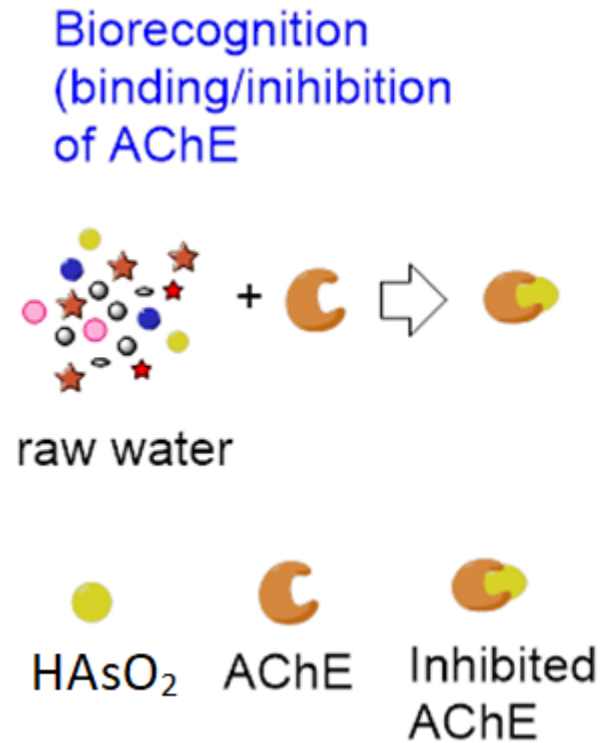


Testing an AChE sensor for arsenic analysis with groundwater samples from Shepley's Hill Landfill

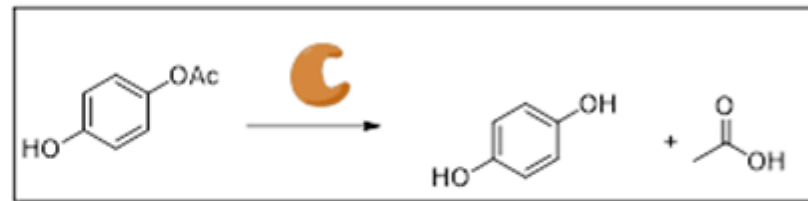
Tao Li, Robert Ford

Land Remediation & Technology Division (LTRD), Center for Environmental Solutions and Emergency Response (CESER), Office of Research and Development, US EPA, Andrew W. Breidenbach Environmental Research Center, 26 W. Martin Luther King Dr., Cincinnati, OH 45268

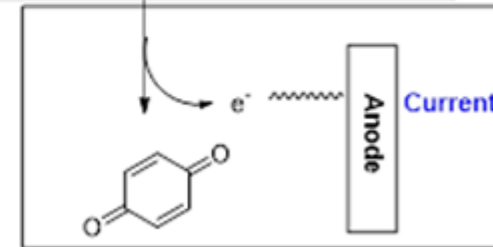
Scientific principle for Arsenite (As(III)) determination based on AChE inhibition



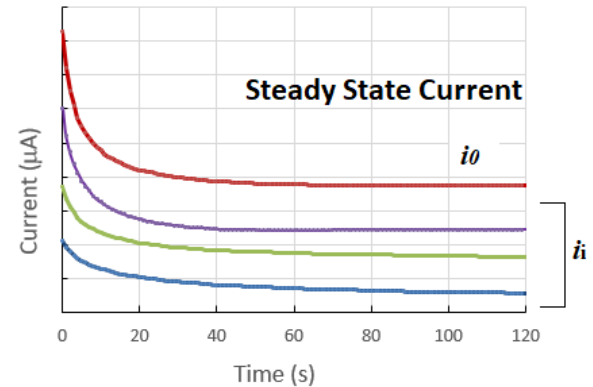
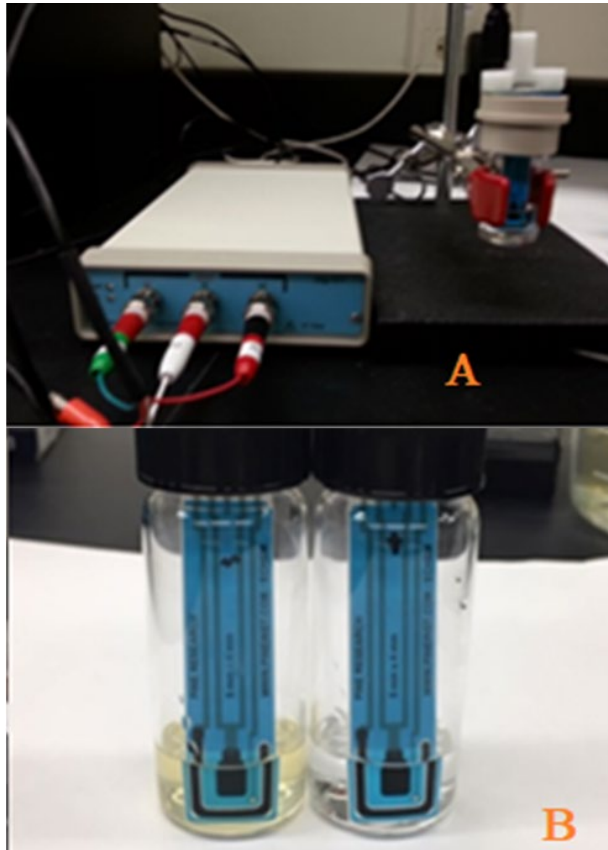
Reaction 1 Biochemical reaction Enzyme activity report with amperometry



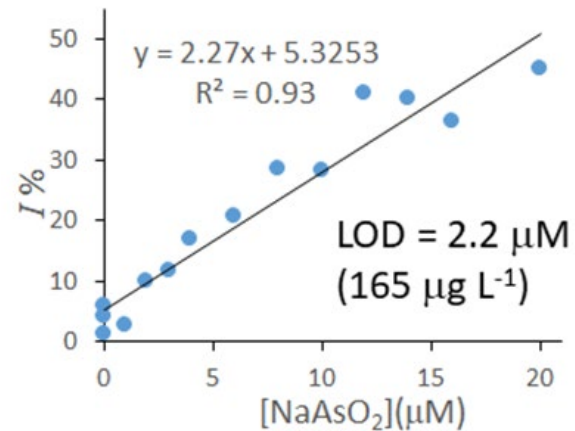
Reaction 2 Electrochemical reaction



Technology for the prototypic enzyme sensor – amperometric assay with screen printed electrode



$$I\% = \frac{A_0 - A_i}{A_0} 100\%$$



Advantages
cheap, disposable, matrix tolerant (???), field deployable.

Disadvantages:
low sensitivity (Freshwater chronic aquatic life criterion 150 ppb). Total iAs: to be developed

Roadmap to develop a practical sensor as guided by Technical Readiness Level

TRL	Characteristics	Activity	Deliverable
1 ✓	Scientific approach for As sensing	Options: chemical vs physical, biol vs non-bio, cellular vs biomolecule	AChE inhibition by As(III), mechanism of As(III) inhibition. % Inhibition = [As(III)]
2 ✓	Technology concept for sensing	Design reactions that links AChE activity decrease to transduction, transduction mechanism (physical vs chemical)	4-Acetoxyphenol hydrolysis followed by anodic oxidation of phenol product, characterization of the two reactions
3 ✓	Proof of the technical concept with a sensor prototype	Prototype (AChE immobilized on the anode of carbon SPE) development and characterization. Analytical method development.	Technology: A streamlined method to fabricate AChE electrodes; Scientific fundation: Kinetic mechanism for As(III) inhibition of the immobilized AChE. Application: a protocol for As(III) analysis
4 ✓	Technology characterization /validation in lab	Sensor product characterization, analytical method characterization, analytical method validation	Sensor: specification and stability, Analysis: dynamic range, linearity, LOD/ LOQ, selectivity, accuracy and precision (RSD) as a function of [As(III)].
5 ?	Technology validation in relevant environment	Understand field sampling need, evaluate characterizable variables in field samples, stability of field samples. Resource for the validation of field samples. Plan for the validation. Testing sensors to verify the hypotheses and identify issues with the technology and analysis developed in Stage 4.	Analysis of spatio-temporal variation of physical and chemical properties of water samples, arsenic concentration range. Developing sample preservation method. Fitting sampling for speciation into the in-field plan. Evaluate sample stability, sensor accuracy, precision.
6 ??	Technology demonstration in relevant environment	Characterize needs in field survey, Characterize lifecycle of the arsenic sensor, evaluate required resource and barriers to field deployment, identify issues with analysis in field.	-
7 to 9 ???	Prototype - α prototype - product	-	-

Q1: What did we know about the groundwater samples to be used for sensor evaluation?

