Probing the Intrinsic Ability of Particles to Generate Reactive Oxygen Species and the Effect of Physiologically Relevant Solutes

Suzanne Paulson[1], Cort Anastasio[2], Alam Hasson[3]

[1]Department of Atmospheric and Oceanic Sciences, University of California at Los Angeles

[2]Land Air and Water Resources, University of California at Davis

[3]Department of Chemistry, California State University at Fresno

Outline

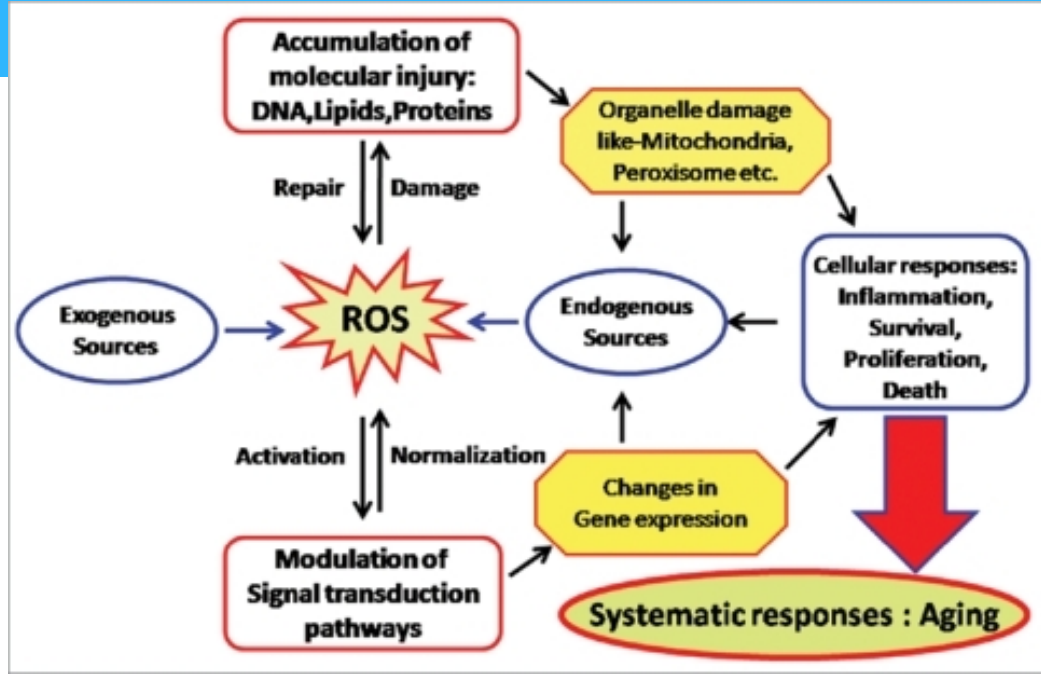
- Introduction
- Brief Description of Field Campaigns
- Laboratory Results
- Field Results:
  - Quinones
  - DTT
  - OH & H_2O_2
- Conclusions
- Acknowledgements

Reactive Oxygen Species

- $O_2 + e^- \rightarrow O_2^-$
- $O_2^- + 2H^+ + e^- \rightarrow H_2O_2$
- $H_2O_2 + e^- \rightarrow OH^- + OH$

Other ROS: HO_2, ROOH, ClO^-, $O(^1D)$, ONOO etc.

Three types of ROS



- Endogenous ROS
- Exogenous ROS:
  - H_2O_2, ROOH etc. present in the particles when they are inhaled "prompt ROS"
  - ROS produced by components in the particles once they are inhaled "generated ROS"
- There are lots of health endpoints. Different health outcomes may be sensitive to different forms of ROS
- There appears to be some overlap in chemical components that cause exogenous ROS formation and elicit endogenous ROS formation.

Field Campaigns

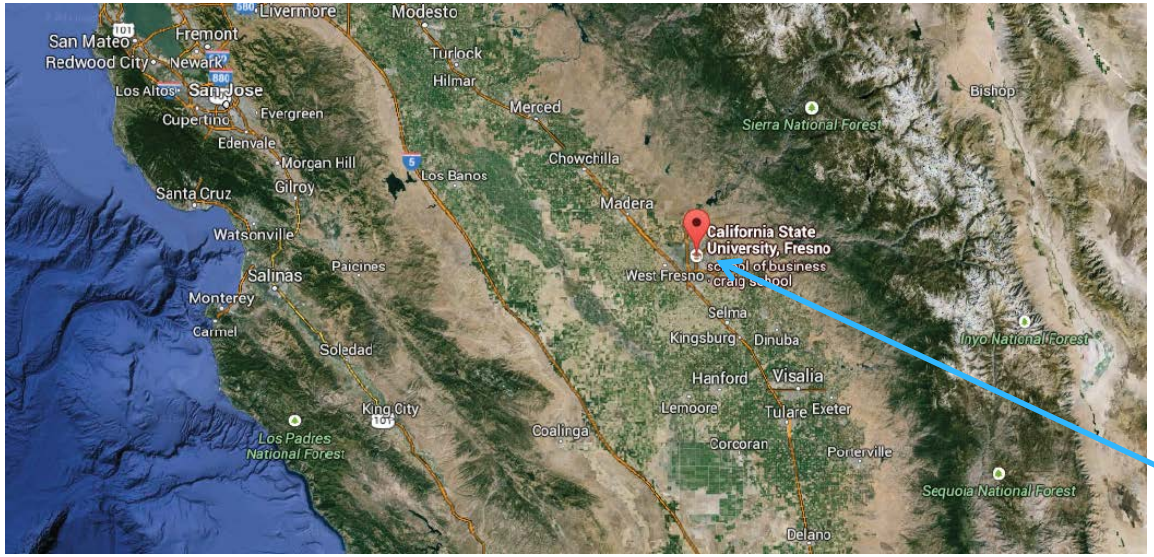
Field Measurements

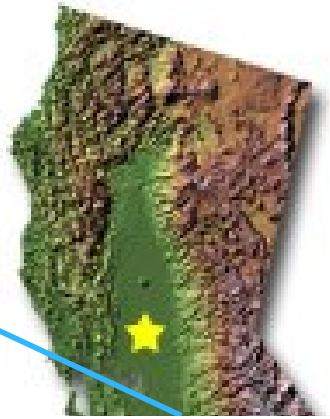
- Winter in **Fresno**, CA → Urban/Agricultural/ Residential wood burning (= Biomass burning)
- Summer in **Claremont** (Los Angeles) → urban/ photochemical SOA
- Samples collected on filters (hi-vol and 47 mm)
- Morning (6 hours), afternoon (6 hours)

