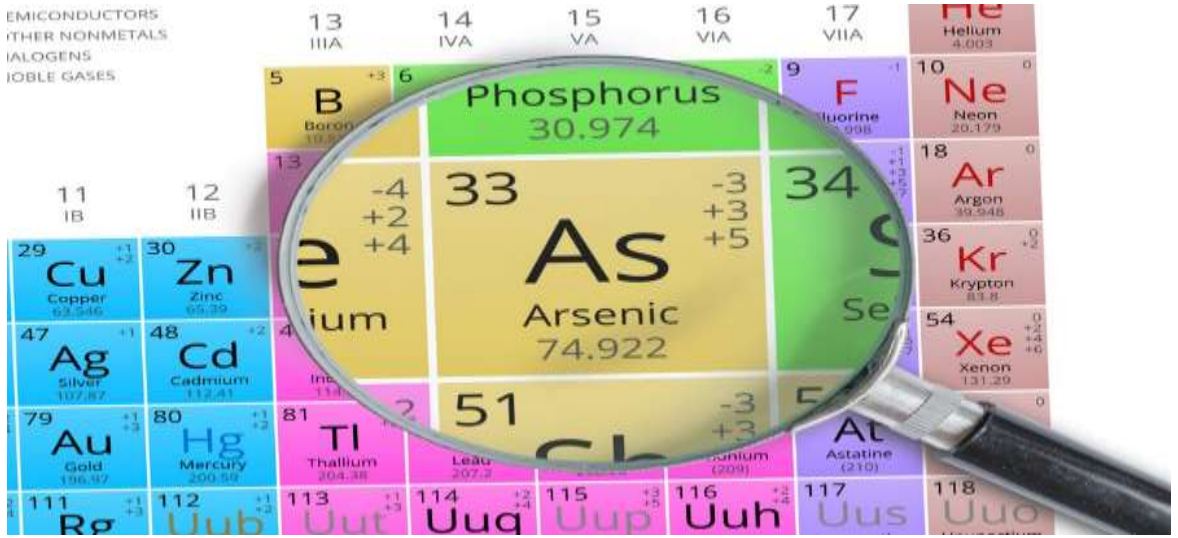
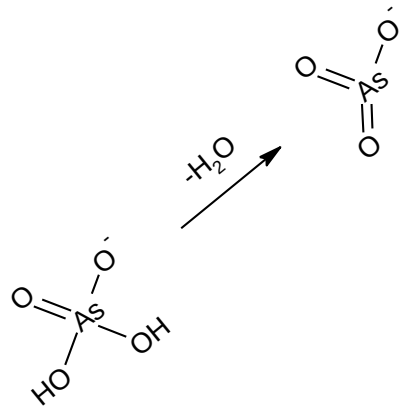
Arsenic Bioremediation in Water – Detection by HPLC-MS





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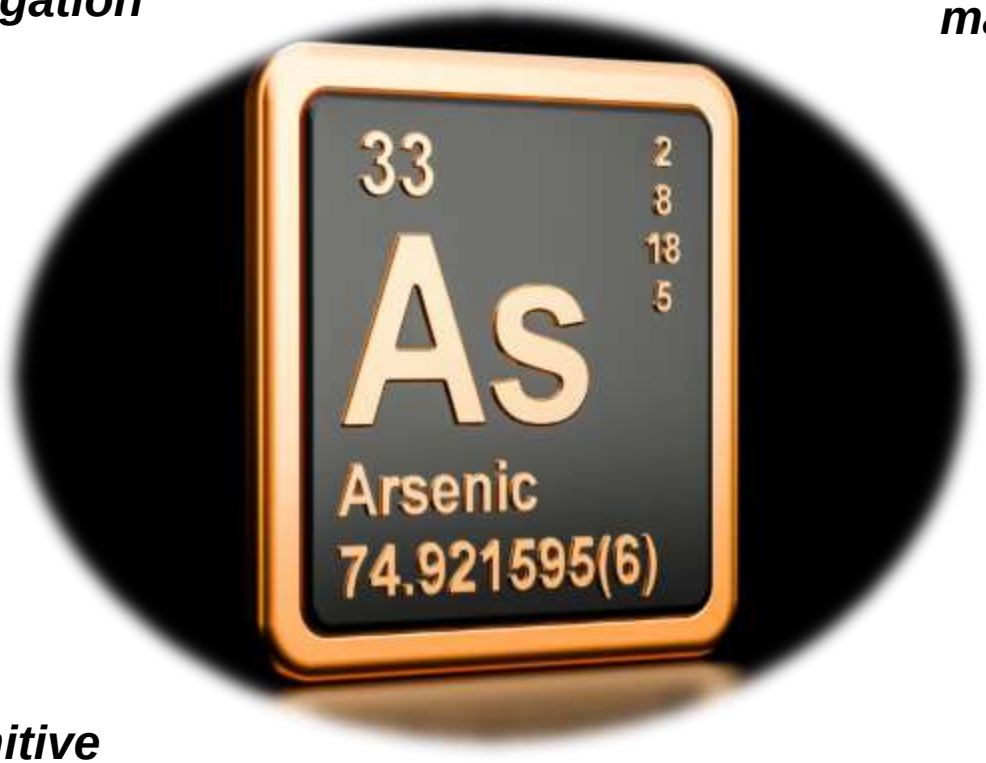
Some Facts - World Health Organization

Exposure through contaminated ***drinking water, food preparation, crops irrigation and tobacco smoking***

One of WHO's 10 chemicals of ***major public health concern***



Long-term exposure can cause ***cancer and skin lesions***

Highly toxic in its ***inorganic*** *form*

Negative impacts on ***cognitive development*** *and increased* ***deaths*** *in young adults*

Naturally present at ***high levels in the groundwater*** *of a number of countries*

United Nations Environment Programme (UNEP)

The coral reef sponge *Theonella swinhoei*

- Wide Indo-pacific distribution
- **High Microbial** Abundance (HMA) sponge (40% tissue volume)

- *T. Swinhoei* acts as a **hyper-accumulator** of arsenic (As) (8,600 µg g^{-1}) - **highest recorded** in any organism from a similar environment

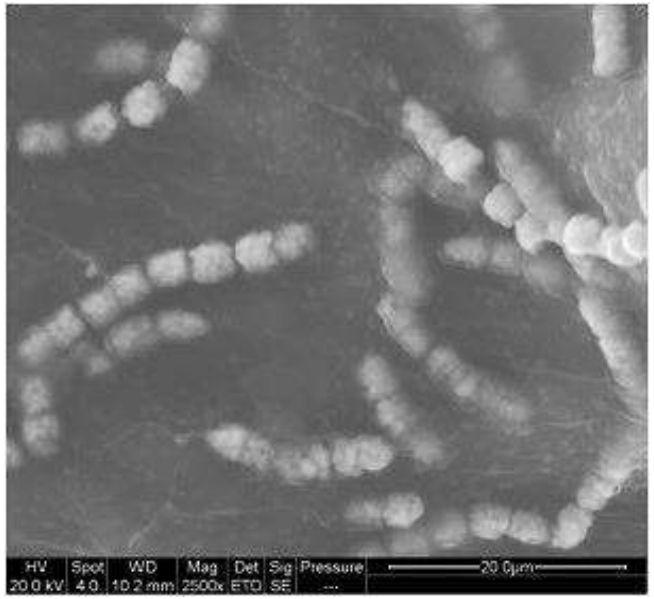
- Much of the arsenic was found in the **bacteria-enriched fraction**

- Bacterium may act similar to a **detoxifying organ** for its host, the sponge

Arsenic reducing bacteria

- Sponge associated bacteria transform Arsenic **pentavalent $As^{(V)}$** to **trivalent $As^{(III)}$,** yielding energy

- Assimilated to organic forms (less toxic)
- In cell mineralization to Calcium Arsenate (Pharmacolite)
- Precipitation with Sulfur or Iron Oxide



