

ATX level in the whole sea fig (µg/kg)
ATX level (µg/kg)
Sea fig individuals

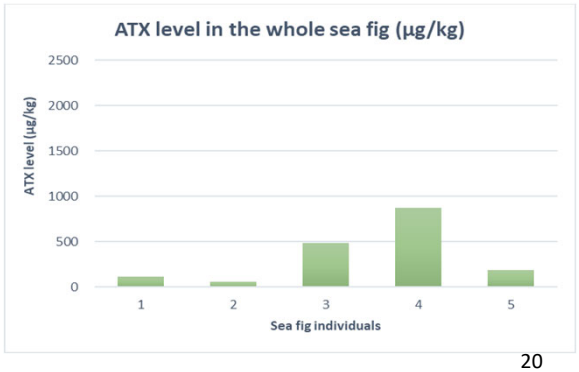

Var
Le Département


Amount of ATX (µg)
ATX (µg)
Sea fig individuals
Liquid
Flesh

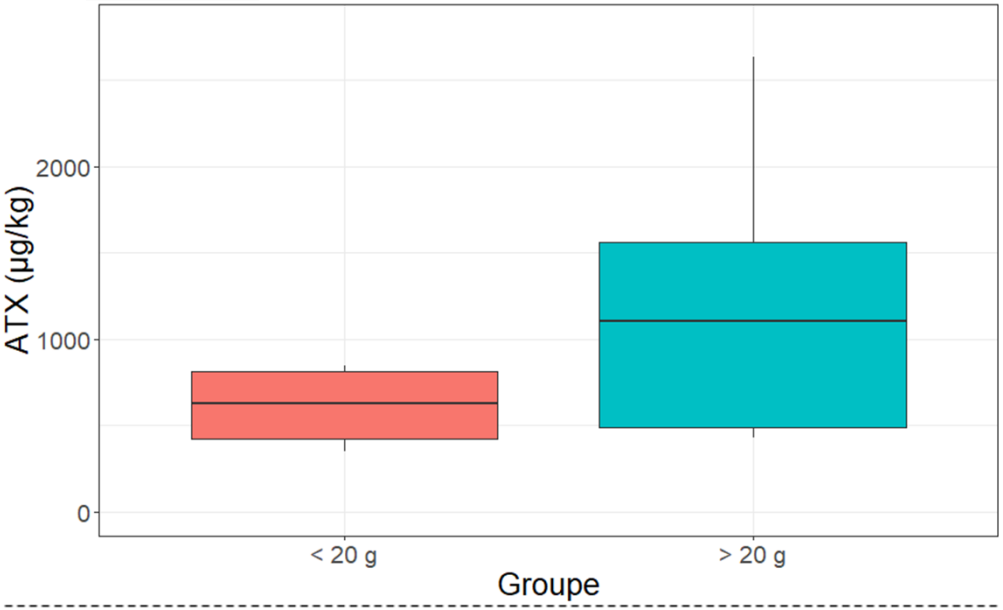

ATX level in the whole sea fig (µg/kg)
ATX level (µg/kg)
Sea fig individuals


Hérault
Hérault


Amount of ATX (µg)
ATX (µg)
Sea fig individuals
Liquid
Flesh

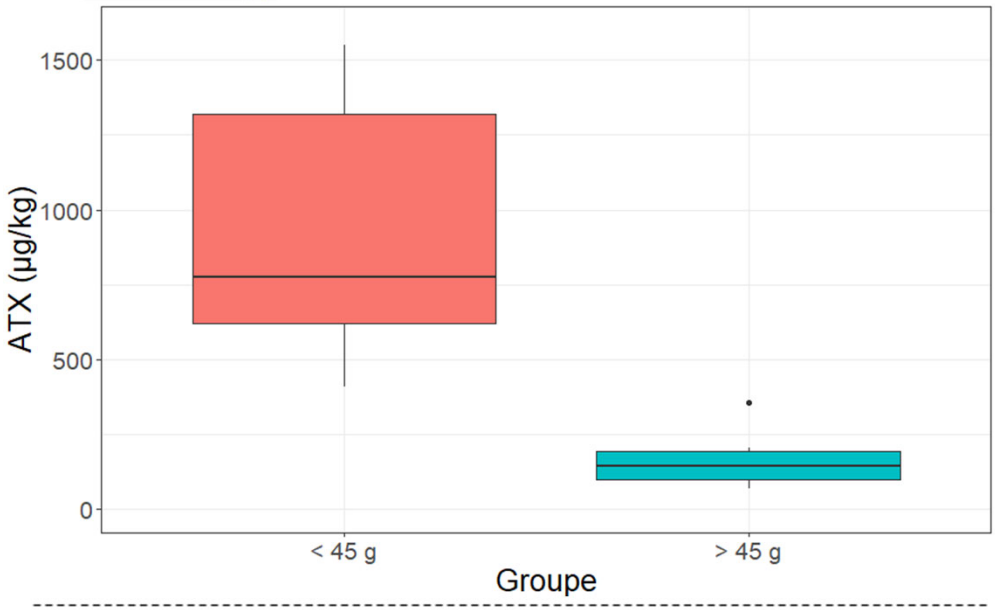

ATX level in the whole sea fig (µg/kg)
ATX level (µg/kg)
Sea fig individuals

- 9 individuals
- Total weight of the animals 12.4 – 38.4 g
- Mean = 24.7 g, SD = 9.0 g
- 2 groups: < 20 g and > 20 g