Overnight (12 hours). Overnight samples had ~ 2x mass

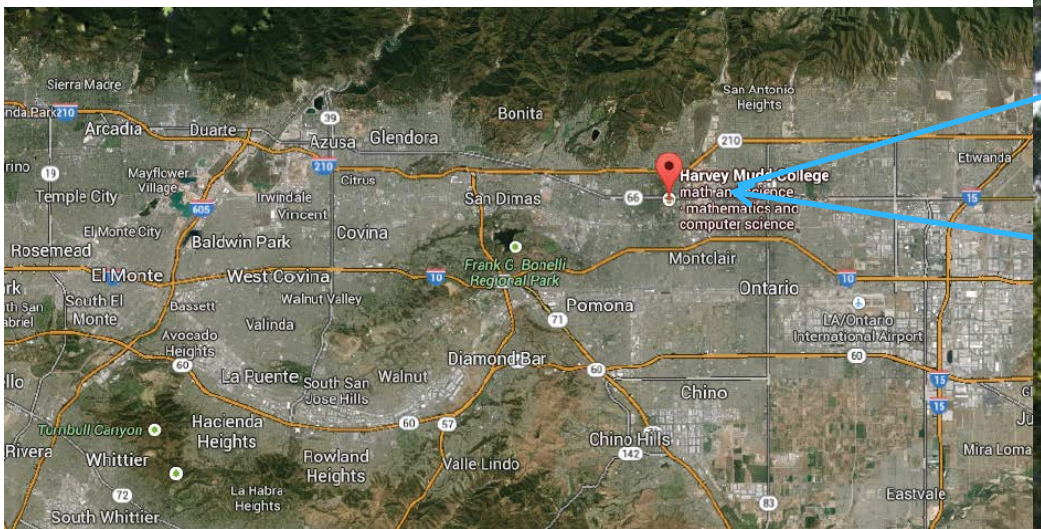
FIELD CAMPAIGNS

Fresno





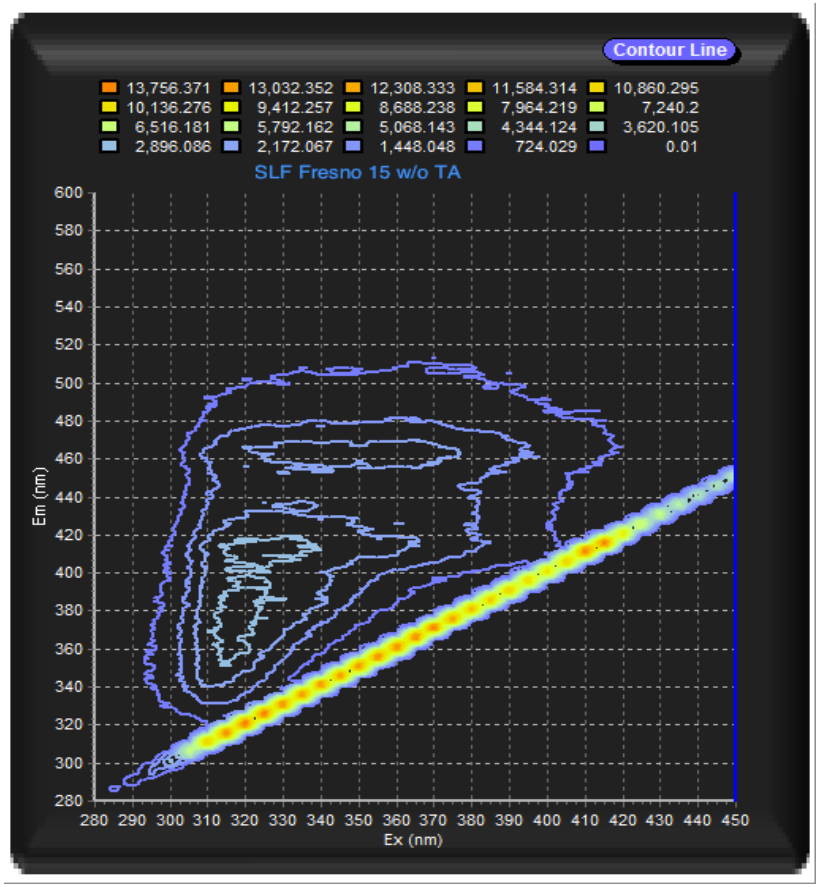
Claremont



Assays

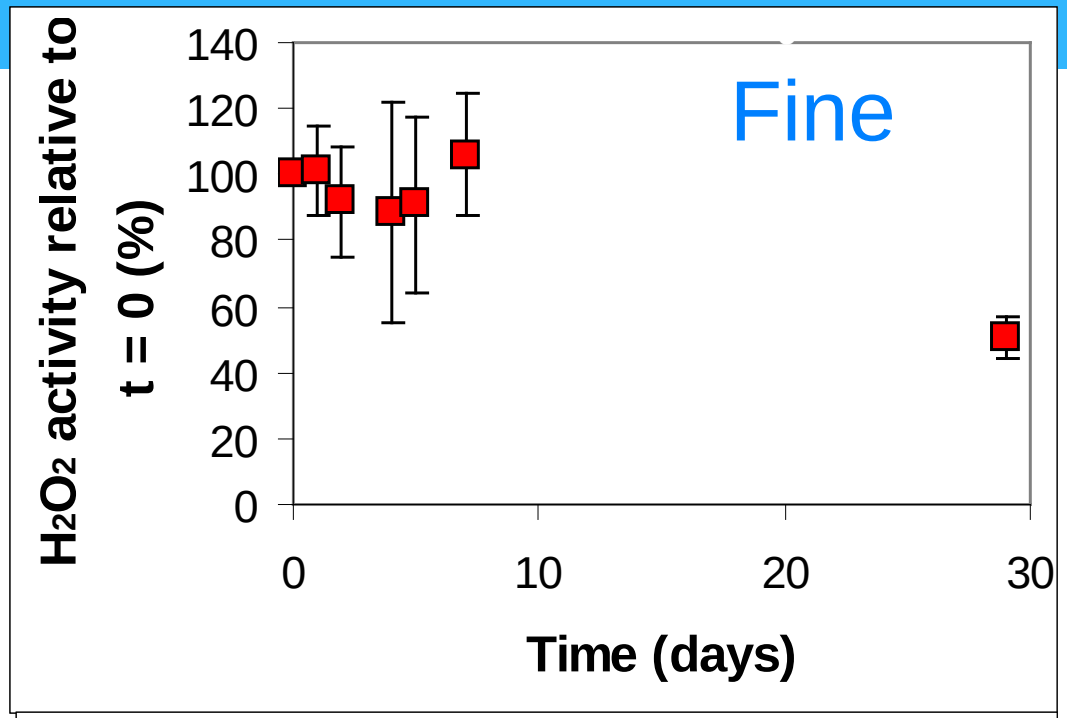
- Measure ROS and species believed to generate ROS:
  - OH (mostly on site)
  - DTT
  - H_2O_2
  - Quinones
  - Soluble Transition metals with ICP-MS
  - Soluble Fe(II), Fe(III) (mostly on site)
  - Brown Carbon (UV abs. on filters)
  - Mass

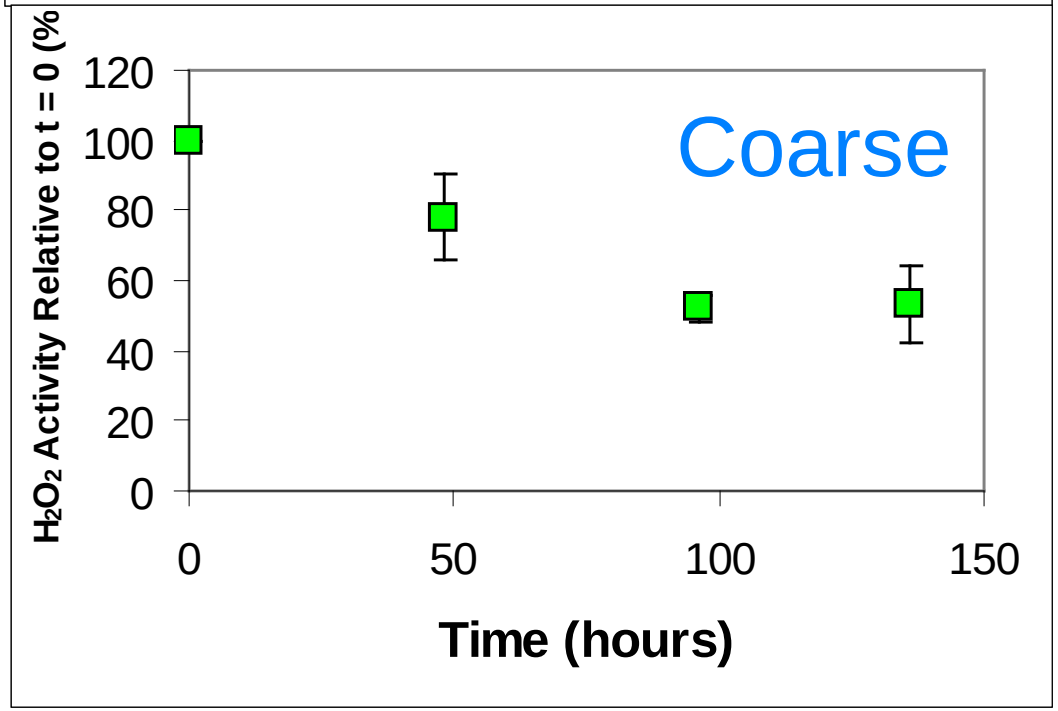
3-D Fluorescence scan of Jan 19 2013 Fresno overnight sample



Signal Persistence Over Days to Weeks

(Wang et al., 2012)

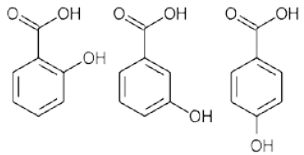


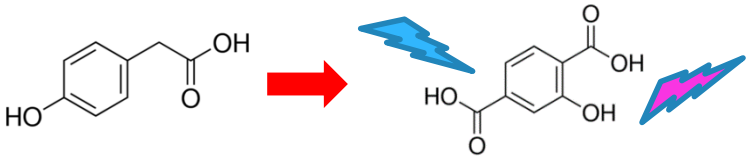


Measuring Exogenous ROS

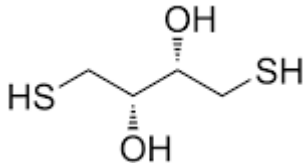
Many ROS Assays: OH, H_2O_2 and DTT were used here.

- OH (benzoate, terephthalate)

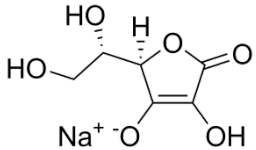




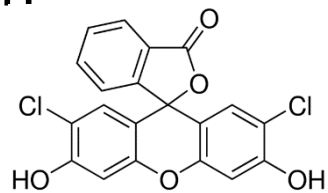
  - OH is the most reactive ROS, responsible for lipid peroxidation, DNA damage and protein oxidation
- H_2O_2 (horseradish peroxidase)
  - An intermediate in ROS chemistry; assay measures steady-state concentration instead of cumulative formation.
- DTT consumption
  - Mimics biological reductants, is oxidized by ROS-generating PM components (e.g., metals and quinones)



- Ascorbic acid consumption



- Electron Paramagnetic Resonance
- Dichlorofluorescin



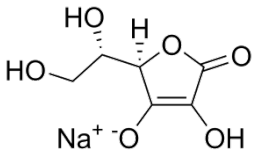
Several Extraction Solutions for ROS

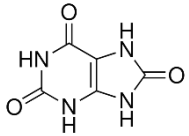
We mostly use “Simulated Lung Fluid” or “SLF”:

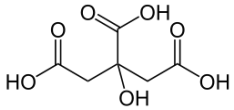
- pH 7.4 /Phosphate
- NaCl
- Lung Antioxidants & Citrate,

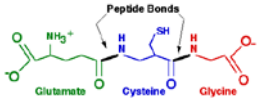
  100 – 300 µM

  - Ascorbate, reductant
  - Glutathione, chelator
  - Urate
  - Citrate, protein mimic, chelator



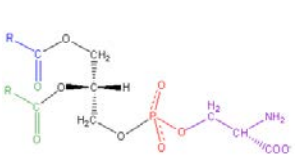


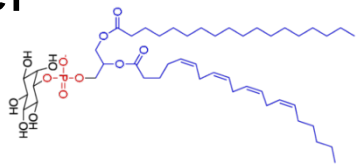




Also used:

- Concentrated phosphate buffer
- Lung Lavage Fluid
- Water @ pH 3.5





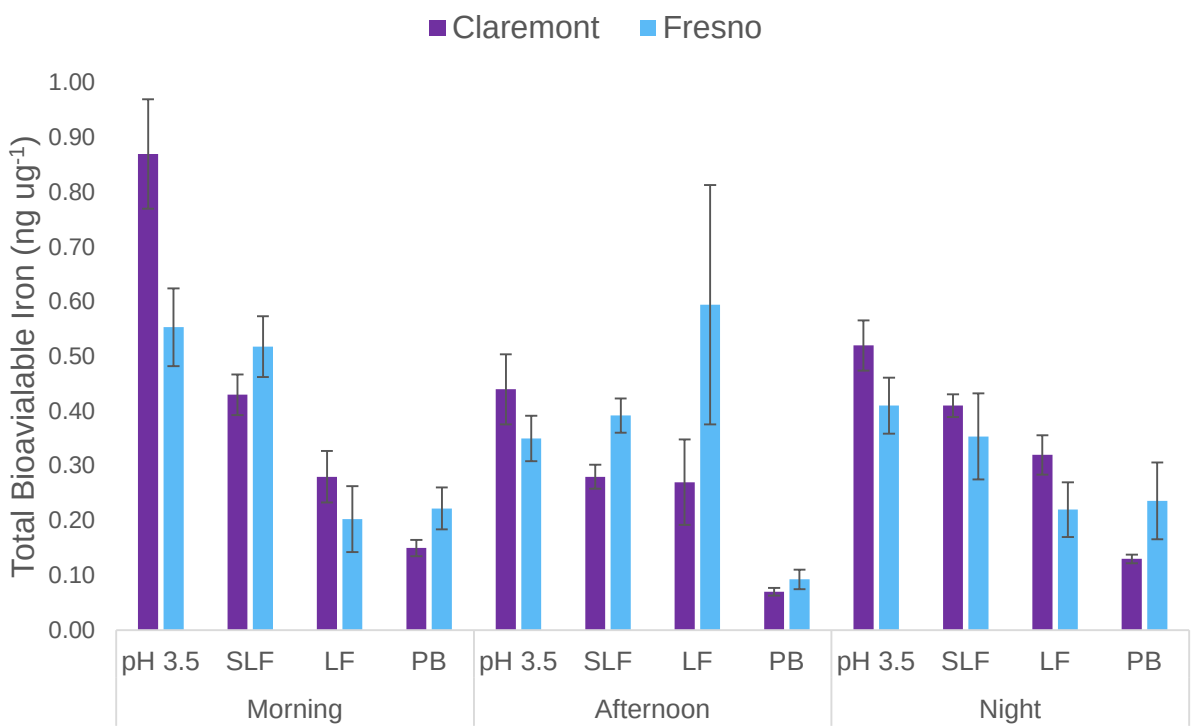
Iron solubility is strongly dependent on extraction solution; more so for Claremont than Fresno

pH 3.5: water @ pH 3.5

SLF: Simulated lung fluid (salt, ascorbate, citrate, urate, glutathione)

LF: Bronchioalveolar Lavage Fluid (each bar from a different patient)

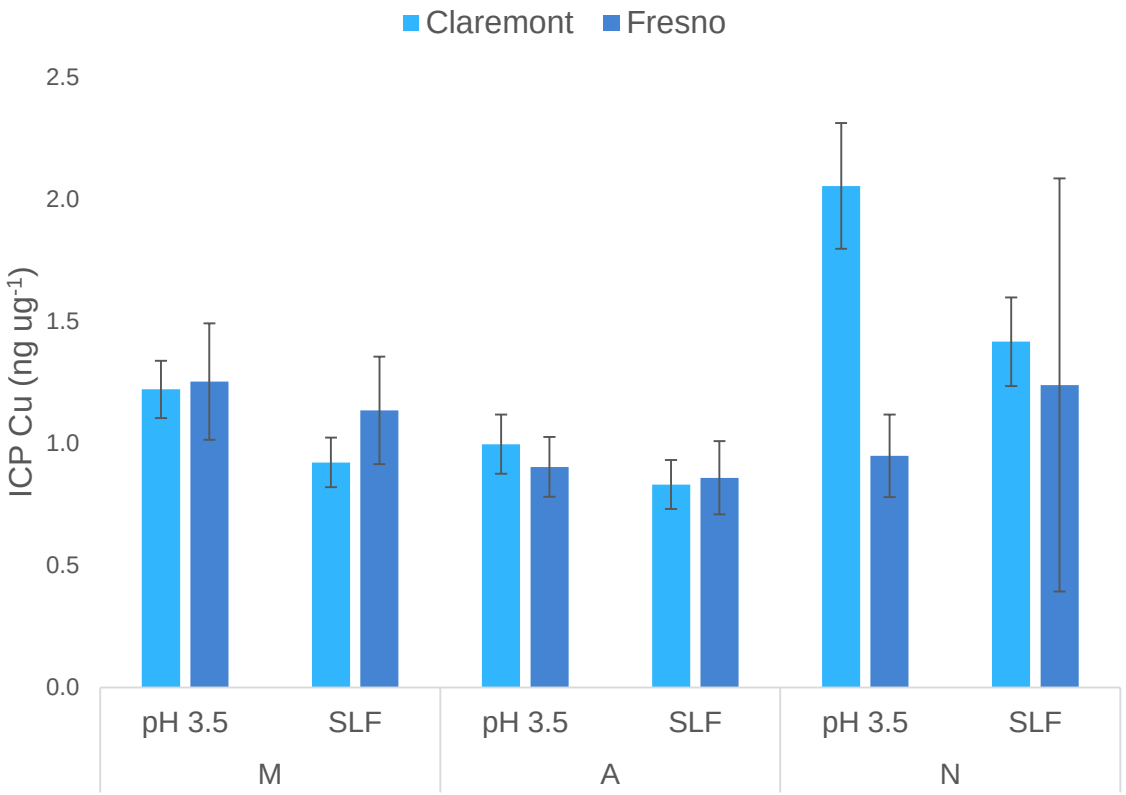
PB: phosphate buffer (10 mM)



Copper solubility appears to be less solution dependent, potentially due to effective chelation of copper by the antioxidants.

pH 3.5: water @ pH 3.5

SLF: Simulated lung fluid (salt, ascorbate, citrate, urate, glutathione)

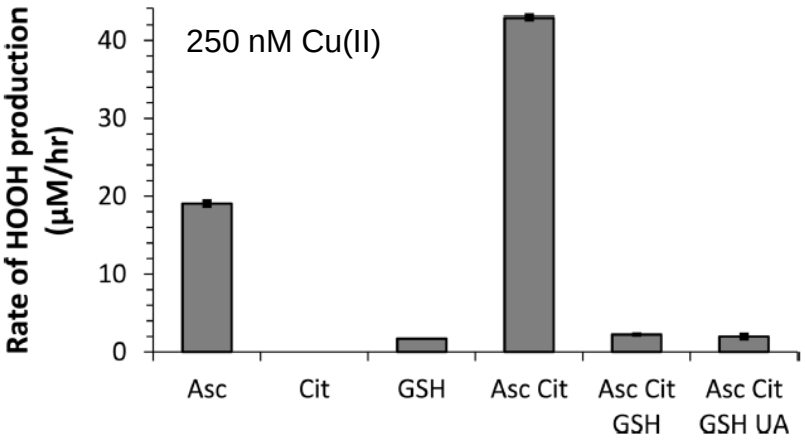


LABORATORY WORK ON HOOH AND OH

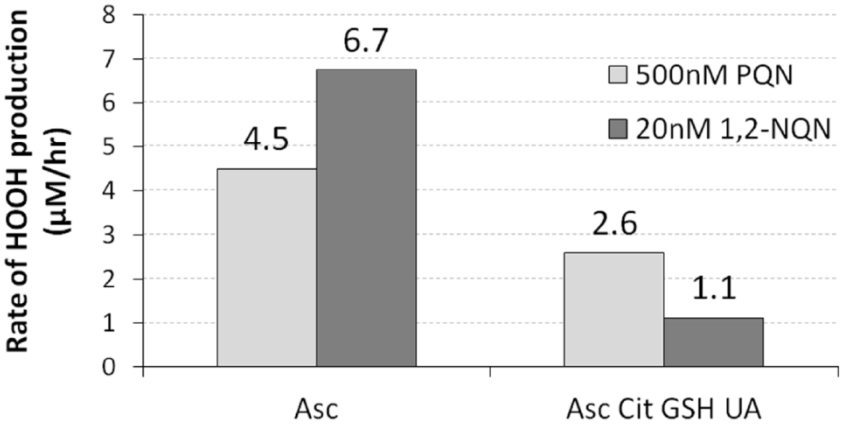
Impact of SLF Composition on HOOH Production

Charrier et al., ES&T, 2014

- Goal
  - Past work: antioxidants in SLF have large impact on OH production
  - Here we examine the effect of antioxidants on HOOH
  - Antioxidants: Asc (200 μM), Cit (300 μM), GSH (100 μM), UA (100 μM)



- Results
  - Copper (top)
    - Asc is major reductant (e^- source)
    - GSH suppresses activity; Cit enhances
  - Quinones (bottom)
    - Asc is required as reductant
    - Adding other 3 antioxidants suppresses activity, which is surprising