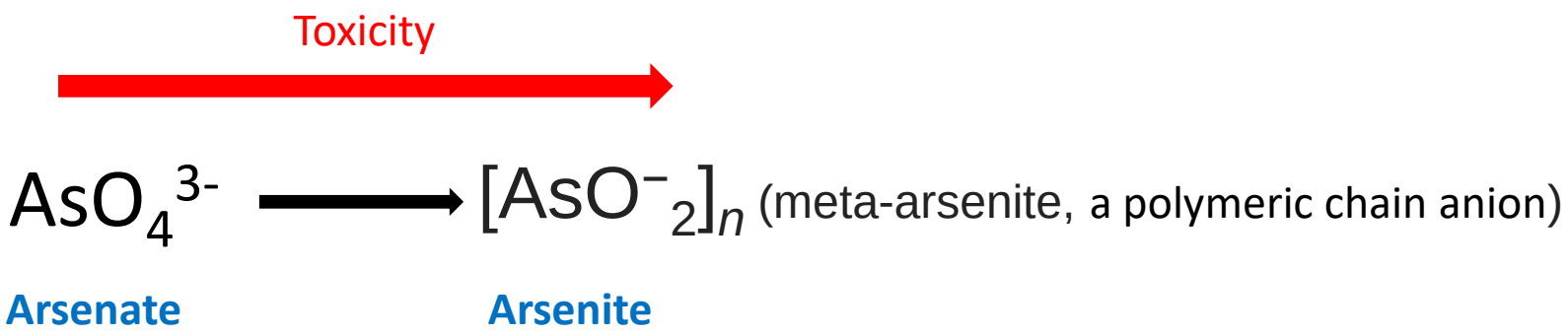
(Keren et al. 2017)

107 – Pseudovibrio ascidisceicola
106 – Alteromonas macleodii
39 – Pseudovibrio denitrificans
18 – Pseudovibrio denitrificans

Research objectives

- Developing HPLC-MS method for separation and quantification of As^{V} and As^{III} in saline water matrix.

- Study the bioremediation effect of the selected bacteria on As^{V} and As^{III} concentrations.



Common methods

Separation techniques

- Ion Chromatography
- Anion exchange HPLC
- Ion Pair RP HPLC
- Capillary electrophoresis (CE)
- Chelating agents

Detection techniques

- ICP-MS
- ESI-MS
- Conductivity?

http://analyteguru.com

Chromatographic method - column

Thermo Fischer Hypercarb™ column:

Based on Porous Graphitic Carbon (PGC), known but non-common since the 1980's.

- **Exceptional retention of very polar analytes**
- Separation for structurally-related substances
- pH stability from 0 to 14, and are not affected by aggressive mobile phases
- Ideal solution for high-temperature applications

https://www.thermofisher.com/il/en/home/industrial/chromatography/liquid-chromatography-lc/hplc-uhplc-columns/hypercarb-hplc-uhplc-columns.html

Chromatographic method - QbD

Conditions:

System: *Agilent 1100 HPLC with QTOF "premier" ESI-MS*

ESI-MS mode: *Negative*

Column: Hypercarb 150x2.1mm, 5µ + pre-column

Temp: 30°

Injection vol: 10µL

Mobile phase:

Solution B – 5mM Ammonium Formate buffer at pH=4.0

Solution C – Methanol (MEOH)

Solution D – Acetonitrile (ACN)

Standards:

Arsenate – 0.15mg/mL; Arsenite – 0.10mg/mL

#	Composition	Flow
1	B:D (50:50)	0.5
2	B:D (50:50)	0.25
3	B:D (75:25)	0.5
4	B:D (75:25)	0.25
5	B:D (25:75)	0.5
6	B:D (25:75)	0.25
7	B:C (50:50)	0.5
8	B:C (50:50)	0.25
9	B:C (75:25)	0.5
10	B:C (75:25)	0.25
11	B:C (25:75)	0.5
12	B:C (25:75)	0.25
13	100%B	0.5
14	100%B	0.25

Chromatographic method - QbD

<u>Additional studied parameters:</u>

1. Ammonium Formate vs. Ammonium Acetate
2. Ionic strength (25mM-5mM)
3. Column Temperature (40°C-10°C)
4. Additional flow rates (0.4-0.2mL/min)
5. Injection volumes (5-100µL)