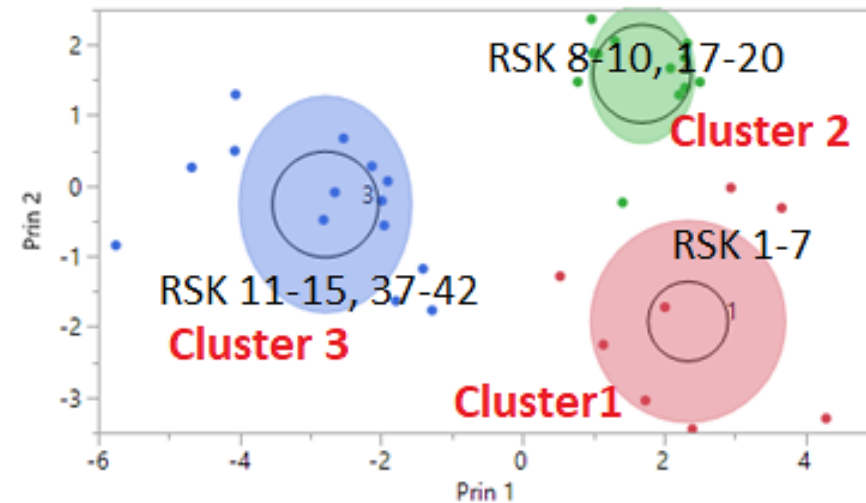
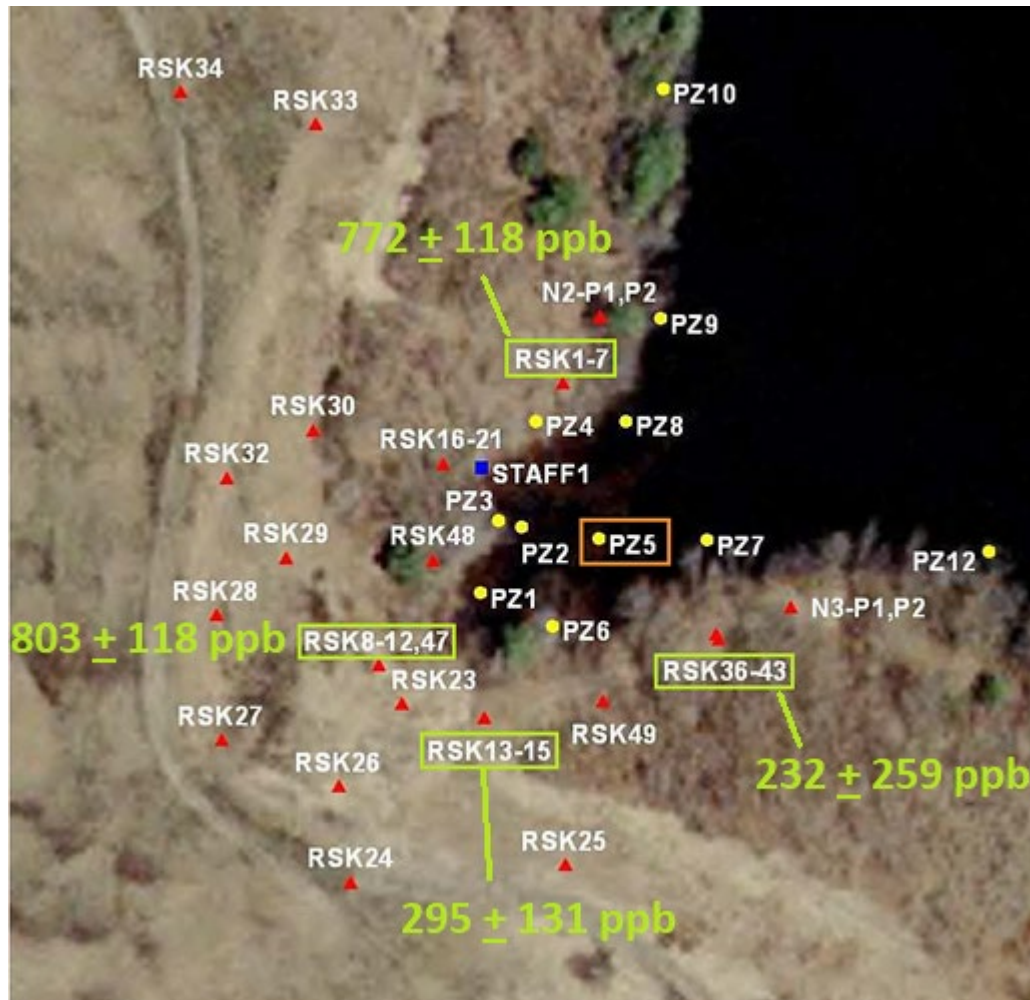
Arsenic pollution in Shepley's Hill Landfill Site



Image cropped from Google Map (17Apr23)

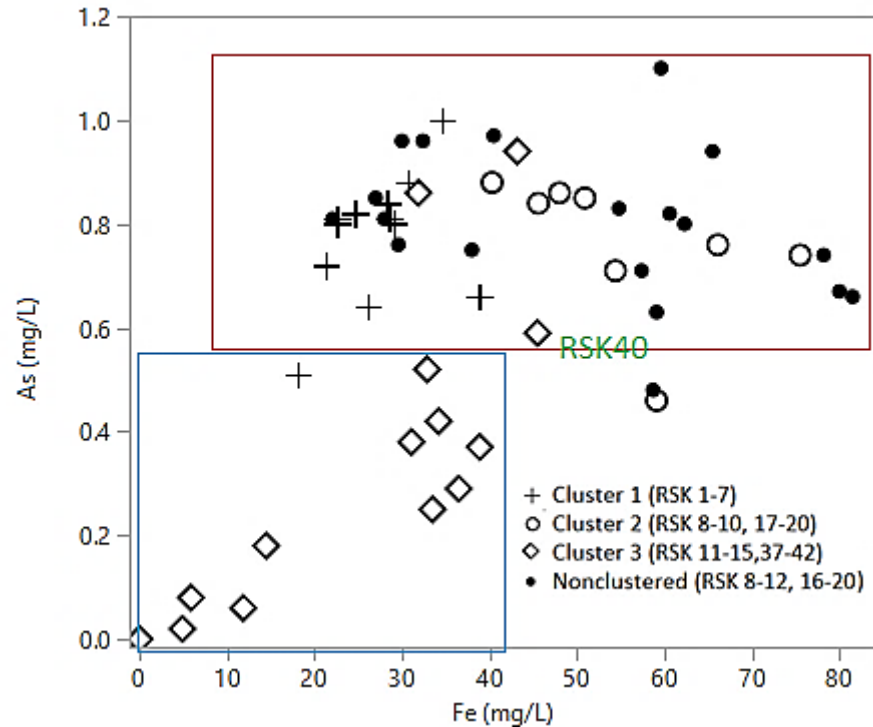
- *Cause of As pollution:* a landfill of waste, with a portion buried below water table. Landfill leachate mobilized geogenic As by reductive dissolution, listed as Superfund site in 1989
- *Landfill Area:* 84 acres, *Plow Shop Pond Area:* 30 acres.
- *As in groundwater:* Average flow $10.76 \text{ m}^3/\text{day}$, average [iAs] 430 ppb adjacent to Red Cove, estimated discharge of 17 Kg year^{-1} to the Red Cove portion of Plough Shop Pond.
- *Mitigations:* impermeable cap (increased As level), pump-and-treat (landfill-north 2006), hydraulic barrier wall next to Red Cove (2012), Red Cove sediment removal (2013).
- *Desirable:* high resolution iAs survey (spatial and temporal), As migration and sequestration mechanism, refined conceptual site model, iAs analysis on as-needed base (mitigation evaluation, events-related, incidents, etc). Cost-efficient remediation.