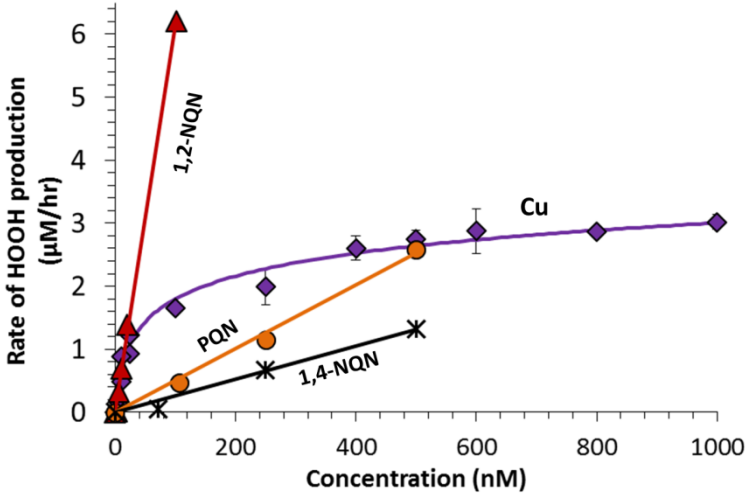


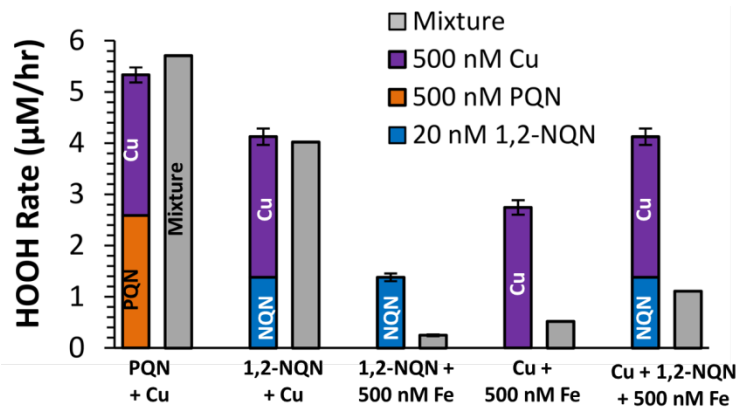
- Bottom line: Composition of SLF has large effect on ROS formation
  - We use all four antioxidants (Asc, Cit, GSH, UA) as the standard condition

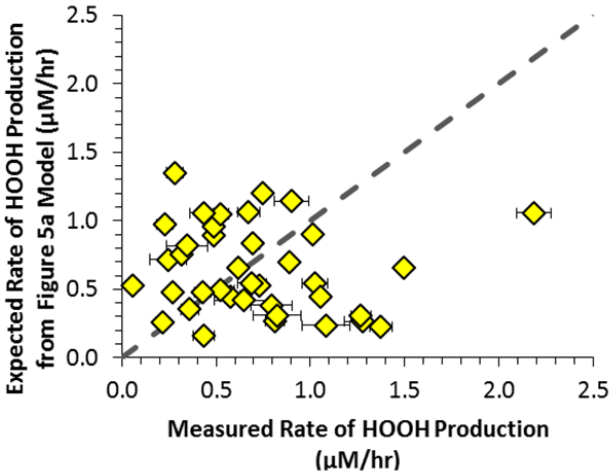
HOOH Production

Charrier et al., ES&T, 2014

- Goals
  - Quantify HOOH production from metals & quinones in surrogate lung fluid (SLF)
  - Examine HOOH production in mixtures
  - Estimate which redox-active species are most important for HOOH in ambient PM
- Results
  - Cu and three quinones make HOOH
    - Other metals tested (Fe, Mn, Co, V, Ni, Pb, Zn, Cd, and Cr) do not make HOOH
  - HOOH from Cu and quinones is additive
  - Fe suppresses HOOH production
    - Suppression in lab samples can be modeled
    - Ambient PM results (Fresno samples from ARB/EPRI project) do not fit lab model
    - Due to PM organics altering metal reactivity?
  - Will discuss importance of individual species for HOOH production later



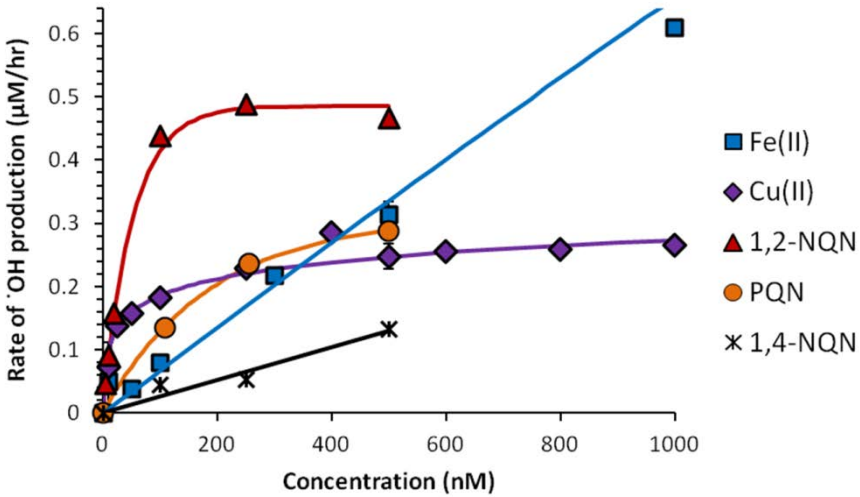


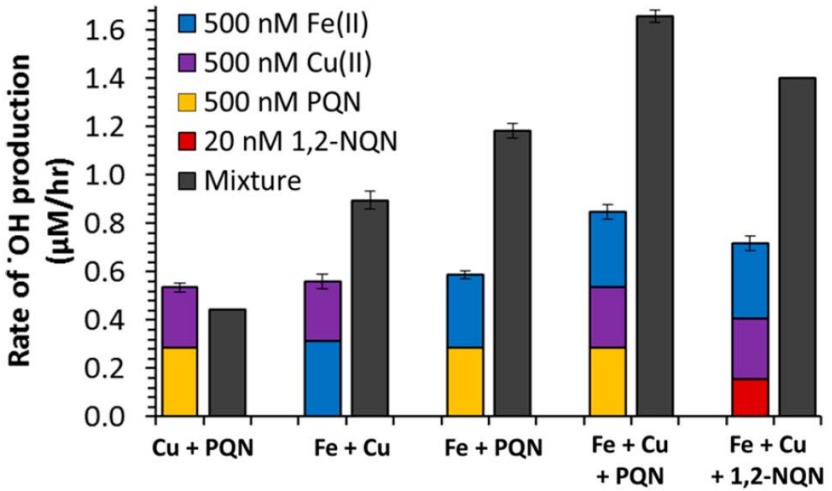


OH Production from Metals & Quinones

Charrier and Anastasio, ES&T, 2015

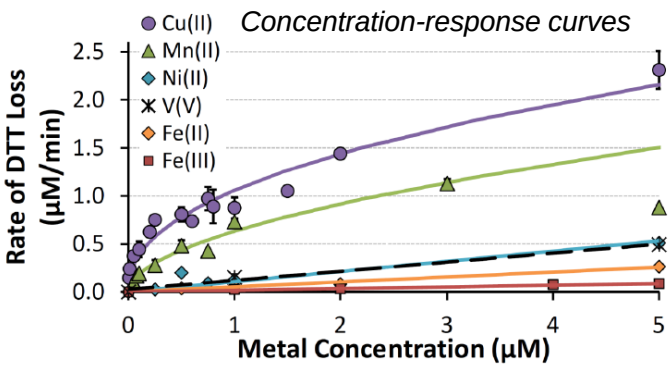
- Goals
  - Quantify OH production by metals and quinones in SLF
  - Examine OH production in mixtures
  - Estimate which redox-active species are most important for OH in ambient PM
- Results
  - Fe, Cu and three quinones make OH
    - Other metals tested (Mn, Co, V, Ni, Pb, Zn, Cd, and Cr) do not make OH
  - Mixtures
    - OH from Cu and quinones is additive
    - Fe gives synergistic OH, up to ~2x
    - We can model synergy in lab samples
    - Fe synergy in ambient PM extracts generally follows lab model
  - Importance of species for OH shown later



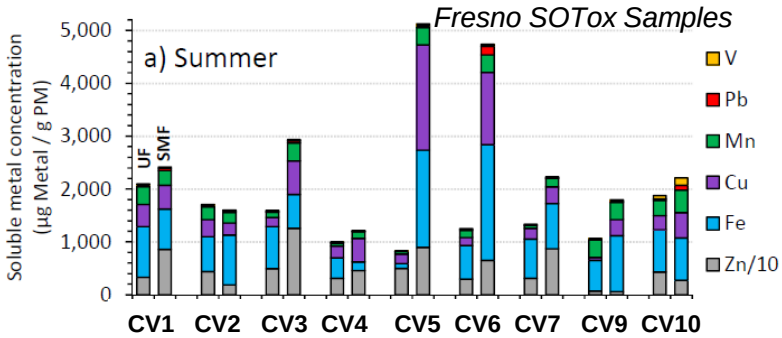


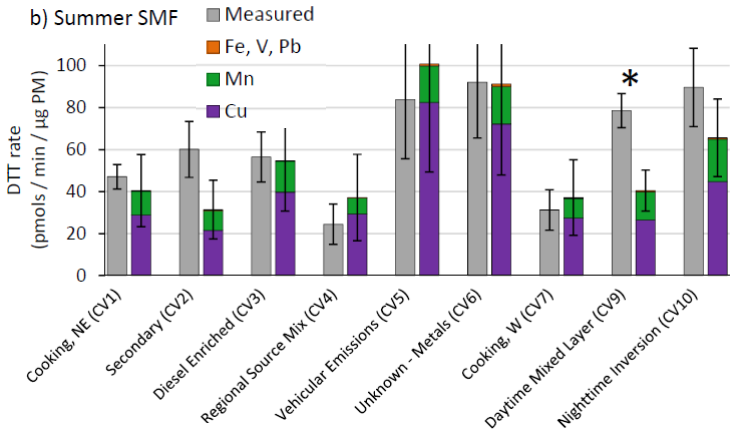
“Mechanistic” Approach to Identifying Redox-Active Species in PM Extracts