Chromatographic method - Optimum

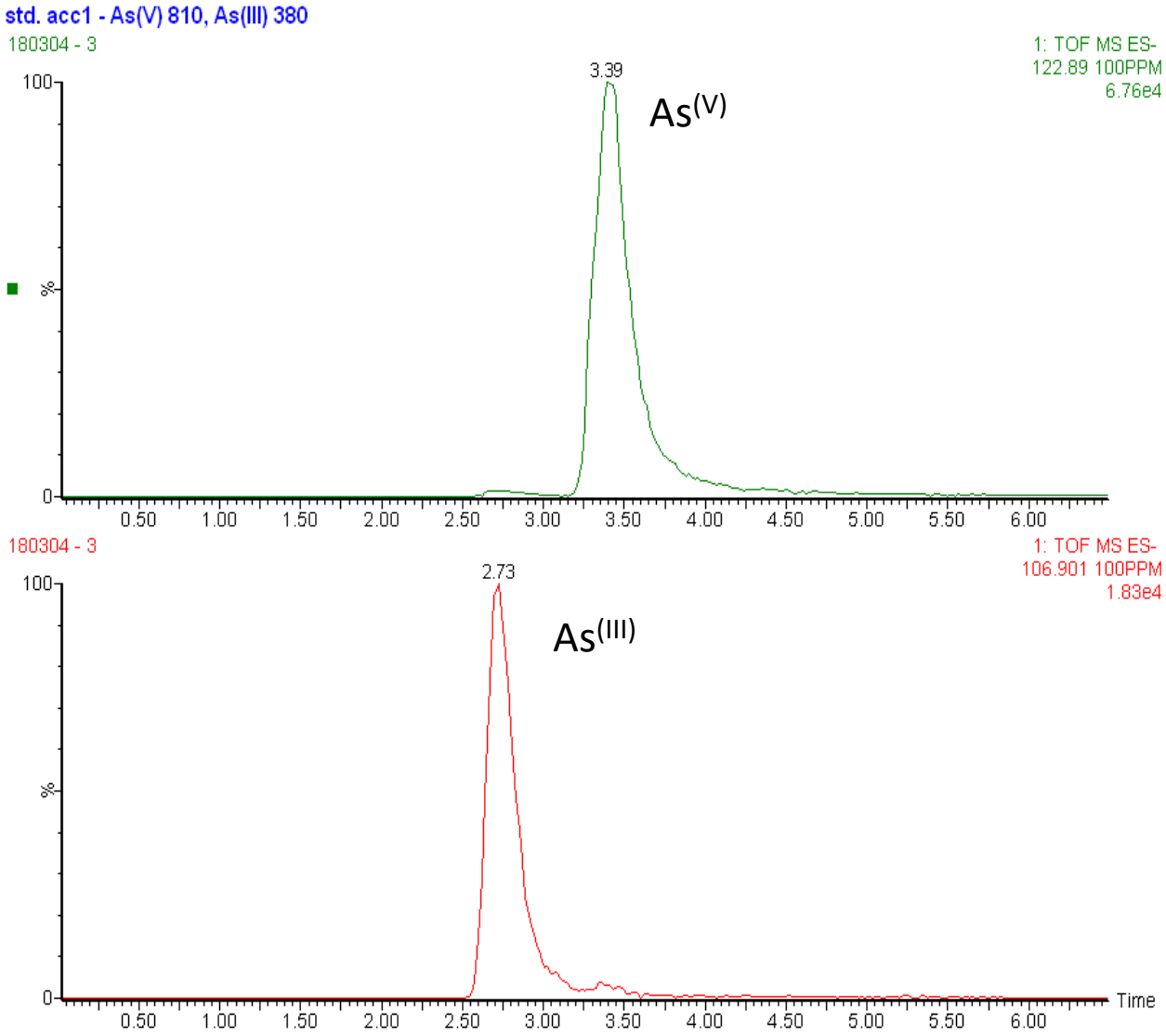
Column Temperature: 15°C

Mobile Phase: Isocratic mode –

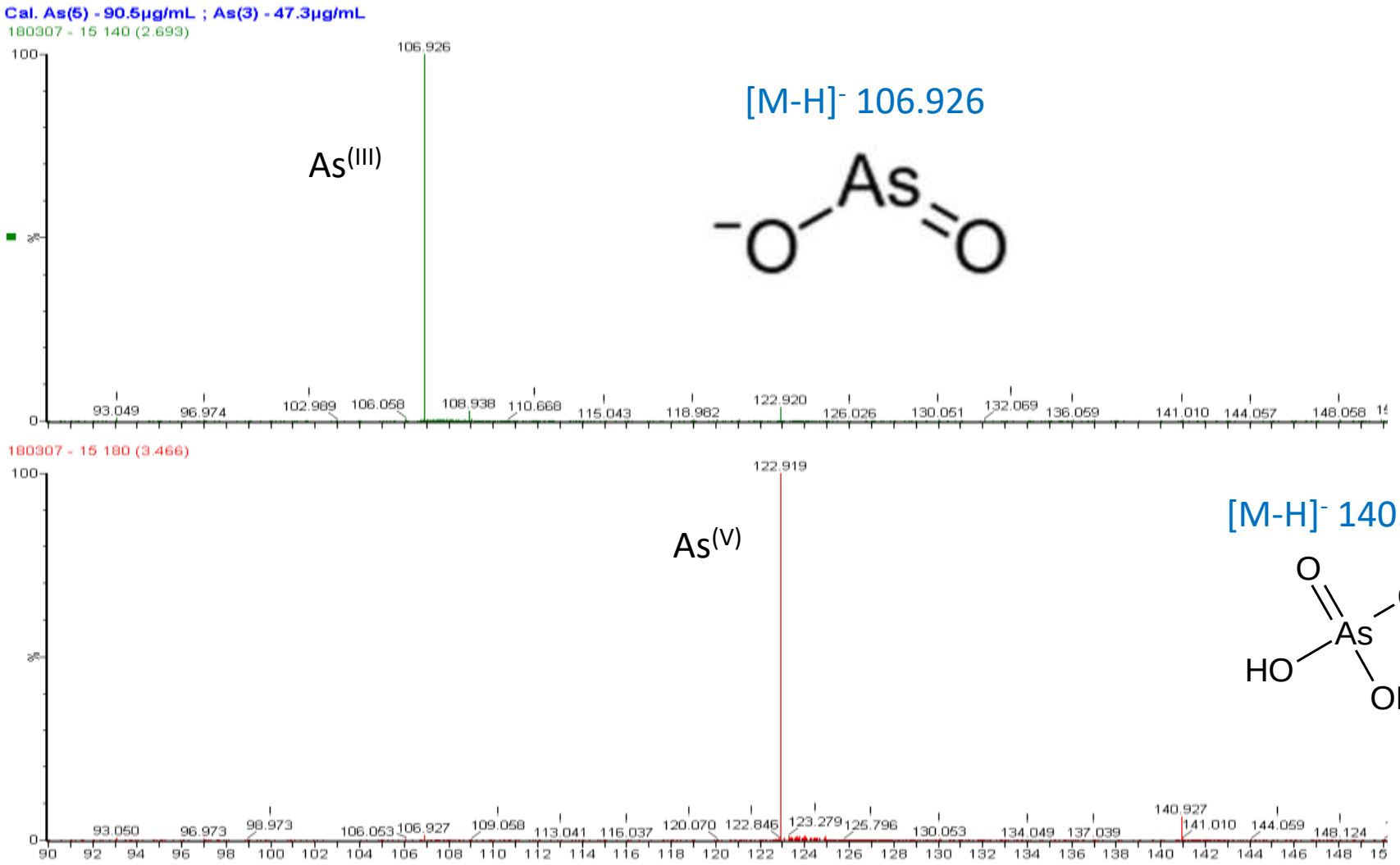
5mM Ammonium Formate:Acetonitrile (70:30)