Groundwater Sample from Red Cove area and clustering (R. Ford. et al: Final Report Arsenic Fate, Transport and Stability Study Groundwater, Surface Water, Soil and Sediment Investigation Fort Devens Superfund Site Devens, Massachusetts)



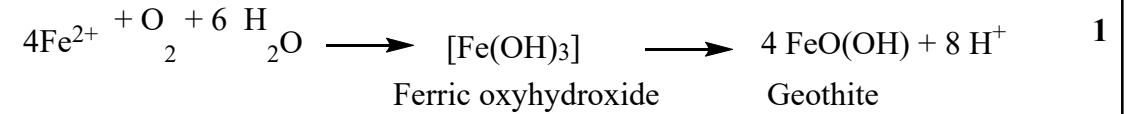
- Monitoring wells (RSK) of different depths around Red Cove. Piezometers (PZ) on the edge.
- Surveys of Mar2006-Sept2007: 53 samples from 12 different visits: cations, anions, alkalinity, ammonia, total organic carbon.
- Clustering was based on 12 chemicals in groundwater matrices. Water chemistry strongly depended on the well. Rare variations observed (e.g. RSK 7 fell in Cluster 1 or 2 at different times)

Arsenic sequestration (instability), correlation to Fe(II) oxidation observed in Cluster 3

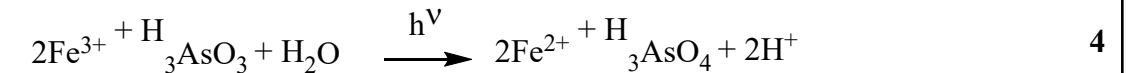
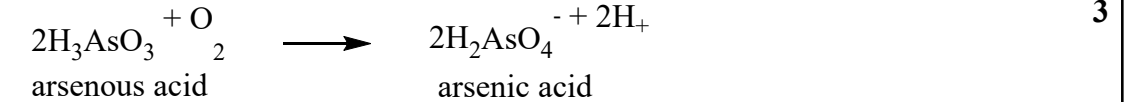


- **Cluster 3:** Correlation of [As] vs [Fe(II)] at low concentrations. Arsenic sequestration depends on Fe(II) oxidation.
- **Cluster 1, 2:** As was more stable in liquid phase, Fe (II) oxidation was less effective to sequester As (due to low O₂?)

Fe(II) oxidation and Rust formation



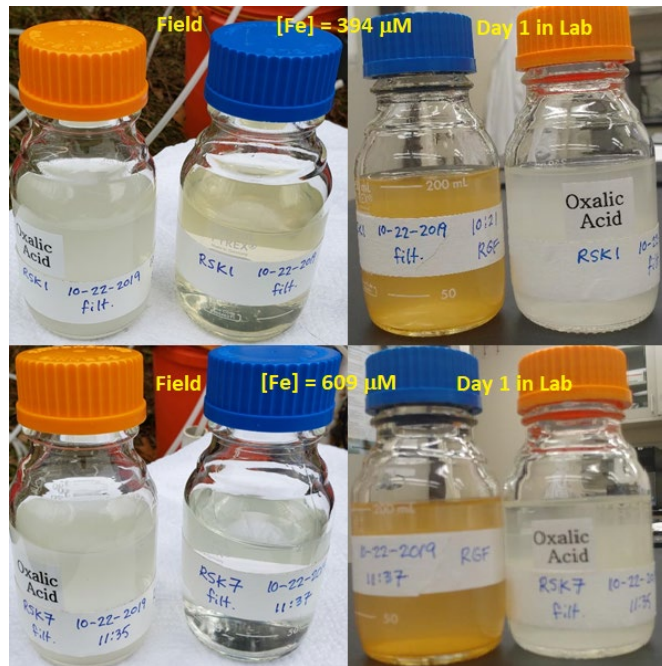
Arsenic (III) oxidation



Adsorption

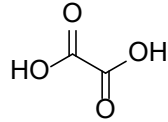


Q2: How to stabilize the iAs sample, and integrate the method in field sampling (small amount of time among many activities, air/light exposure)?

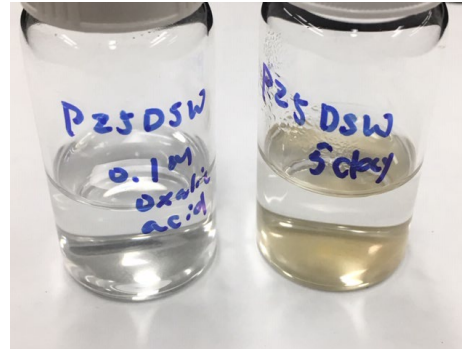


Iron oxidation when exposed to air:
Usually very quick (< 15 min)

Oxalic acid: sample preservation based on acidification + Fe complex formation



Chemical Formula: C₂H₂O₄
Exact Mass: 90.00



PZ25DSW with and without
0.1 M oxalic acid for 3 months
at RT

Impact

- Acidify solution to ~ pH 1.3
- Slow down the oxidations of Fe(II) and As (III)
- Forming complex with Fe(III) – (1-100 mM OA: [Fe^{III}(C₂O₄)₂]⁻, [Fe^{III}(C₂O₄)₃]³⁻)
- May reduce Fe(III) by decarboxylation (hv)
- Calcium oxalate solubility = 0.67 mgL⁻¹

Technology for sample preparation:

- Pre-load sample bottle with oxalic acid
- In field, add a fixed volume of groundwater to the sample bottles (< 10 min)
- Shipping samples to the lab (no chilling)

Reaction	Reaction	Equilibrium constant
1	$\text{H}_2\text{C}_2\text{O}_4 = \text{H}^+ + \text{HC}_2\text{O}_4^-$	$K_{a1} = 5.6 \times 10^{-2}$ (pK _{a1} = 1.25)
2	$\text{HC}_2\text{O}_4^- = \text{H}^+ + \text{C}_2\text{O}_4^{2-}$	$K_{a1} = 6.2 \times 10^{-5}$ (pK _{a2} = 4.14)
3	$[\text{Fe}^{\text{III}}\text{C}_2\text{O}_4]^+ = \text{Fe}^{3+} + \text{C}_2\text{O}_4^{2-}$	$K_d = 3.98 \times 10^{-10}$ M
4	$[\text{Fe}^{\text{III}}(\text{C}_2\text{O}_4)_2]^- = [\text{Fe}^{\text{III}}\text{C}_2\text{O}_4]^+ + \text{C}_2\text{O}_4^{2-}$	$K_d = 6.31 \times 10^{-21}$ M
5	$[\text{Fe}^{\text{III}}(\text{C}_2\text{O}_4)_3]^{3-} = [\text{Fe}^{\text{III}}(\text{C}_2\text{O}_4)_2]^- + \text{C}_2\text{O}_4^{2-}$	$K_d = 3 \times 10^{-21}$ M
6	$\text{Fe}(\text{C}_2\text{O}_4)_{(s)} = \text{Fe}^{2+} + \text{C}_2\text{O}_4^{2-}$	$K_{sp} = 2 \times 10^{-7}$ M ² (solubility 0.97 g/L 25 °C)
7	$[\text{Fe}^{\text{II}}(\text{C}_2\text{O}_4)_2]^{2-} = \text{Fe}^{2+} + 2\text{C}_2\text{O}_4^{2-}$	$K_d = 2 \times 10^{-8}$ M ²
8	$[\text{Fe}^{\text{II}}(\text{C}_2\text{O}_4)_3]^{4-} = \text{Fe}(\text{C}_2\text{O}_4)_2^{2-} + \text{C}_2\text{O}_4^{2-}$	$K_d = 6.6 \times 10^{-6}$ M