- Regression Analysis
  - Linear regressions often used to assess PM components most strongly correlated w/ ROS
  - Can be useful, but has issues
    - Non-redox-active species are often strongly correlated with ROS
    - Some species have non-linear concentration-response curves (e.g., Cu and Mn)
    - Correlation ≠ causation
- Mechanistic Approach
  - We developed method in 2008 and have used it in 7 studies since (DTT, HOOH, OH)
  - Approach accounts for redox activity and concentrations of species (see figures)
  - Uncertainties
    - PM organics might alter metal reactivity
    - Have examined mixtures, but more work to do



Charrier and Anastasio, 2012



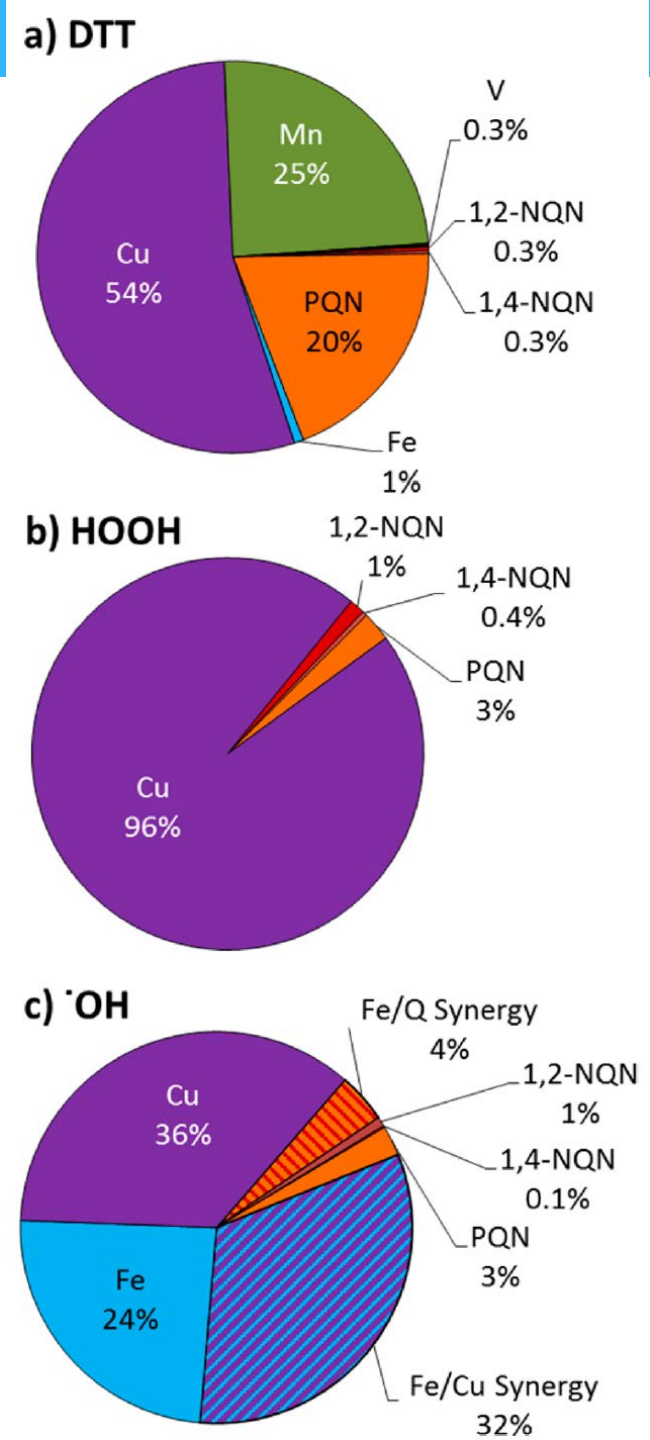


Charrier et al., 2015

Relative Importance of Metals & Quinones for ROS

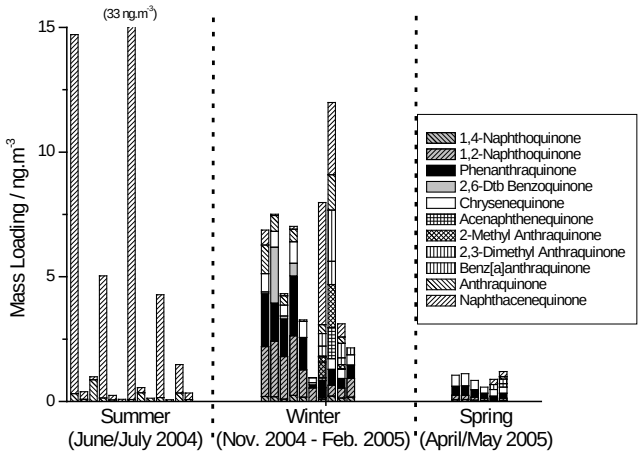
Charrier and Anastasio, ES&T, 2015

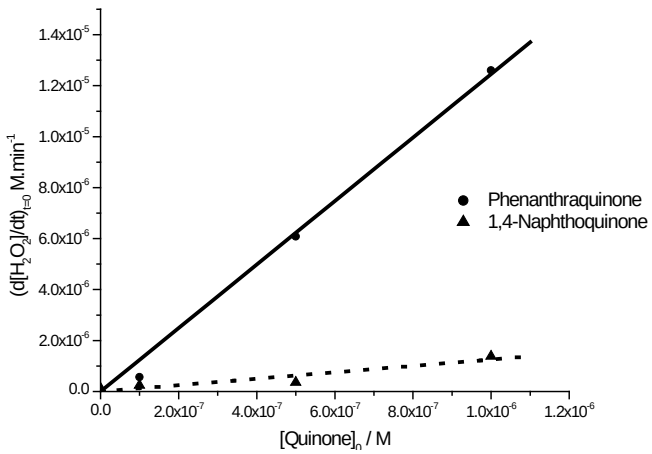
- Apply mechanistic approach to hypothetical $PM_{2.5}$ sample w/ US median concentrations, incl:
  - Water-soluble Fe, Cu, Mn = 7, 2, 2 ng/m^3
  - PQN, 1,2-NQN, 1,4-NQN = 0.3, 0.02, 0.1 ng/m^3
- Cu is the major player
  - Cu, Mn & PQN dominate DTT loss
  - Cu dominates HOOH formation
    - But impacts of PM organics might be significant
  - Cu, Fe and Fe-Cu synergy dominate OH formation
- Cu and the California REL
  - There is *in vitro*, *in vivo*, and epidemiological evidence that Cu is very redox active and toxic
  - Current REL: 100 µg/m^3. Unlikely health protective.
  - OEHHA is revisiting the Cu REL

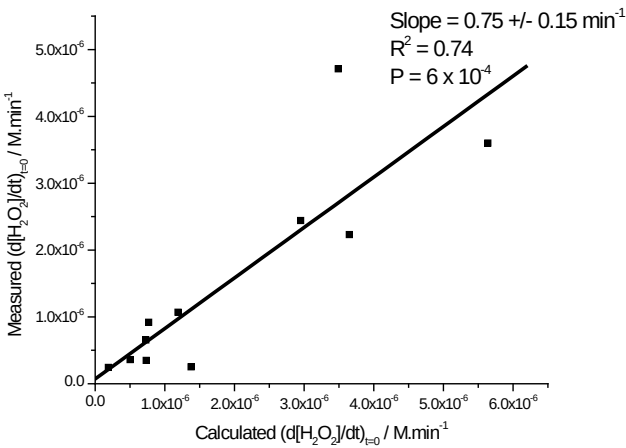


Quinones

Background and Goals







- Redox-active Quinone mass loadings in Fresno up to > 10 ng m^{-3}.
- Quinones accounted for H_2O_2 generation from PM extracts in the presence of diothiothreitol (DTT).

Primary Goals

- Quantify quinone levels in PM extracts to enable their contributions to ROS generation to be evaluated.
- Identify origins of quinones (if their contributions are significant)

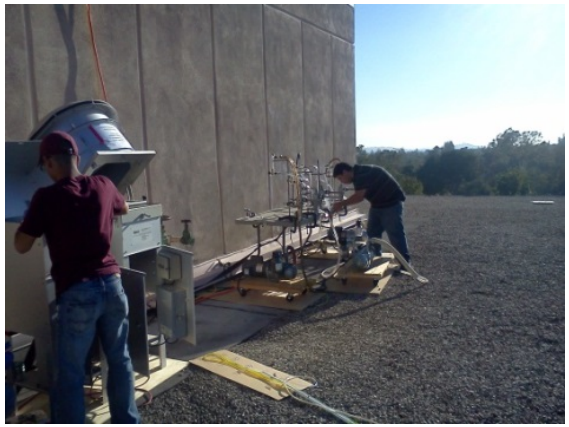
Overview

- Sampling Sites
  - **Claremont (07/26/12-08/13/12). 56 Samples**

    Organics mostly anthropogenic; significant photochemical component.

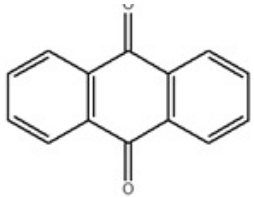
  - **Fresno (01/15/13-01/30/13). 45 Samples**

    More primary, biogenic organics.