Flow rate: 0.2mL/min

Injection Volume: 5µL



MS Spectra

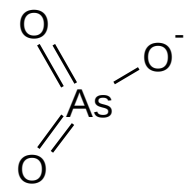


$[M-H]^-$ 106.926

$[M-H]^-$ 140.927

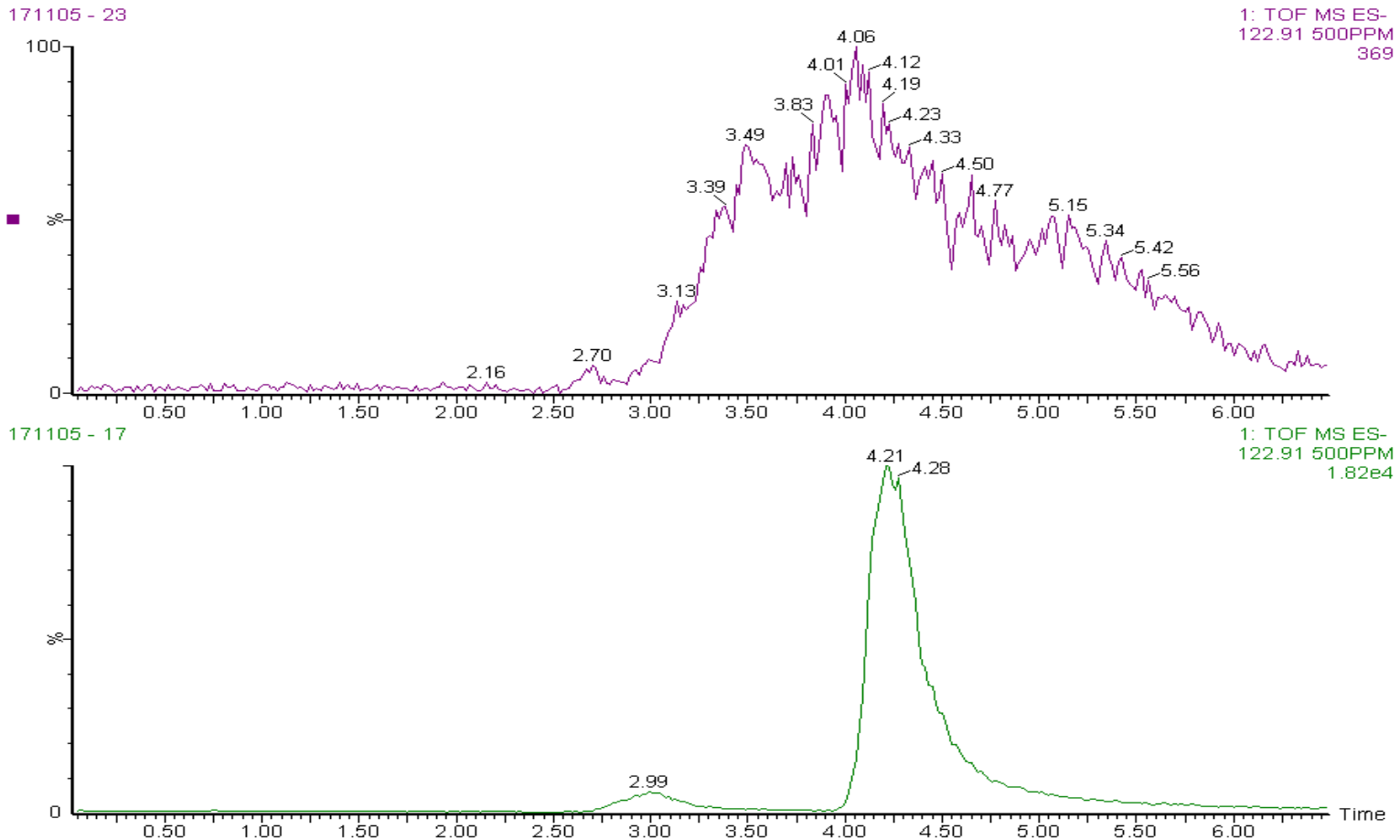
$-H_2O$

$[M-H]^-$ 122.919



Analysis of real matrix – signal problem

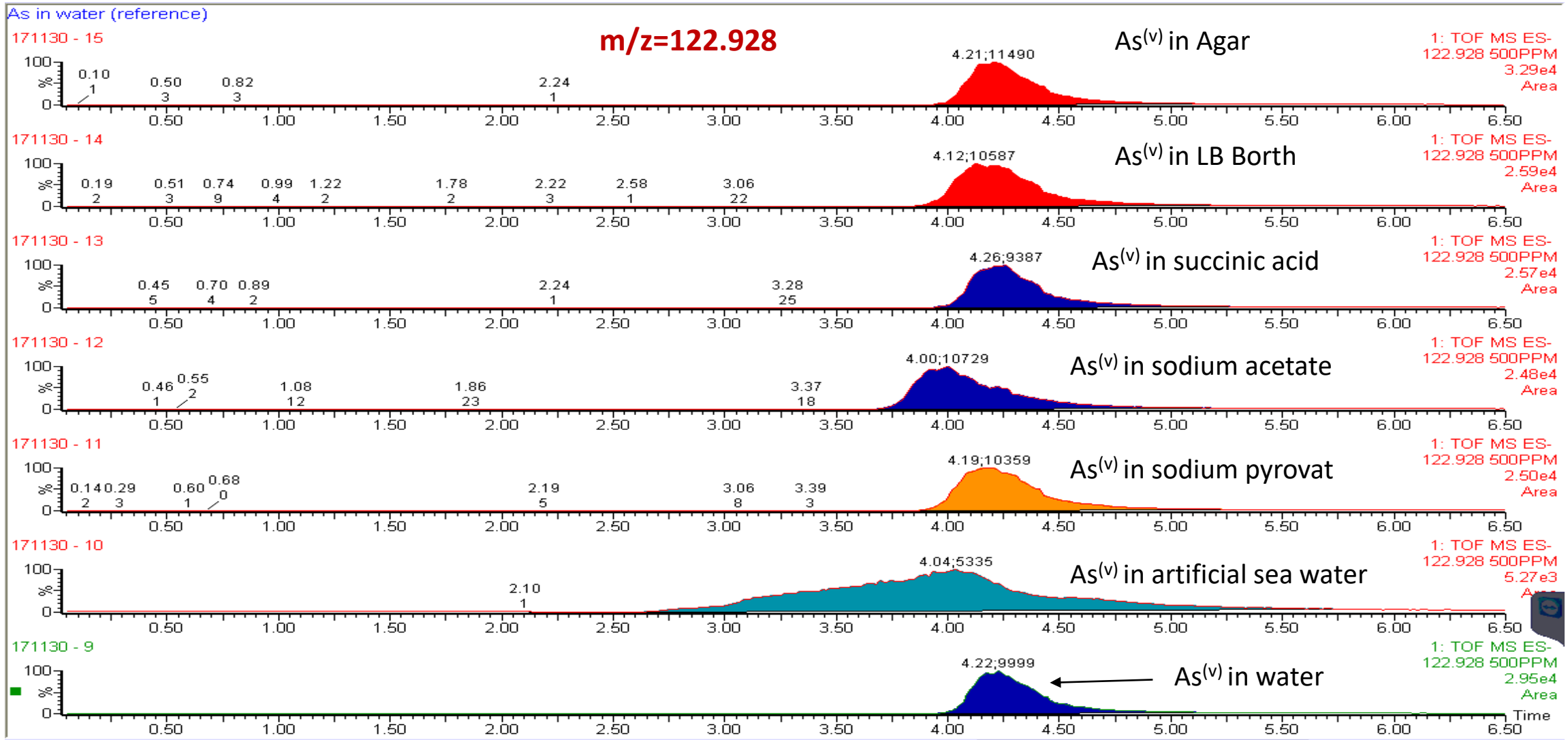
Spiked sample Vs. Standard



Sample (after filtration) contains:

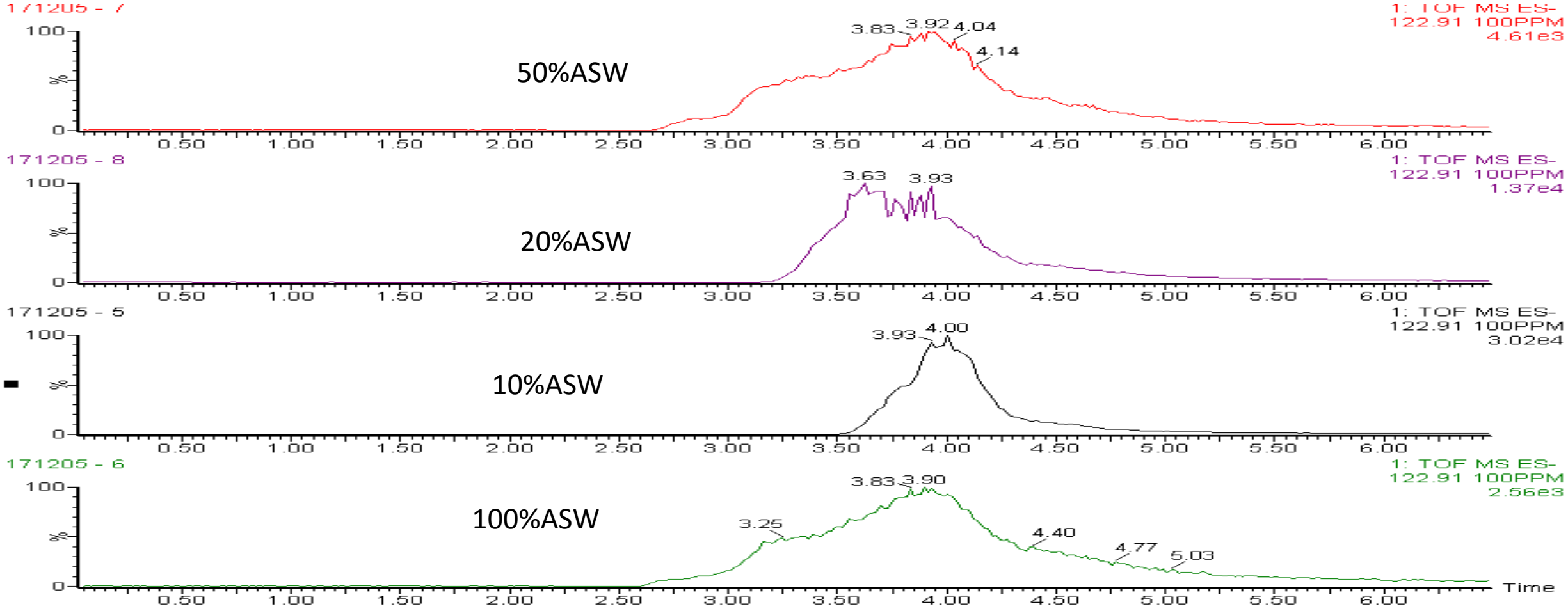
- Sodium Pyrovat
- Sodium Acetate
- Succinic Acid
- Vitamin solution (LB Borth)
- Agar
- Artificial Sea Water (ASW) - 35ppt (part per thousand)=3.5%