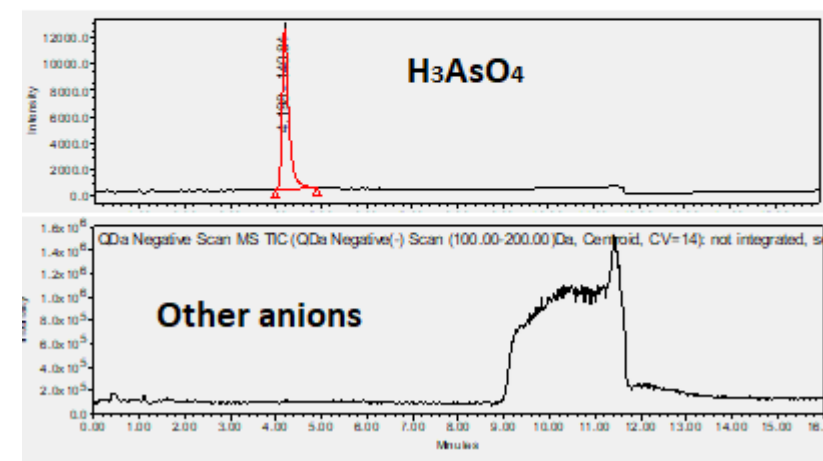
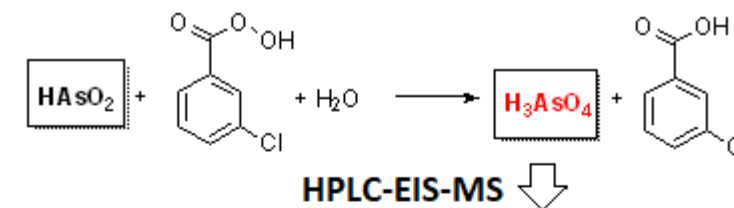
iAs is stable for 15 days in 5 mM Oxalic acid as measured with an HPLC-EIS-MS method (May 2022)

Recovery of As (V) (%)

Sample	As(V) (μM)	As(III) (μM)	Day 1	Day 2	Day 3	Day 4	Day 8	Day 15
1	0.2	0.8	100	90	110	94.5	94.5	95
2	0.5	0.5	92	98	106	92.2	93.2	98.8
3	0.8	0.2	97.5	102.5	98.8	100.5	96.3	100.8

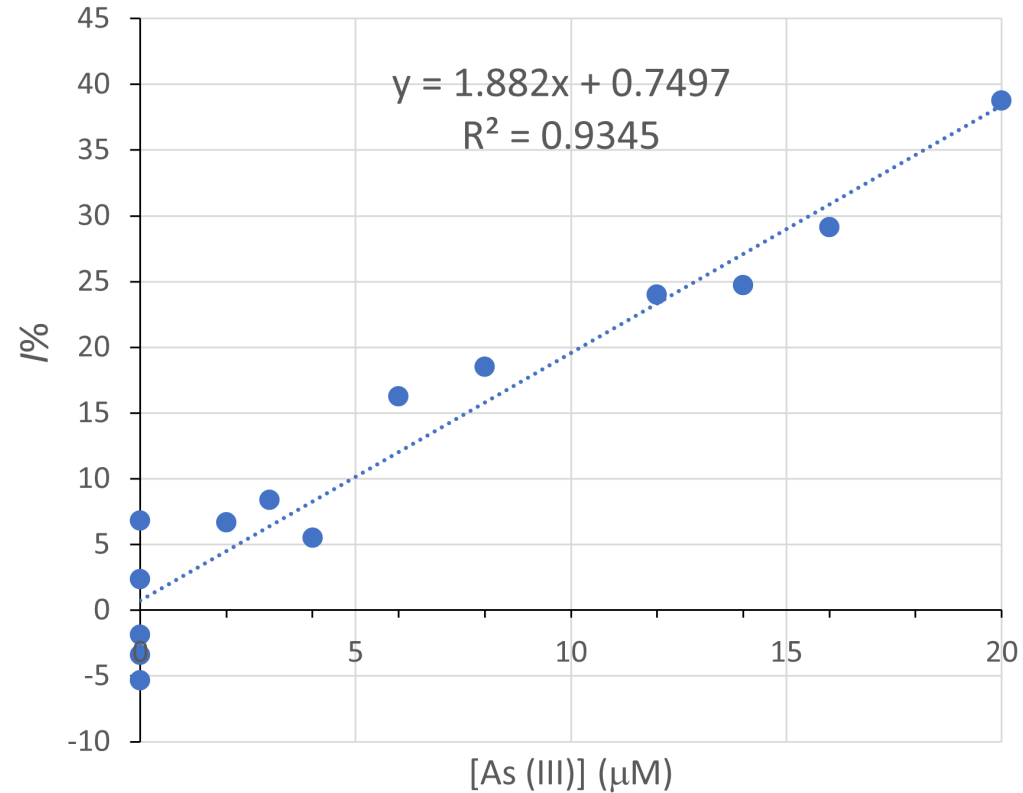
Recovery of iAs (%)

Sample	As(V) (μM)	As(III) (μM)	Day 1	Day 2	Day 3	Day 4	Day 8	Day 15
1	0.2	0.8	102	97	105	95.8	99.4	102.7
2	0.5	0.5	100	92	103	104.9	94.1	103.5
3	0.8	0.2	95	88	101	105.9	97.3	106.1



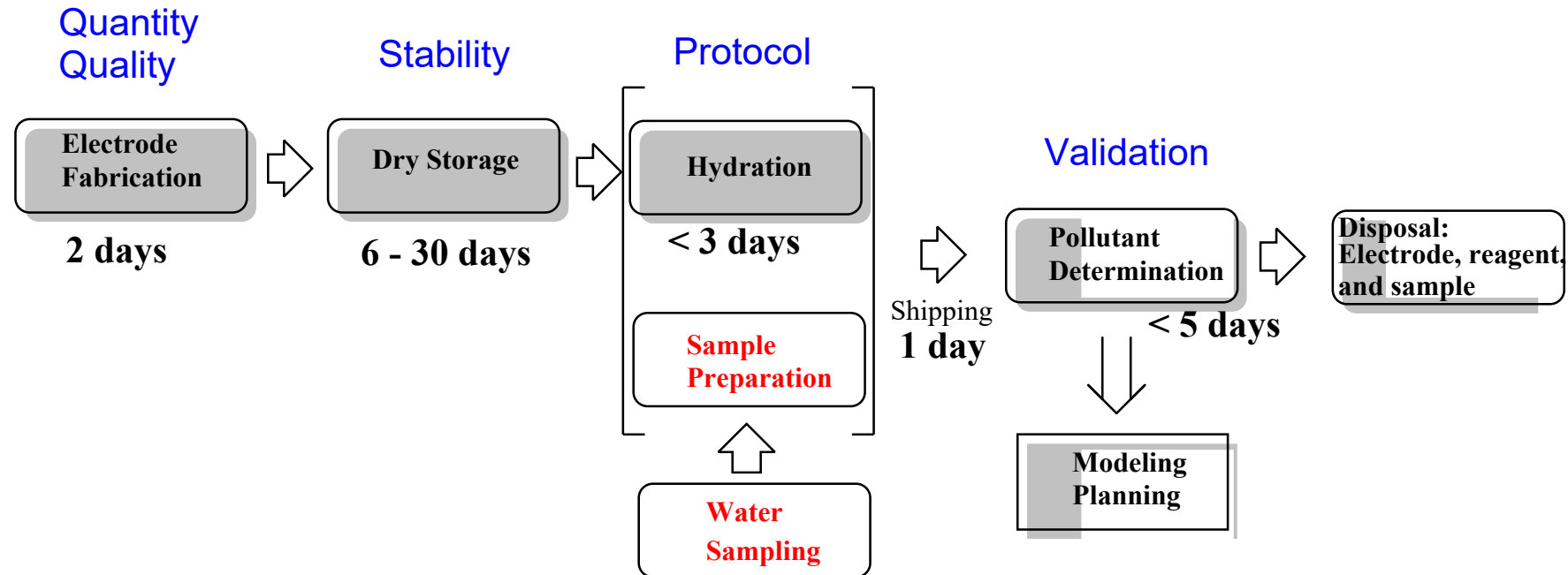
Stabilization with oxalic acid in Fe-containing matrix is yet to be evaluated