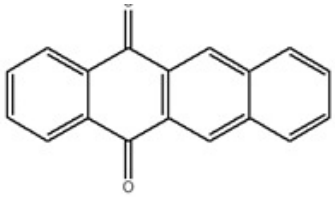


- Procedure
  - Samples collected with Hi-Vol PM2.5 sampler on 8 x 10 inch Teflon-coated filters and PUF sampler.
  - 3 samples/day: Morning (6 hr), Afternoon (6 hr), Overnight (12 hr)
    - Filter sections extracted with SLF and then organic solvent; PUFs extracted with organic solvent. Organics quantified by GC/MS.
    - Sections provided to UCLA and UC Davis for ROS assay and other chemical measurements.
    - (Sections used in rat alveolar macrophage ROS assay.)

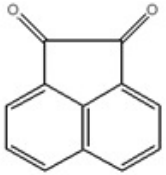
Quinones



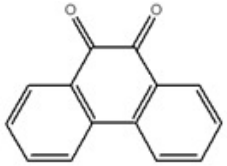
Anthraquinone



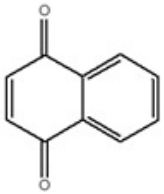
5,12-Naphthacenequinone



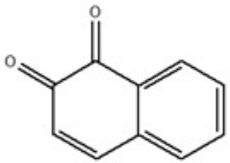
Acenaphthenequinone



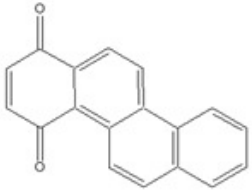
Phenanthraquinone



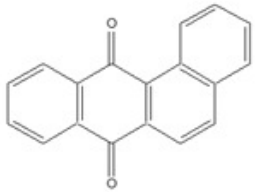
1,4-Naphthoquinone



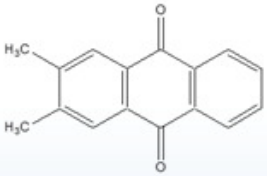
1,2-Naphthoquinone



1,4-Chrysenequinone

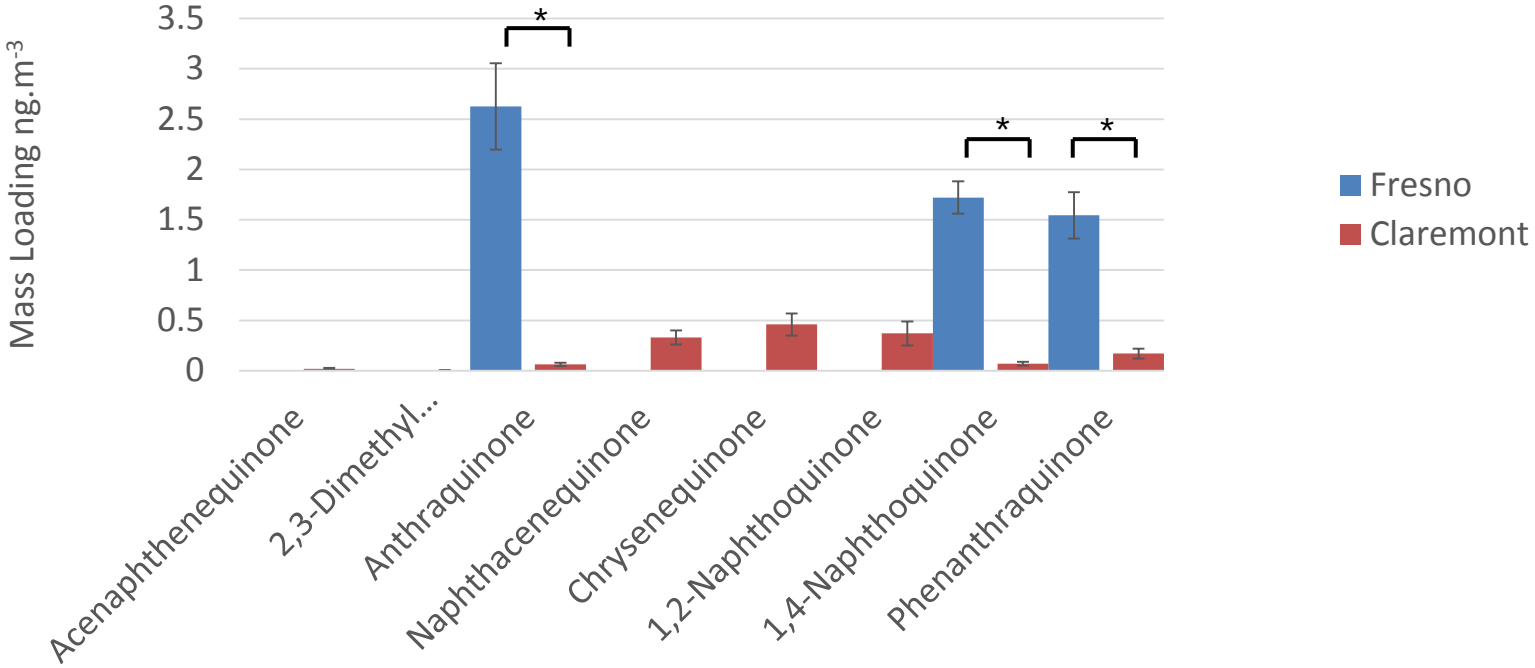


Benz[a]anthracene
-7,12-dione

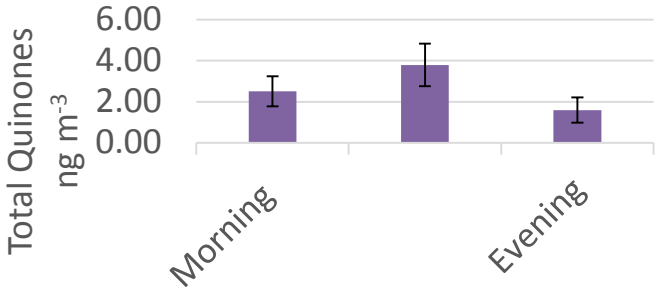


2,3-Dimethyl Anthraquinone

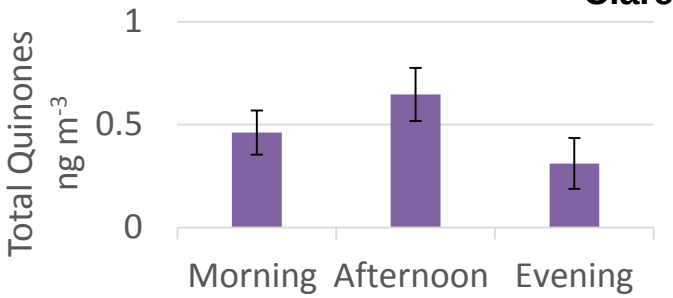
Summary of Results



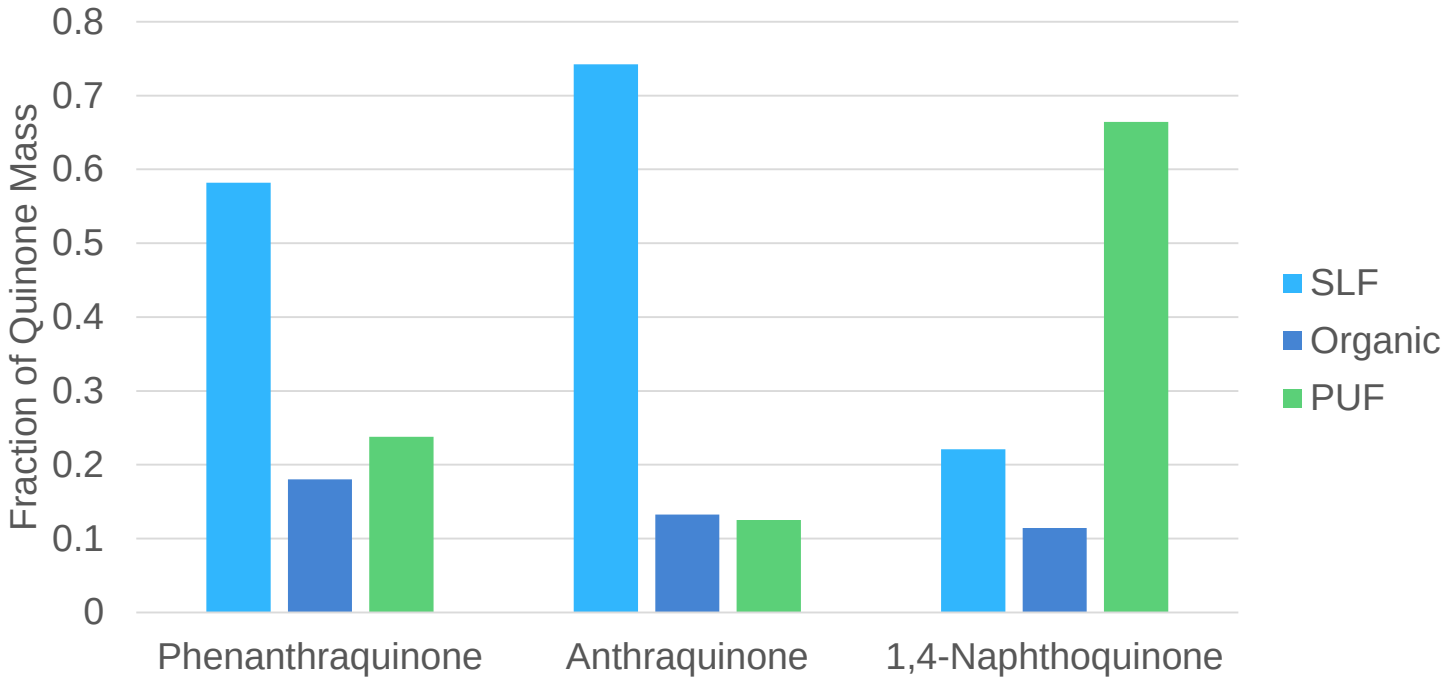
Fresno



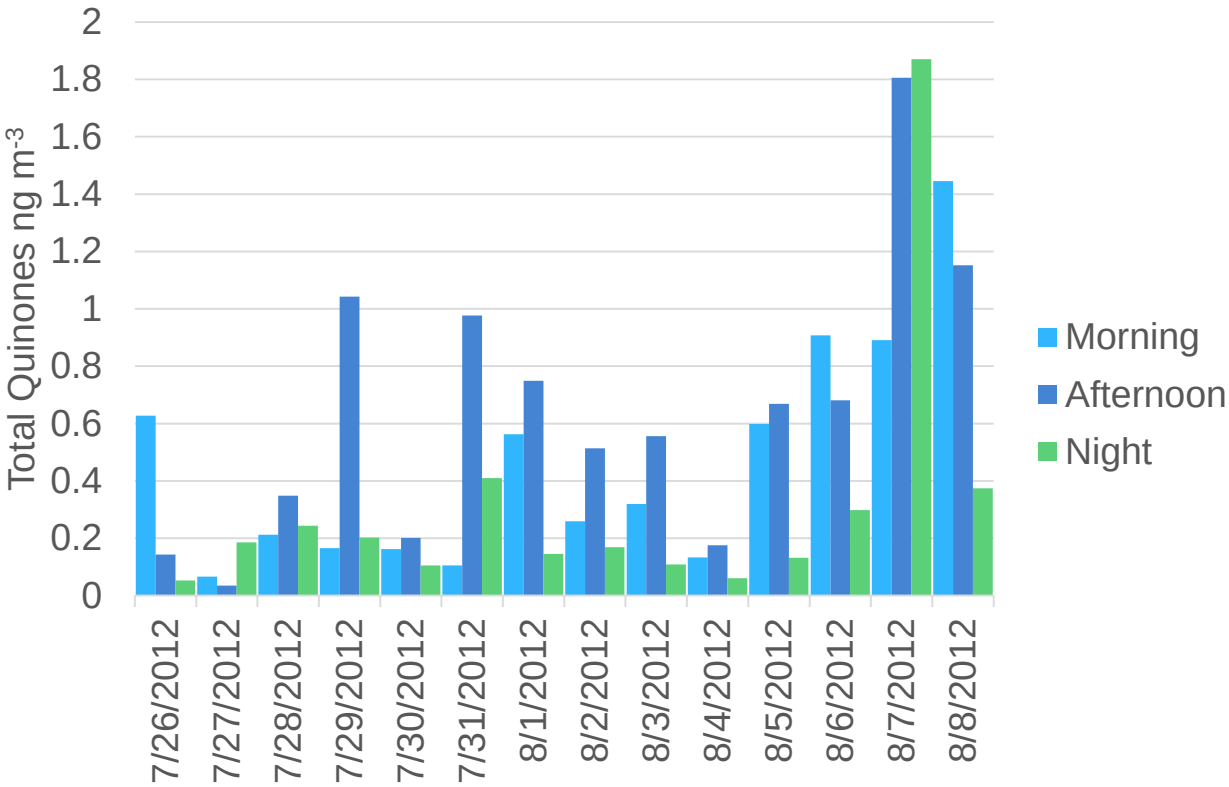
Claremont

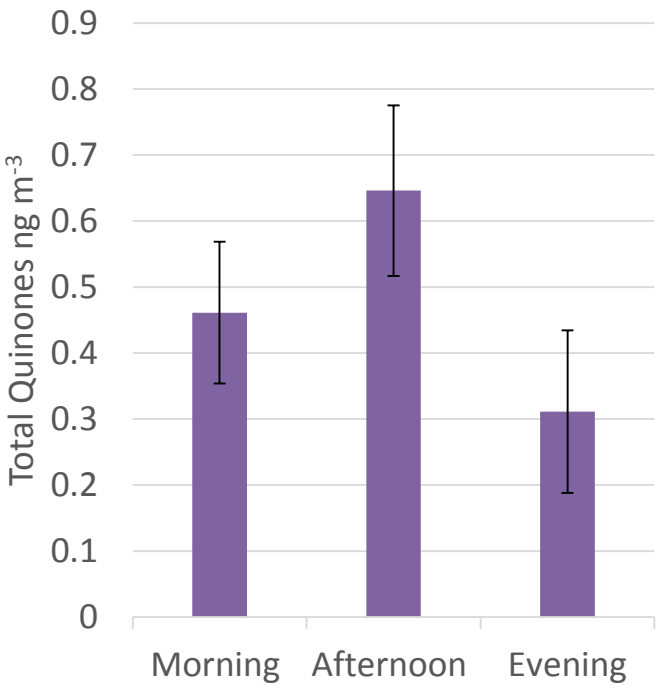


Distribution of Quinones



Claremont – All Quinones