Groupe	N	Minimum	Maximum	Moyenne	Ecart_type	Q25	Mediane	Q75
< 20 g	4	349.3	847.2	612.9	250.7	424	627.5	816.3
> 20 g	5	432	2632	1243	905.8	486.7	1102	1562

- 12 individuals
- Total weight of the animals 20.7 – 86.6 g
- Mean = 49.0 g, SD = 21.9 g
- 2 groups: < 45 g and > 45 g



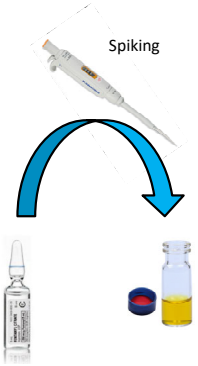
Groupe	N	Minimum	Maximum	Moyenne	Ecart_type	Q25	Mediane	Q75
< 45 g	6	406.2	1549	929.6	473.5	619.8	776	1321
> 45 g	6	67.1	355.6	166.9	104.9	97.1	143.6	193.4

Conclusion : These preliminary results show that it is not possible to generalize about the existence or not of a correlation between the size of the sea figs and their contamination level. Several factors come into play.

Suspicion of the presence of hATX in 1/5 sea fig of the Thau lagoon (LC-MS low resolution)

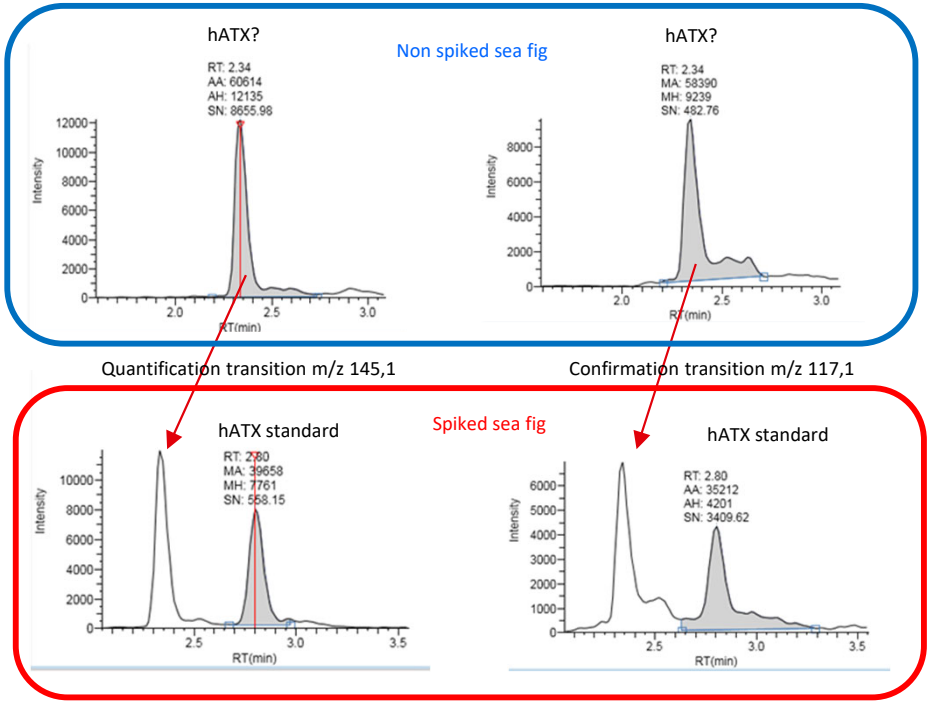
- Specific transitions of hATX
- But RT < that of the hATX standard

TSQ Vantage
(low resolution)



hATX standard

API 5600 Qtof
(high resolution)



- Different HRMS2 fragmentation spectra for the hATX standard and the violet
- However, some low mass fragments are common → not hATX but related compound?

Conclusions and perspectives

- ✓ Evidence of ATX-a in violets involved in TIAC cases (low and high resolution)
- ✓ High variability of contamination of samples (194 - 1240 µg/kg)High variability of contamination between individuals, but no correlation between size and contamination level
- ✓ Need for further investigations to know if ATX-a is indeed responsible for the intoxication cases
- ✓ What about contamination of other marine organisms?

Results published in May 2020 in an A+ journal (IF 4,379)

marine drugs

MDPI

Article

First Evidence of the Presence of Anatoxin-A in Sea Figs Associated with Human Food Poisonings in France

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