Individual ingredients



Lowering salinity

Different ASW concentrations

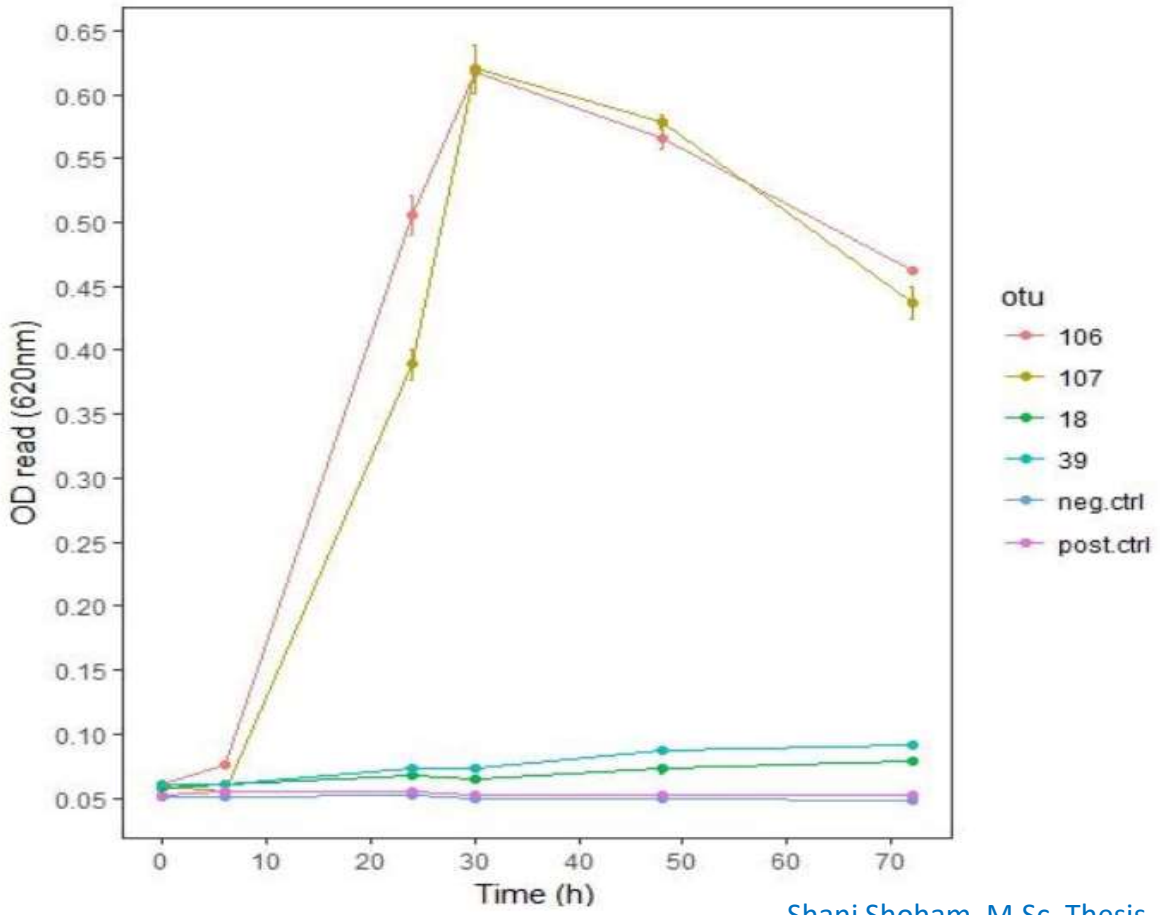


Lowering salinity

Action	Possible outcome
Dilution	Sensitivity problem
Increase Arsenic levels and dilution	Toxicity to bacteria
Reduce salinity in growing matrix	Not suitable for bacteria

www.walmart.com

Bacteria growth in 0.35% salinity matrix



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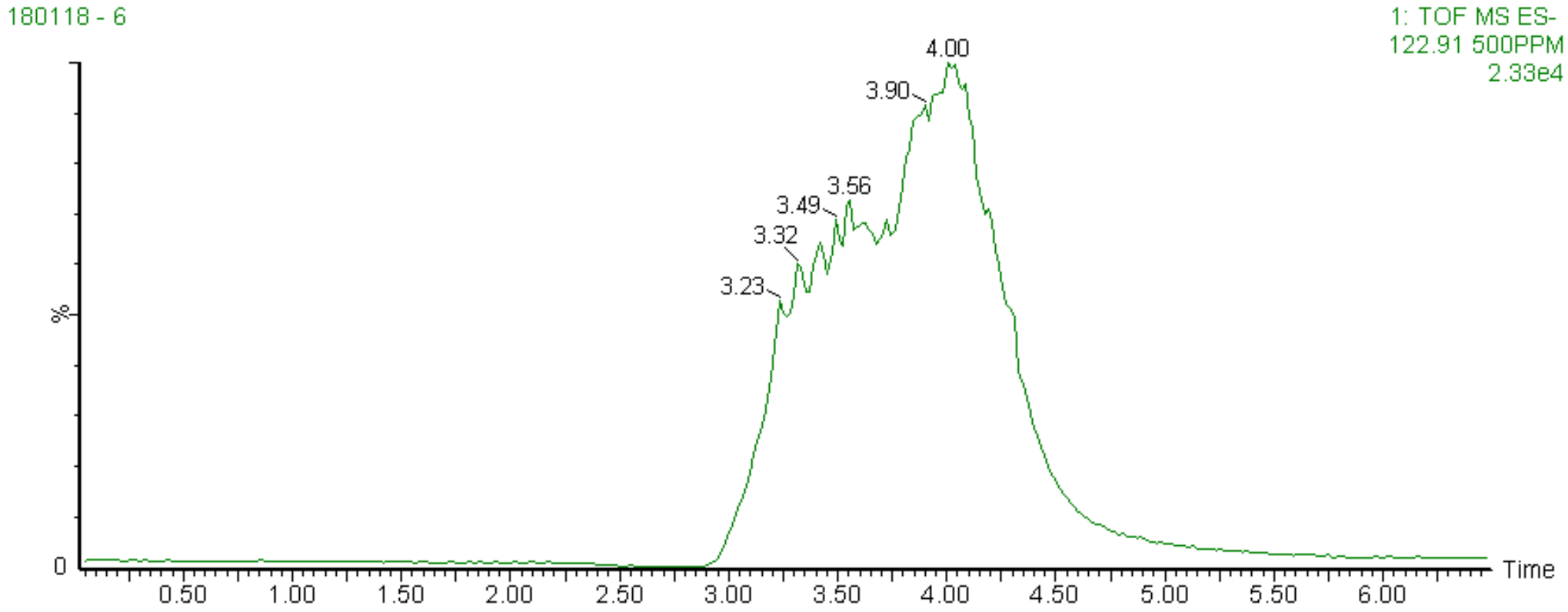
Lowering salinity

$As^{(v)}$ spiked to 0.35% salinity matrix

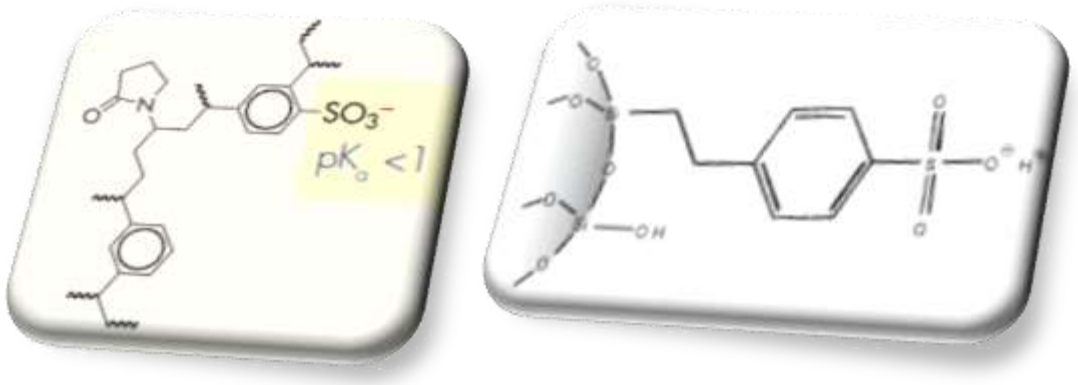


Pass-through SCX SPE

- Originally design to target weakly basic compounds by traditionally **Strong Cations Exchange SPE** procedure

- Here, used as a “pass-through” column to exchange Na^+ ions with H^+
- Therefore, reduces sodium concentration but increases acidity

- Since Arsenic ions are negatively charge, they pass the sorbent without any interaction