AchE sensor in Tris-Oxalate (pH 8.0, 0.1M) buffer



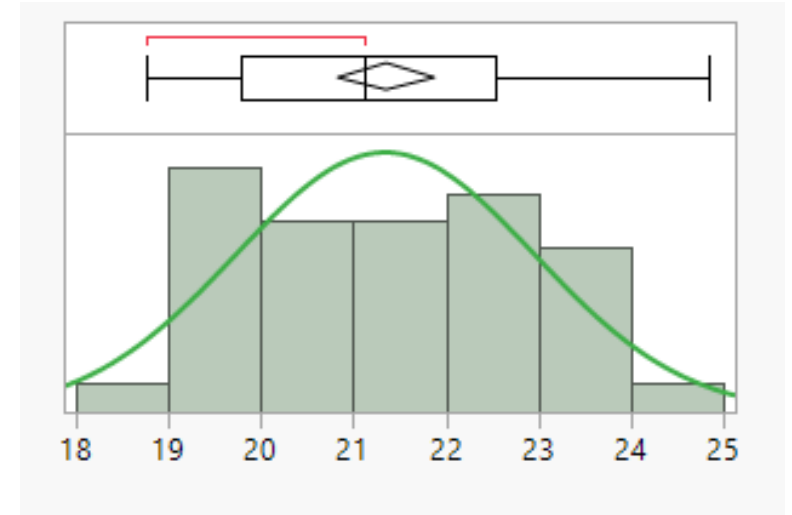
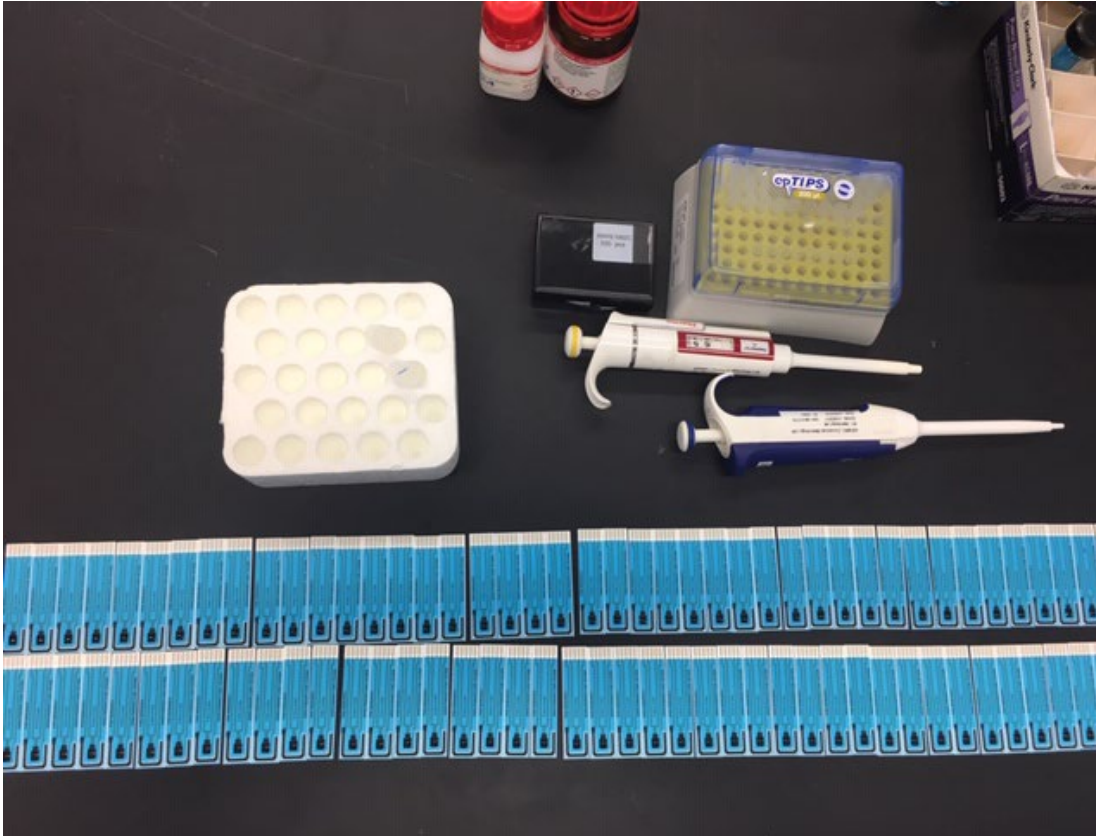
- Sensitivity (Slope): 83% of that in 0.1M, pH 8.0 Tris HCl
- Precision: similar as suggested by R^2
- Calibration needed. [Oxalate] vs sensitivity: not studied
- Potential issues: high RSD in low concentration measurements

Q3: Is the sensor prototype ready for TRL-5 level assessment?



Lifecycle of the AChE sensor and Preparation work for TRL-5 level evaluation

Manual fabrication of AChE sensor, productivity, quality



- Process
 - Stock solution preparation
 - Deposit the enzyme solution on the working electrode
 - Crosslinking with glutaraldehyde
 - Curing/Dry storage
- Productivity: 100 electrodes/batch in 2 days
- Activity of the sensor (16Aug19):
 A_0 , 18-24.8 μA , Mean = $21.3 \pm 1.6 \mu A$, n = 39

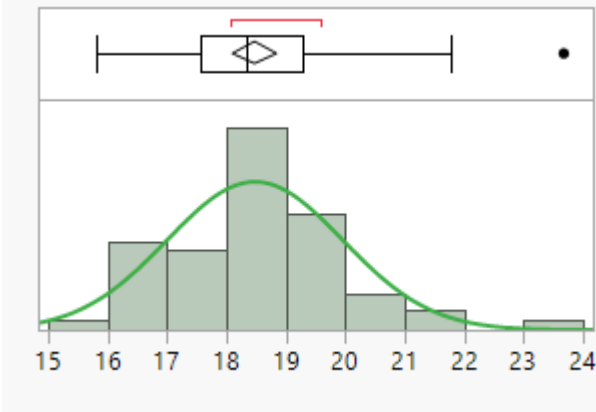
Did A_0 variation cause systematic error? (07Jun29) – yes, but small

Experiment:

The Electrodes (n = 63)

Range: 15.8-23.7 μA

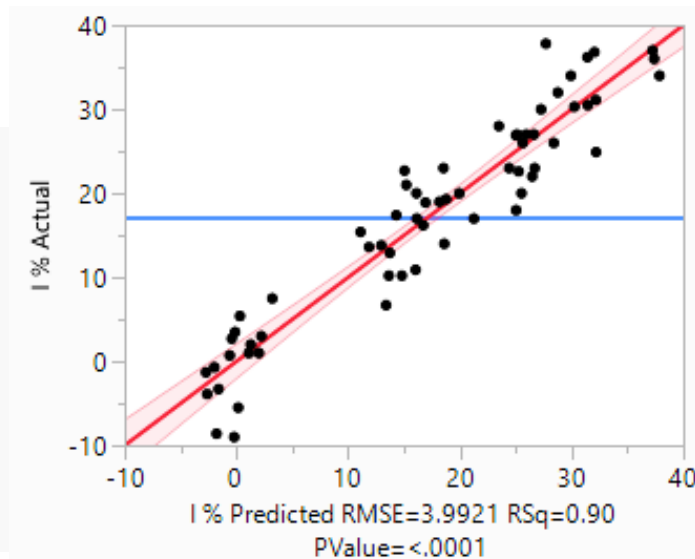
Mean = $18.5 \pm 1.5 \mu\text{A}$



As (III): 0 - 18 μM

Inhibition: -9% – 38%

Model: $I\% = a [\text{As(III)}] + b A_0 + c$



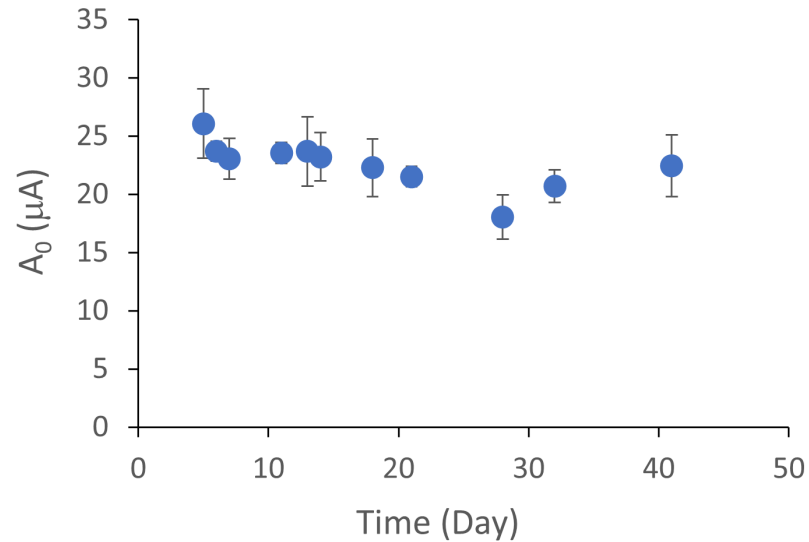
Findings

Source	DF	Sum of Squares	F Ratio	Prob > F
A_0	1	138.0	8.66	0.0046
[As(III)]	1	8423.5	528.56	<.0001

- A_0 did have a significant impact on $I\%$. Higher A_0 correlated to higher $I\%$
- Overall, the impact of A_0 (range 15.8 – 23.7 μA) was 1.6% of that from 0 – 18 μM As(III)
- No significant interaction between A_0 and [As(III)]

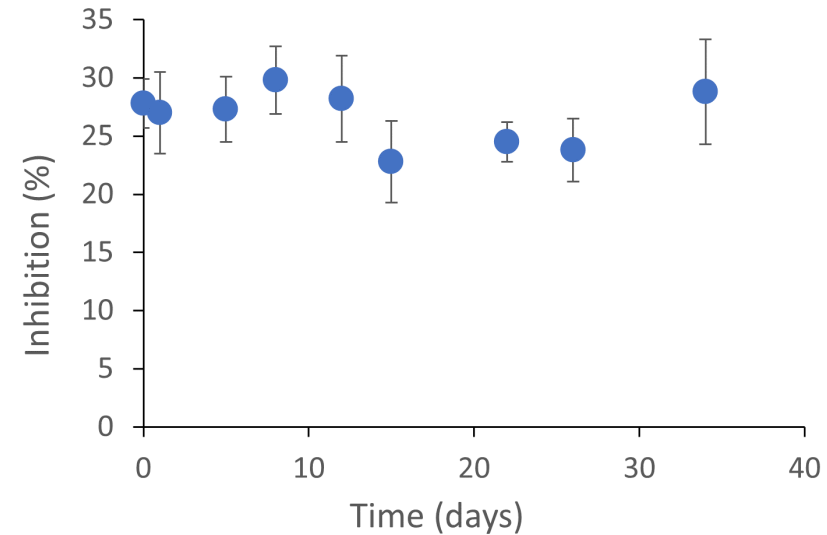
Specification of the electrode: $A_0 > 16 \mu\text{A}$

Storage Stability: sensor activity A_0 and sensitivity to 8 μM As(III)



Dry storage stability evaluated over 41 days,
5-16 sensors measured each time.
Overall A_0 : 16.5 – 30.1 μA , Distribution: normal
Mean A_0 = 23.05 ± 3.06 μA , $n = 77$

Hydration in 0.1 M phosphate-Tris, pH 7 for 24-36 h



Inhibition evaluated with 8 μM As(III).
Overall: Inhibition 18.6 – 35.6%. Distribution: normal
Mean $I\%$ = $26.57 \pm 3.73\%$, $n = 50$

TRL-5 level assessment results (May – Nov, 2019)

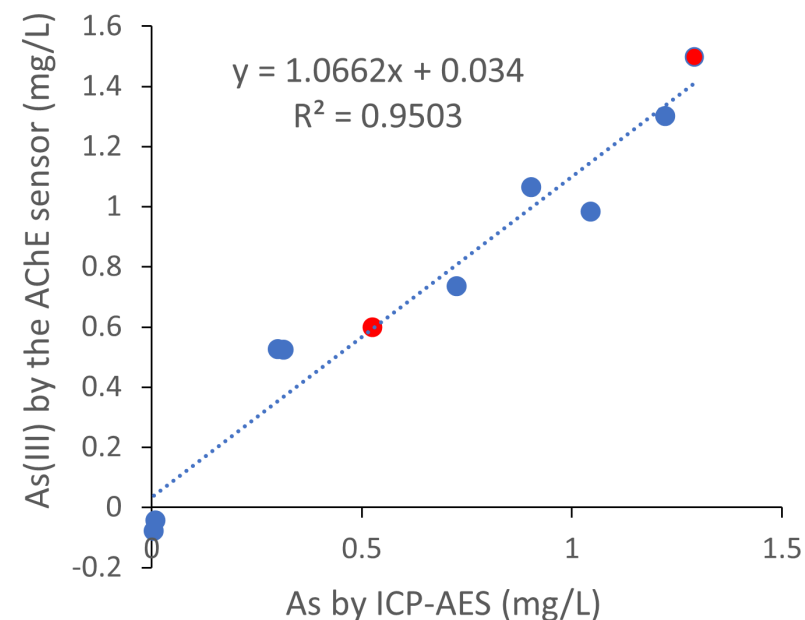
Accuracy evaluation with field samples (14May19)

Field sampling/shipping: Two (GW) from the source of landfill, two (SW) from a piezometer in Red Cove, chilled, overnight shipping.

Samples: standard addition (5 μ M As(III)) to field samples + 2 lab references. Assuming All iAs was As(III).

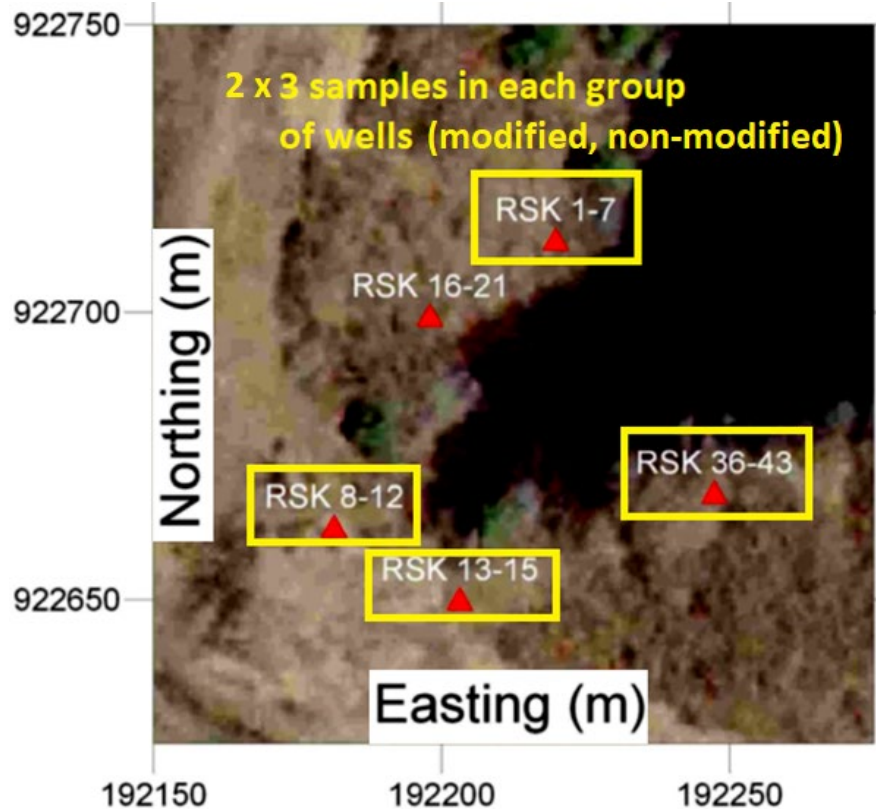
Overall correlation: $R^2 = 0.9503$, Slope = 1.0662 in the range of 0 – 20 μ M (1.5 mg/L) As(III)