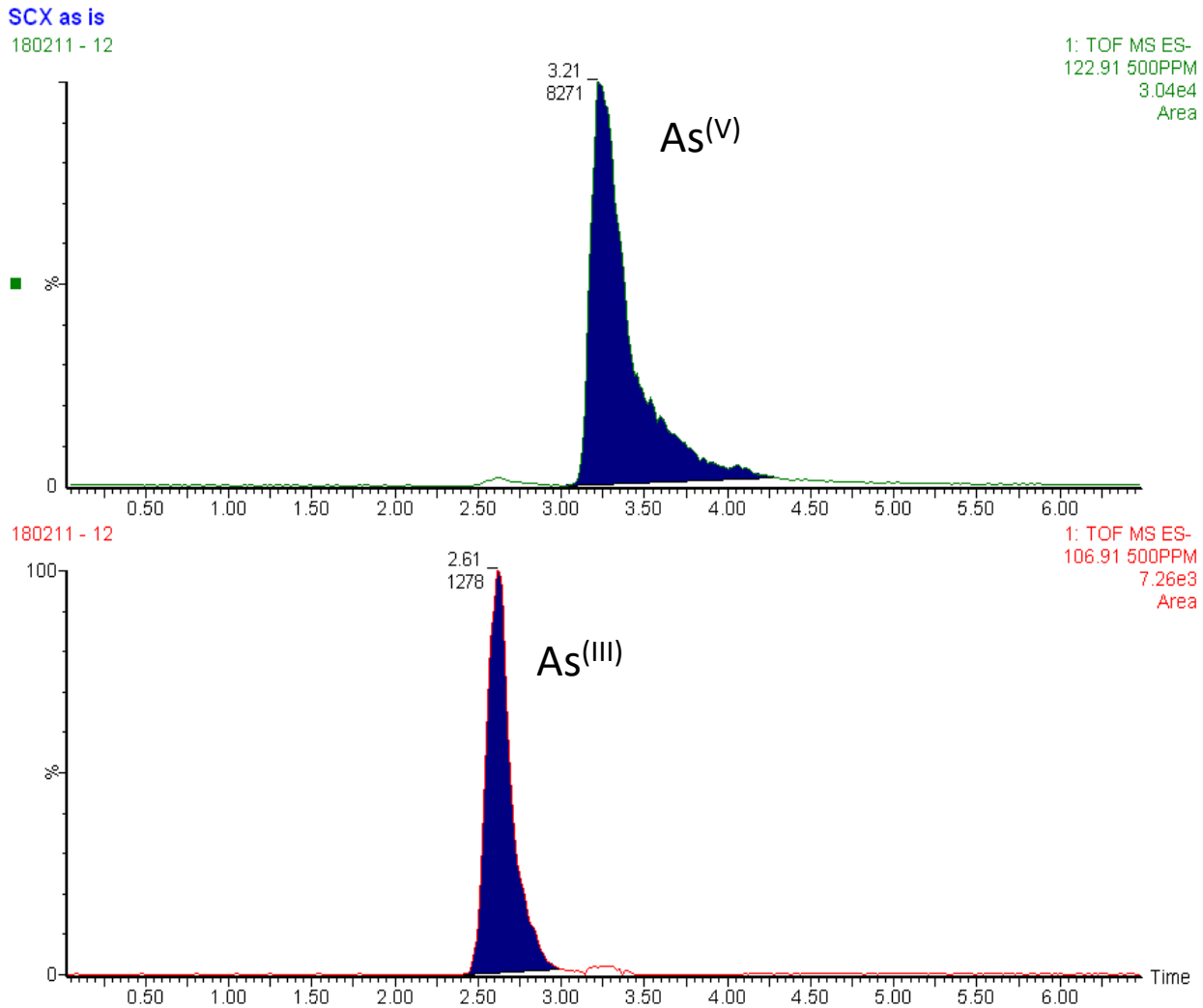
Oasis MCX

Strata SCX

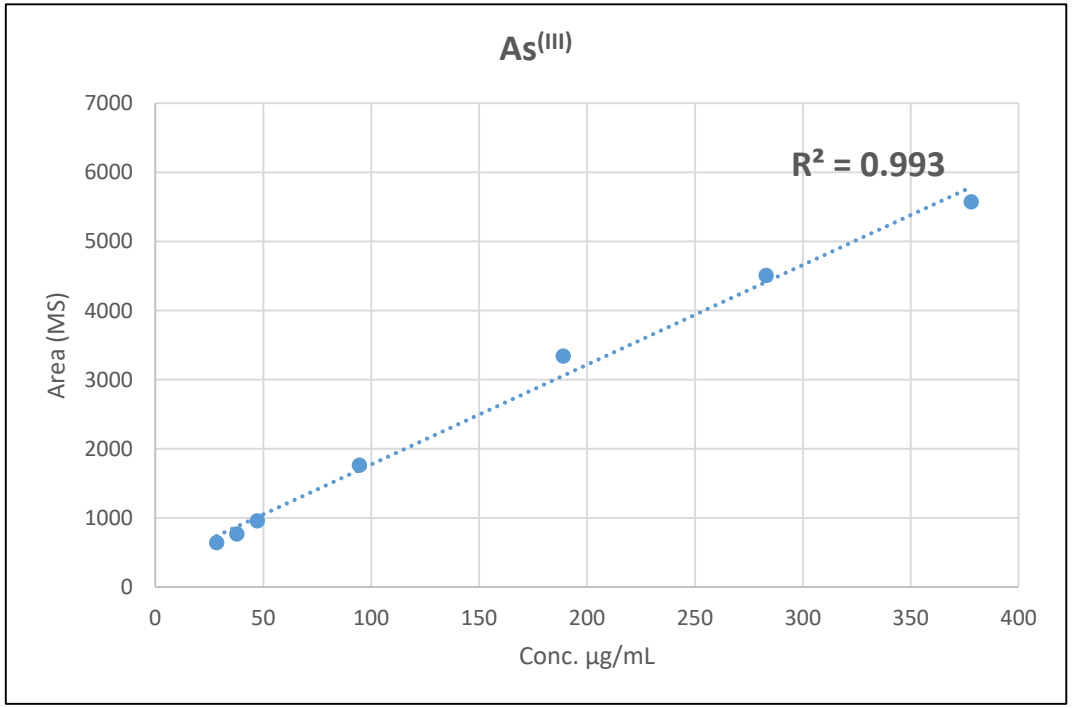
pH effects and adjustments

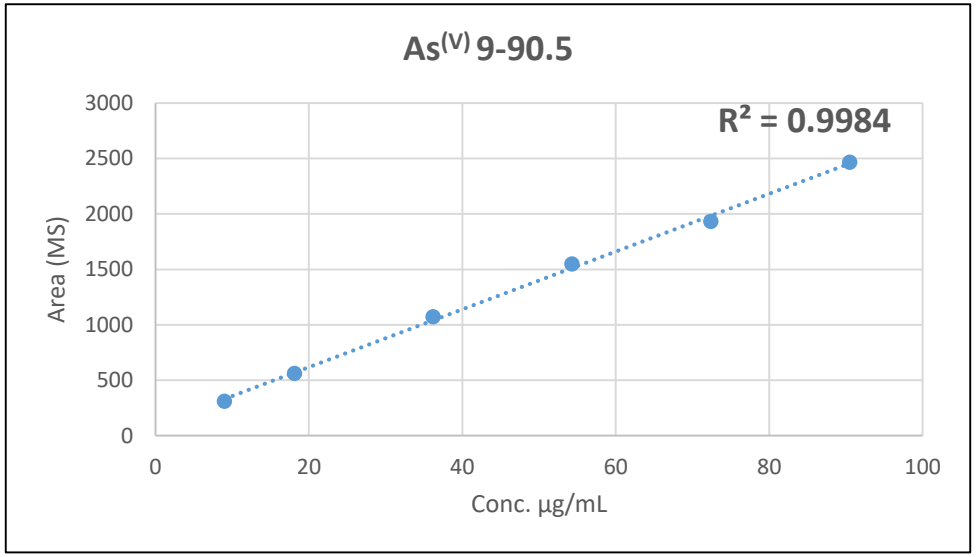
- Sample pH after SCX-SPE was very low (~2), causes peaks widening

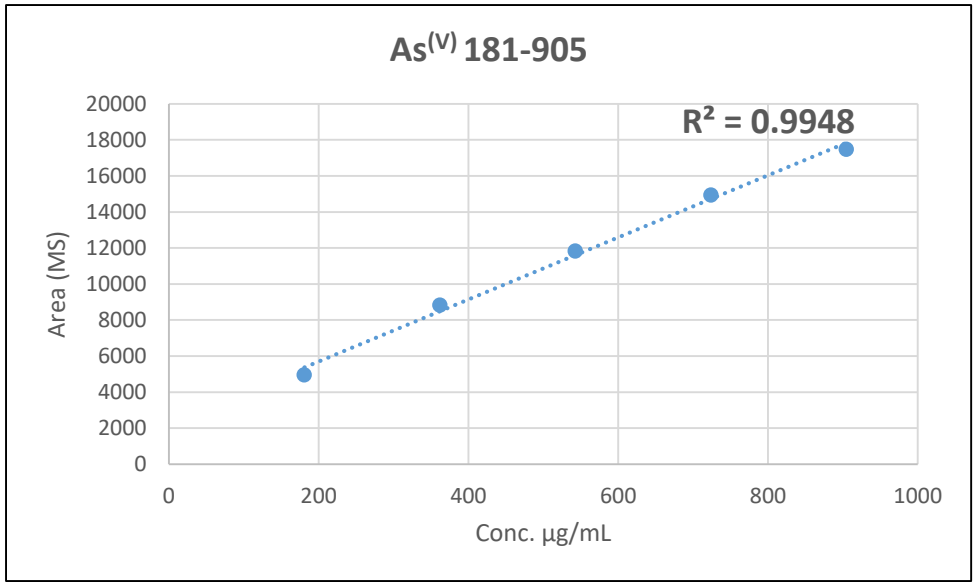
- Dilution 1:1 with 10mM Ammonium Formate at pH=6.4:
  - Increase and stable the pH at ~3
  - More reduction of Na^+ concentrations