Ca, Fe, Mg Mn, Na, and S did not interfere with the accuracy



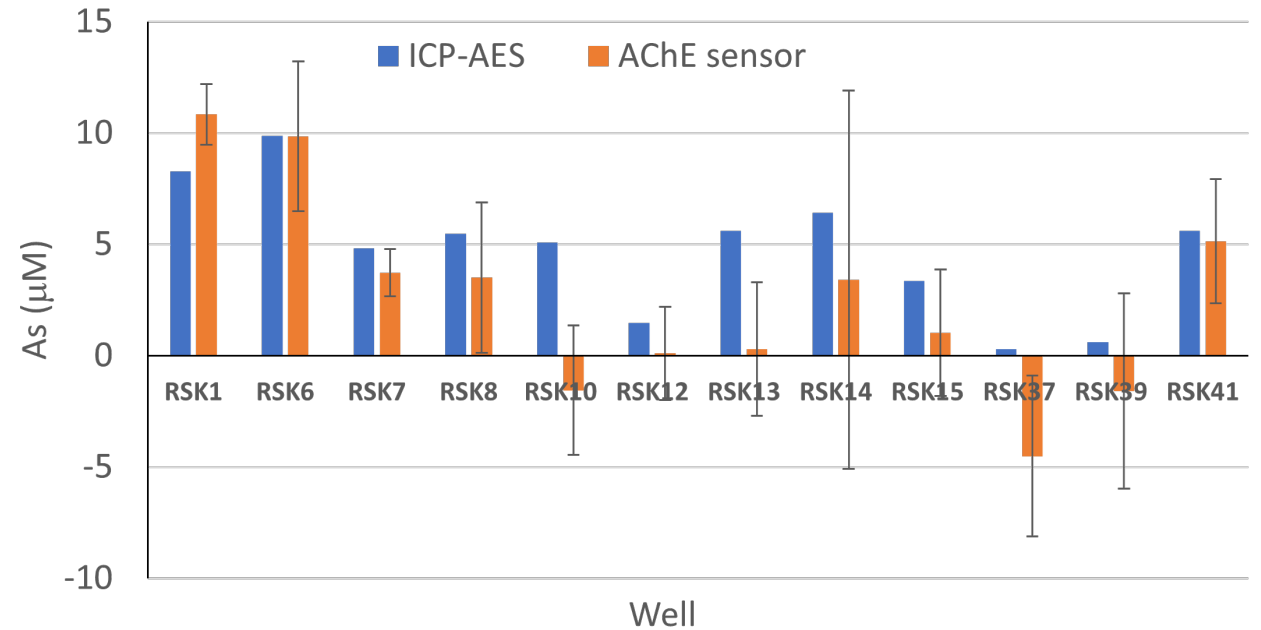
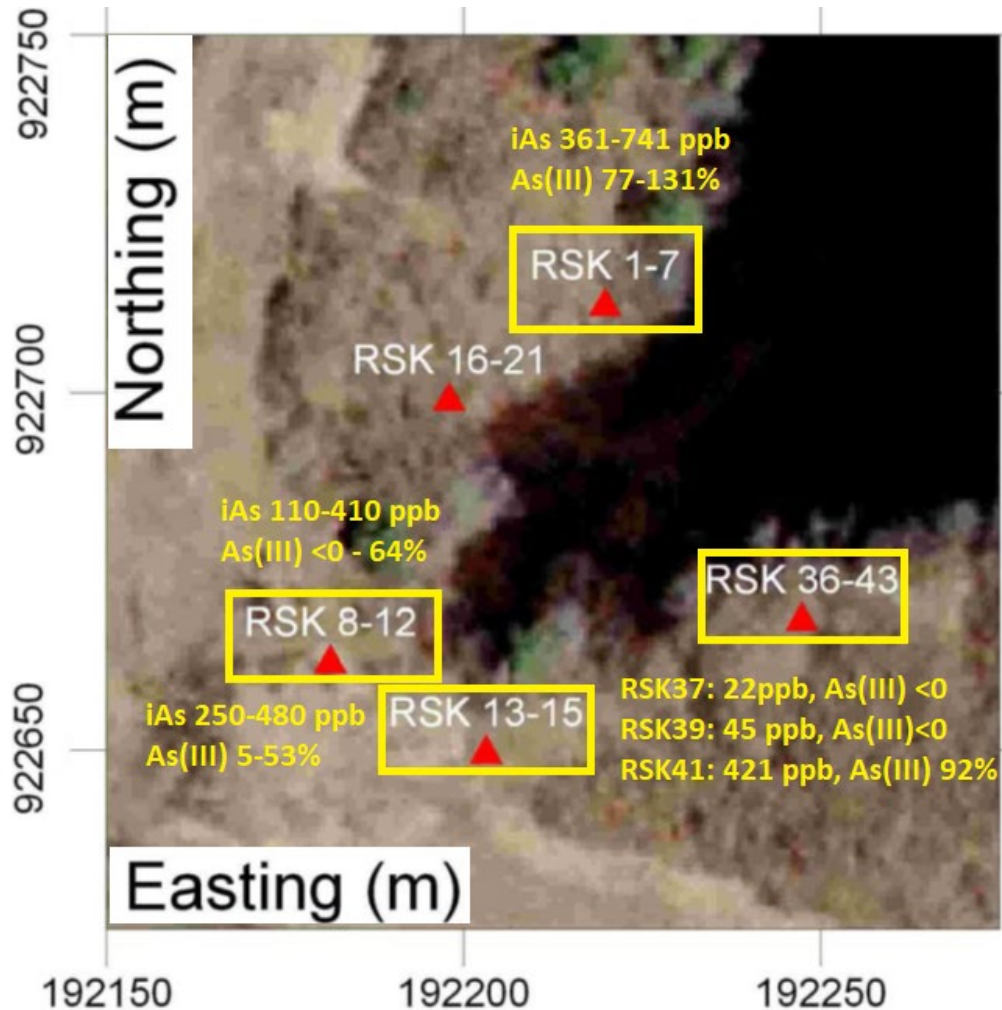
SAMPLE	Source	As (mg/L)	Ca	Fe	Mg	Mn	Na	S
SHP-2016-6B-1	GW	0.726	44.2	0.42	4.72	1.34	161.2	89.6
SHP-2016-6B-1-A	GW+5 μ M	1.045	44.8	0.409	4.78	1.33	162.2	88.4
SHP-2016-6B-3	GW	0.903	23.8	0.052	2.68	0.318	38.5	9.03
SHP-2016-6B-3-A	GW+5 μ M	1.222	24.2	0.06	2.71	0.323	39.2	9.17
PZ5-SSW	SW	<0.005	11.9	0.338	2.05	0.049	35.9	1.91
PZ5-SSW-A	SW + 5 μ M	0.314	12.1	0.324	2.06	0.05	35.8	1.90
PZ5-DSW	SW	0.01	27.6	9.77	3.57	1.12	21.8	0.96
PZ5-DSW-A	SW + 5 μ M	0.301	27.3	9.9	3.53	1.1	22.0	1.04
Lab Ref 8um	Lab std	0.527	0.556	<0.037	<0.134	< 0.007	<0.191	1.85
Lab Ref 20um	Lab std	1.292	0.974	<0.037	<0.134	< 0.007	0.547	1.87

Evaluation with field samples around Red Cove (24-25Oct2019): precision issue, sample preservation, understanding of the As sequestration