Validation parameters - Linearity



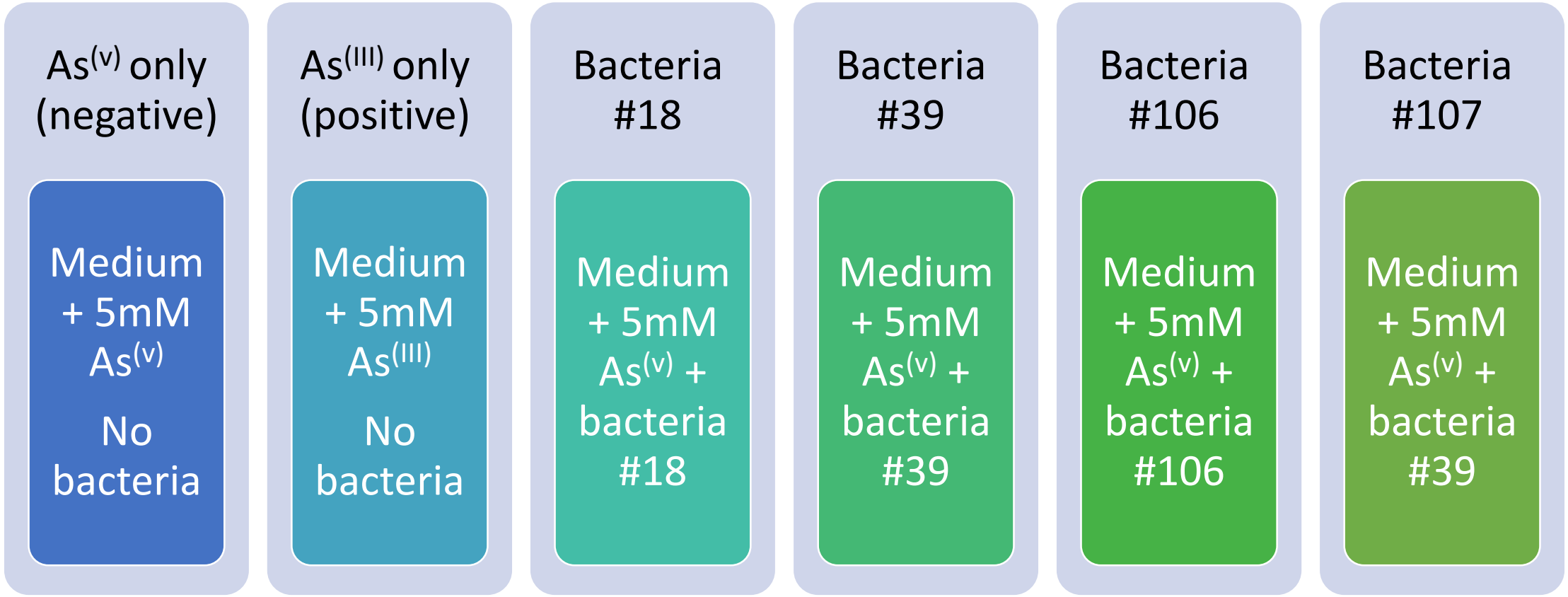




Validation parameters - continue

Parameter	$As^{(III)}$	$As^{(V)}$
LOD	0.03mM	0.02mM
LOQ	0.05mM	0.05mM
Accuracy (as %recovery)	95%	97%
Standard Stability	NMT 48h	NMT 48h