- Two sets of samples (12 each). One set was modified with 46 mM Oxalic acid. The other was not. For As(III) assay,
- Shipping: no temperature control, arrived in 36 h. Rust formation observed in non-modified samples.
- On the day of arrival: Tris was added to adjust the pH to 8.0 with the oxalic acid-modified samples. For non-modified samples, Oxalic acid was added first to dissolve Fe(III), then the pH was adjusted. Both sets were analyzed for As(III) with the AChE sensor.
- Meanwhile, samples were analyzed with ICP-AES for total iAs and other ions

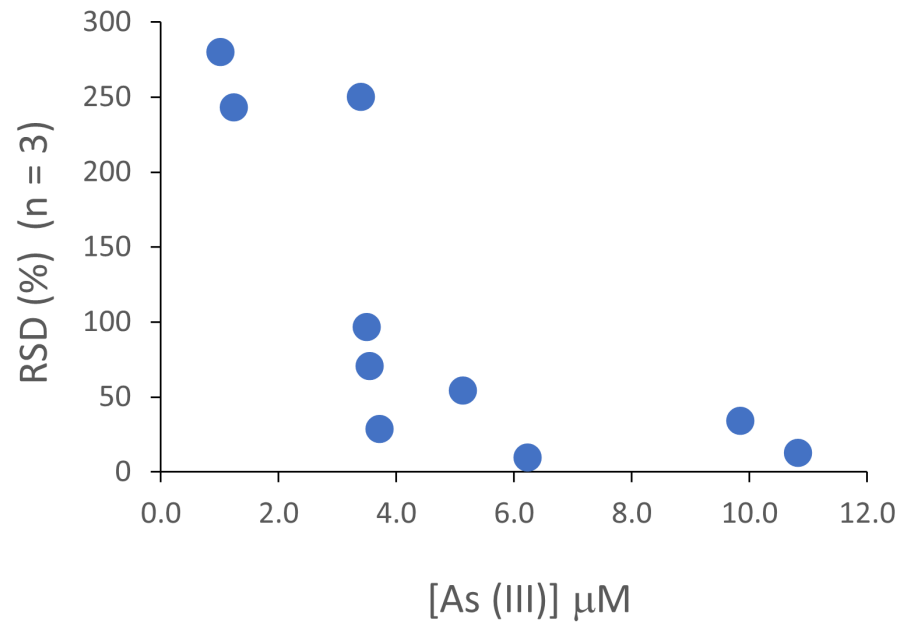
Speciation with ICP-AES (total As) and the AChE sensor (As(III)) with oxalic acid preserved samples



General impression: High As(III) in North (RSK 1-7, Cluster 1), significant variation in West (RSK 8-15, Cluster 2 and 3) and south (RSK 36-41, Cluster 3). RSK 41 deeper than RSK 37 and 39 (less oxygen)

Sensor precision: very poor, especially at low concentration, reliability of results questionable. – main target for TRL 6 stage work

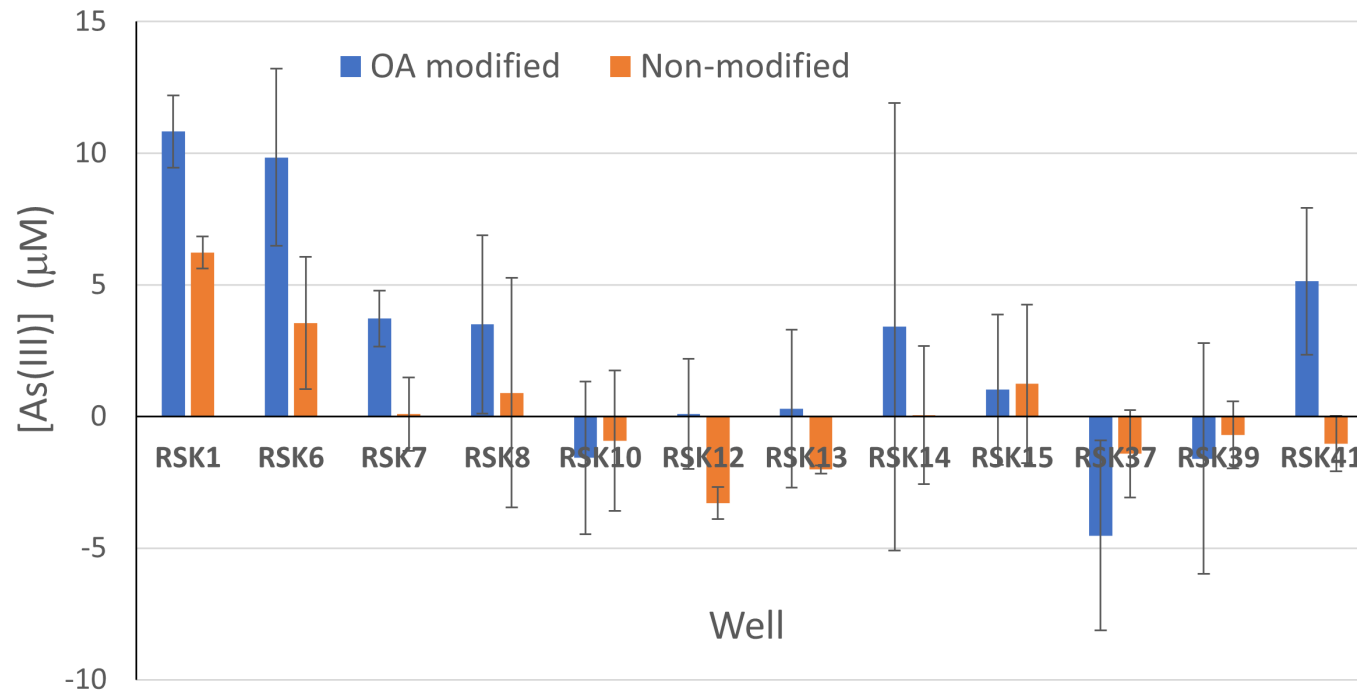
Sensor precision: dependence on [As(III)] and other impact factors



RSD for As(III) determination in 0.1 M Tris-Oxalate, pH 8

Source	Nature of sources	Approach to improve precision
[As(III)]	mechanism of inhibition	pick the best AChE from a pool of options
Sensor fabrication method	Crosslinking with glutaraldehyde	Change immobilization chemistry,
-	Fabrication error (human, equipment)	develop precise fabrication method. Identify/Characterize/minimize systematic errors (such as A_0)
Sensor storage, hydration	stability of immobilized AChE	Re-evaluate and optimize the new methods
Measurement protocol	variation of water matrices	Characterization matrix impact, customize sample preparation, measurement, and calibration

Did oxalic acid help to prevent As(III) oxidation? Can we characterize sample stability as a function of matrices?



- Significantly different based on Pair-wise t-test (n =3). Pair difference: RSK 41 > RSK 7 > RSK6 > RSK1. RSK 41 sample was least stable when exposed to air.
- For most samples: errors too big to tell (low As: RSK 10, 12, 13, 15, 37, 39; medium As: RSK 7, 8, 14)
- Stability can be better characterized with HLPC-ESI-MS

Speciation was apparently stabilized by oxalic acid modification. Comparing stabilized samples with original samples may help to understand the impact of matrix on sample stability

Summary:

- **TRL is an important tool for sensor development:** determine appropriate level for the next step, set goals consistent with product development, plan and allocate resource (preparation > actual test), use findings to revise development plan: check scientific principles in realistic environment, revise technologies, develop key analytical tools (e.g. HPLC-EIS-MS for speciation) to facilitate technology development, etc.
- **Findings in TLR-5 level development:**
 - The enzyme-based sensor tolerates complex matrices. Carbon screen-printed electrode remains a viable technology platform.
 - Oxalic acid modification has helped to stabilize speciation, but more study is needed to understand matrix impact.
 - Current method (pipetting, glutaraldehyde crosslinking) for sensor fabrication is not suitable for TLR-5 and higher level development due to large errors.
 - In Red Cove area, As in the north area was mostly As(III), while in the west and south the speciation was mixture of As(V) and As(III). In the south side, low As mobility strongly correlated to low Fe mobility.

Disclaimer and acknowledgement

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