Samples – Design of experiment

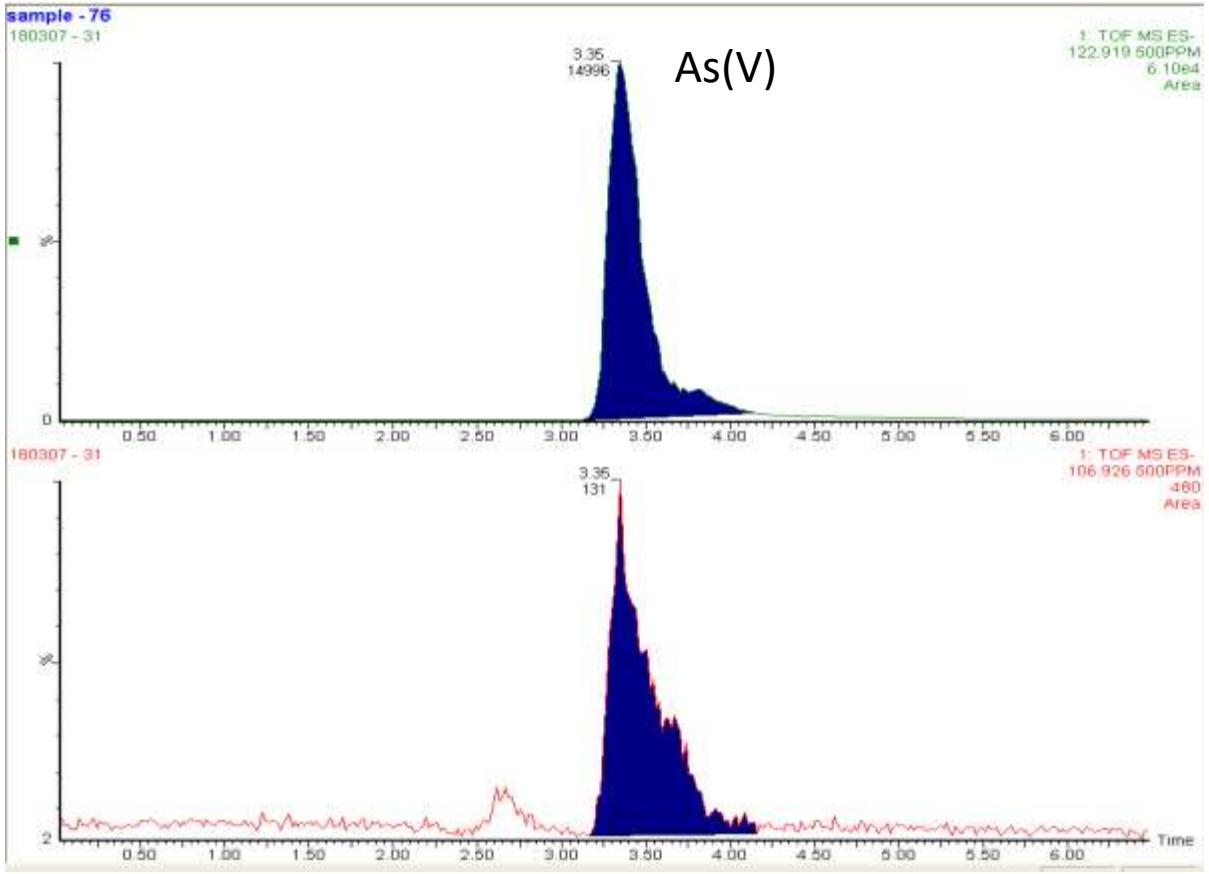


Sampling – 0, 6, 24, 30, 48, 72 hours. Centrifuge and 0.22µ filter

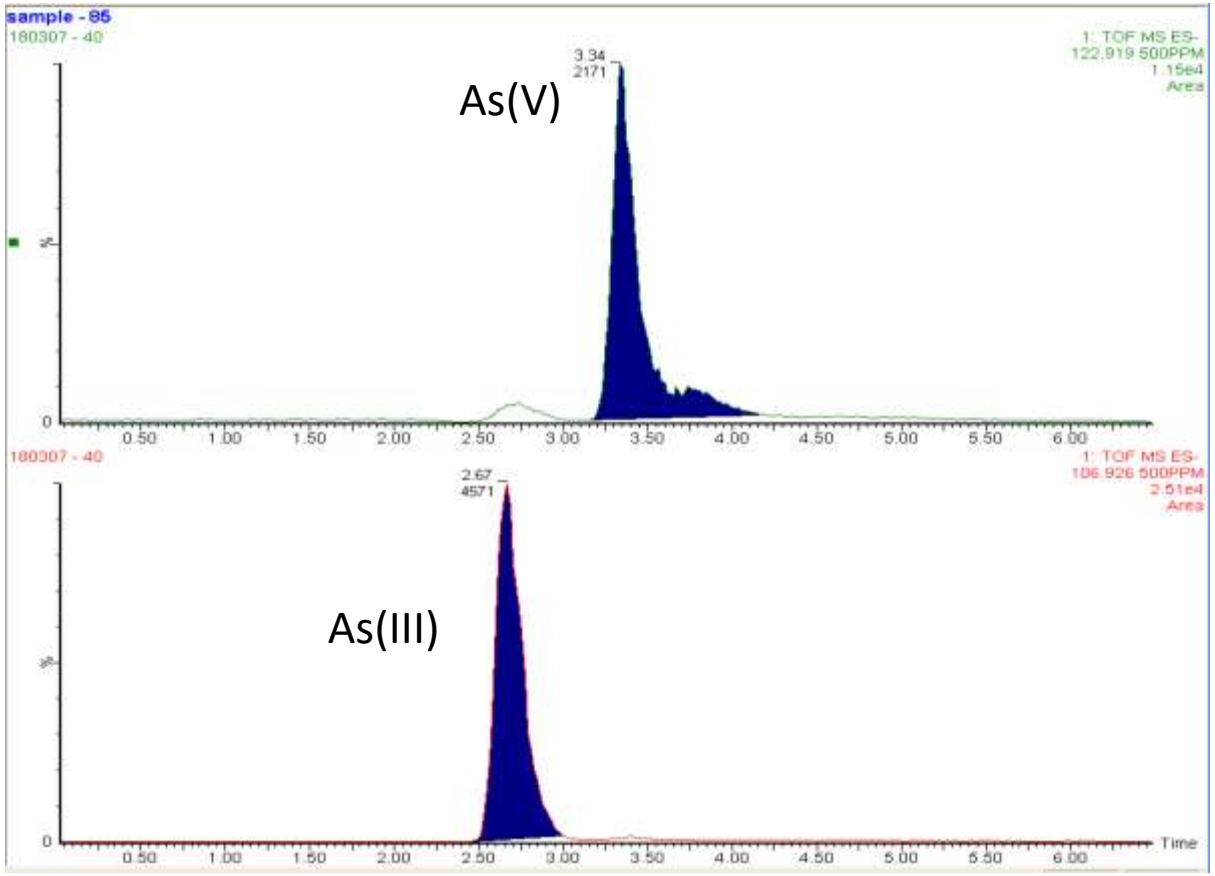
Results

Samples after SCX-SPE cleanup procedure

Negative control: Medium+ As(V) without bacteria after 48h



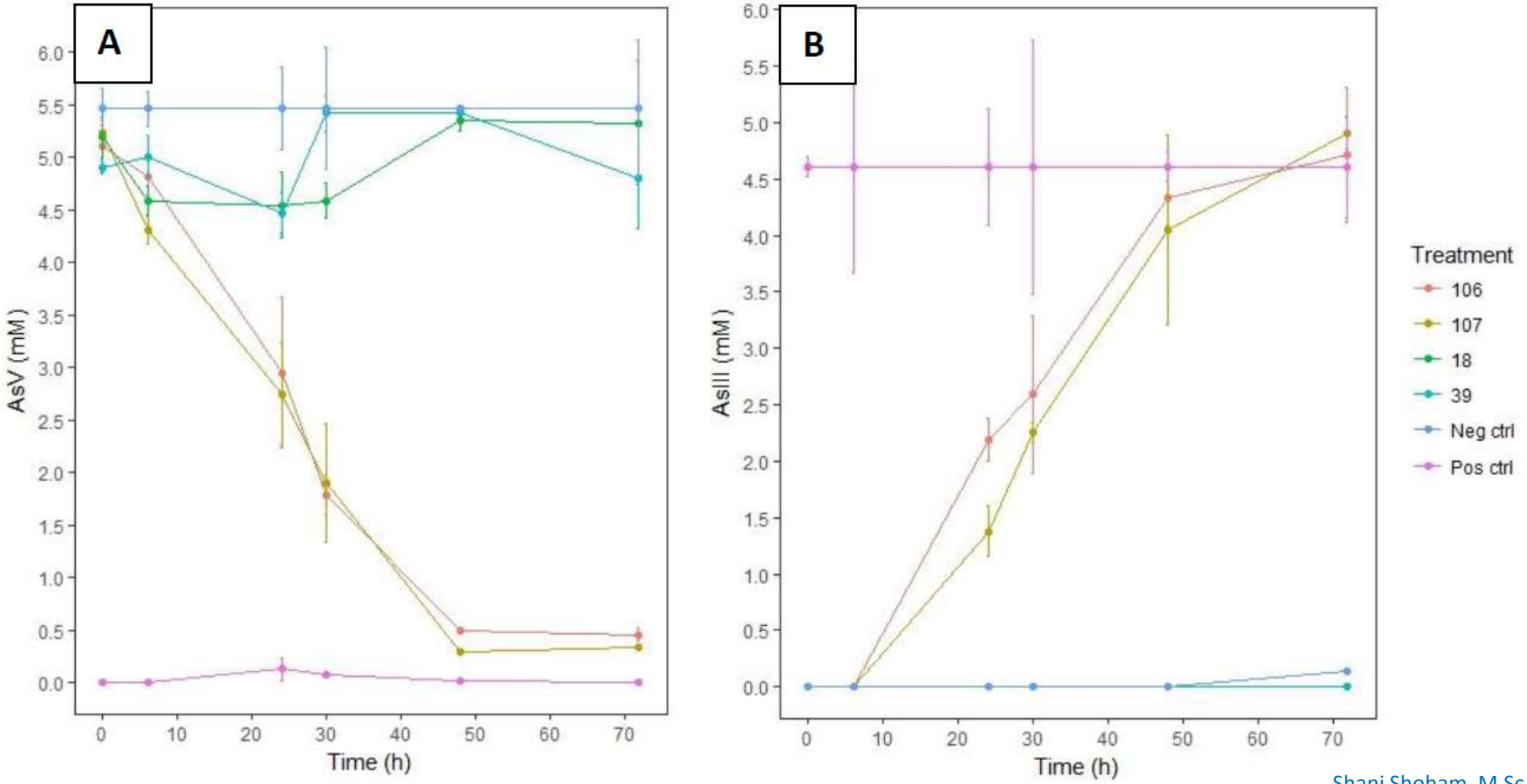
Bacteria sample #106: After 48h (initial only As(V))



Results – 48h Interval

Treatment		As(III) [mM]	As(V) [mM]
Positive control: Medium+ As(III) without bacteria	smp_73	4.54	0.02
	smp_74	4.76	0.02
	smp_75	4.53	0.02
Negative control: Medium+ As(V) without bacteria	smp_76	<LOD	5.50
	smp_77	<LOD	5.45
	smp_78	<LOD	5.44
OUT num **18**	smp_79	<LOQ	5.47
	smp_80	<LOQ	5.30
	smp_81	<LOQ	5.30
OUT num **39**	smp_82	<LOQ	5.45
	smp_83	<LOQ	5.40
	smp_84	<LOQ	5.44
OUT num **106**	smp_85	4.52	0.51
	smp_86	4.06	0.50
	smp_87	4.42	0.50
OUT num **107**	smp_88	4.41	0.29
	smp_89	4.65	0.29
	smp_90	3.08	0.31

Results



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Summary

- HPLC-MS method for separation of Arsenate $As^{(v)}$ and Arsenite $As^{(III)}$ using PGC column

- Sponge bacteria were able to grow and to reduce Arsenic oxidation levels in 0.35% saline water (10% salinity from native environment)
- Formation of $As^{(III)}$ at an average rate of 0.07mM/h

- Further studies on bacteria to isolate more Arsenic reduction mechanisms
- Possible future application for drinking water treatment

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Water Research Center
Tel Aviv University

Questions ??