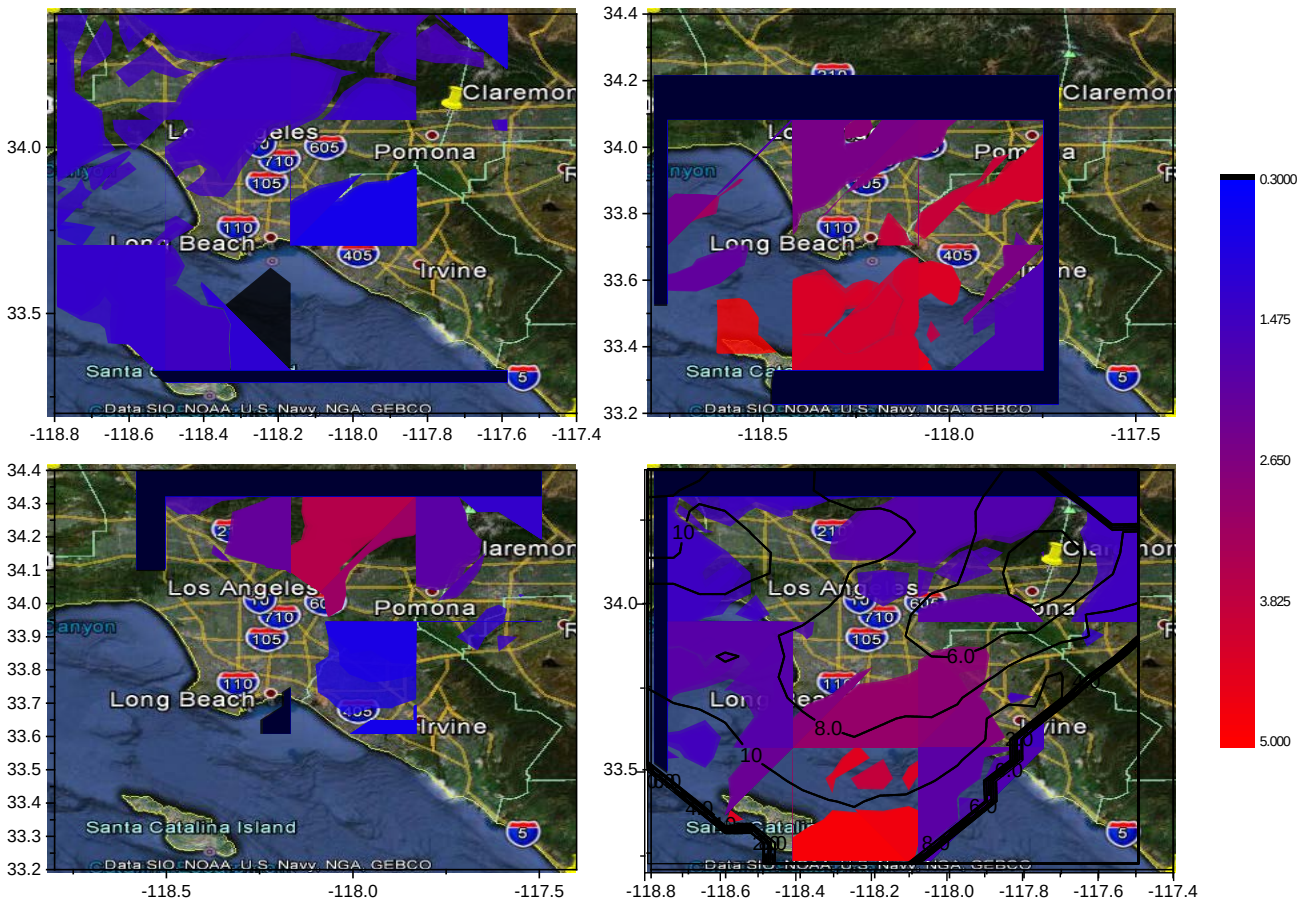




Claremont – All Quinones

Night

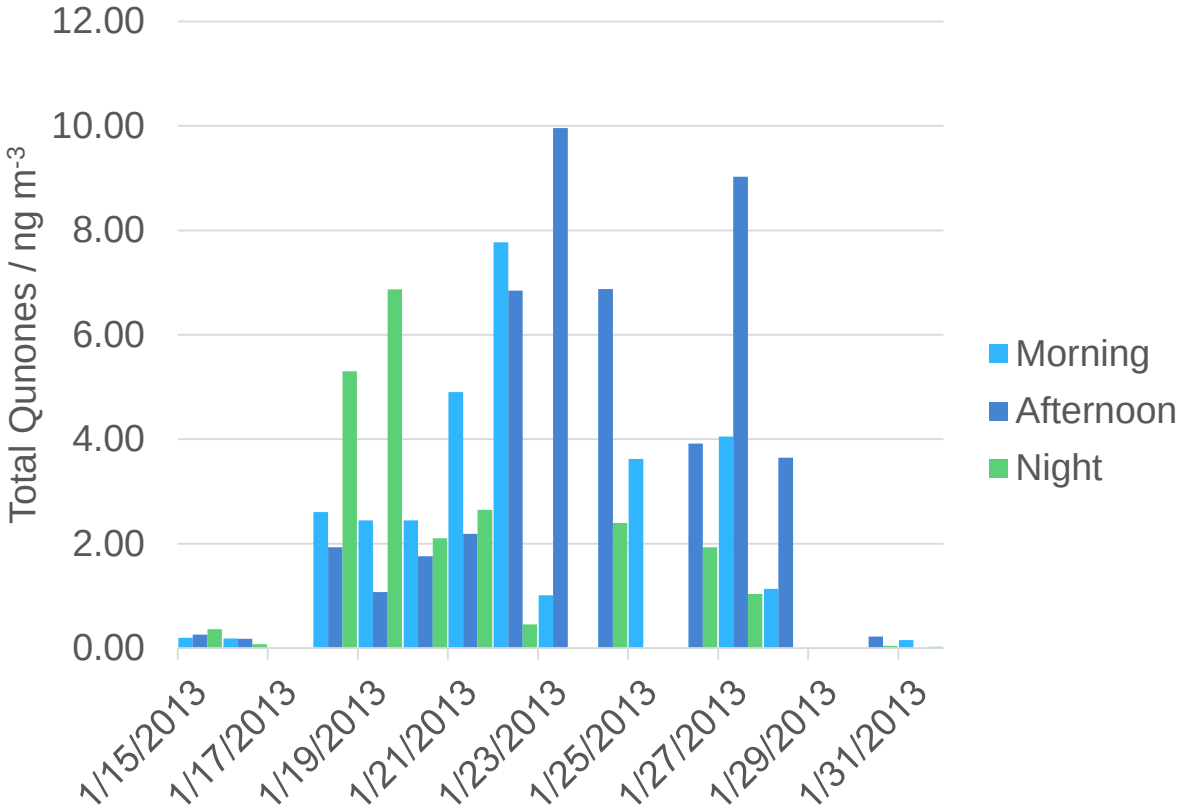
Afternoon

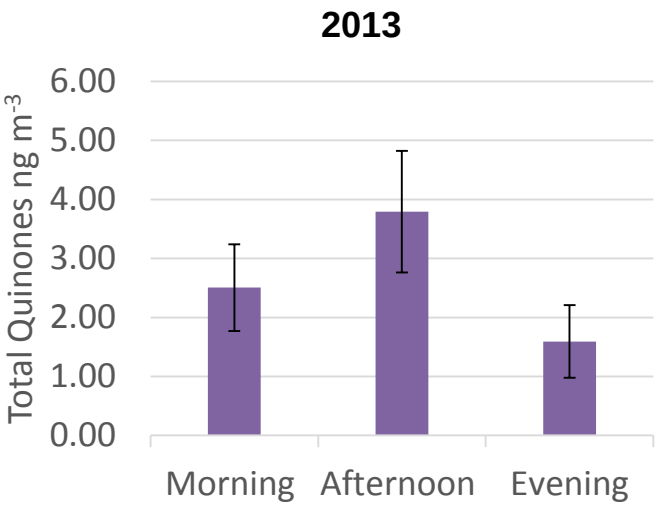


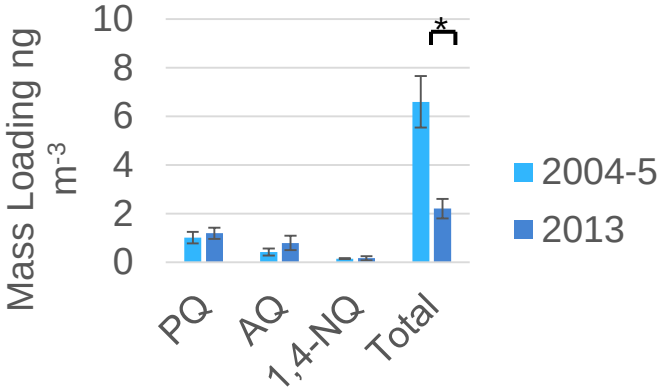
Morning

Overall

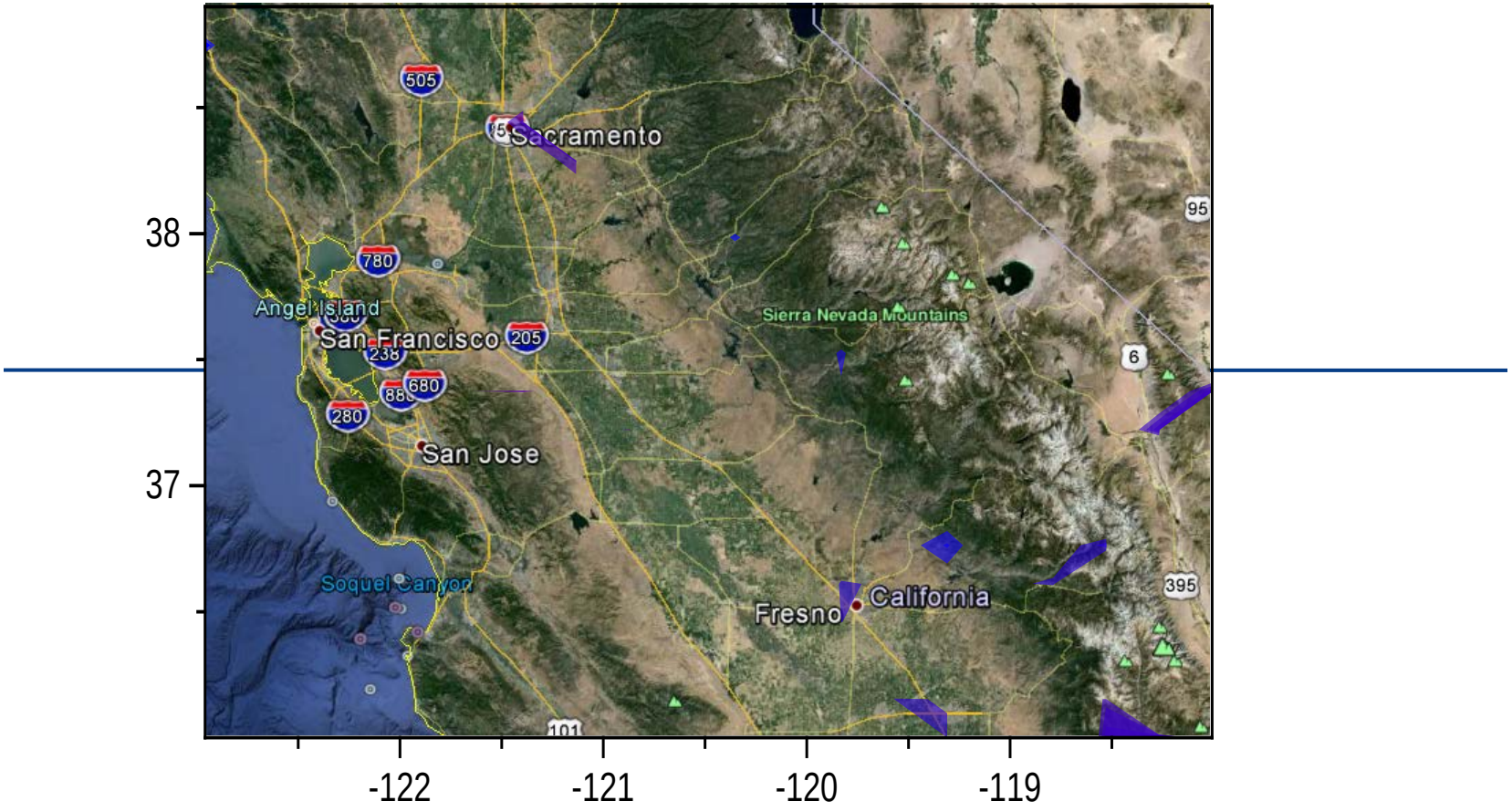
Fresno


12.00
10.00
8.00
6.00
4.00
2.00
0.00
Total Qunones / ng m-3
1/15/2013
1/17/2013
1/19/2013
1/21/2013
1/23/2013
1/25/2013
1/27/2013
1/29/2013
1/31/2013
Morning
Afternoon
Night


2013
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Total Quinones ng m-3
Morning
Afternoon
Evening


10
8
6
4
2
0
Mass Loading ng m-3
*
PQ
AQ
1,4-NQ
Total
2004-5
2013

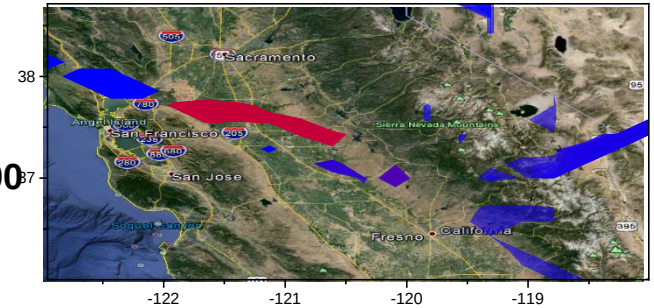
Fresno - Phenanthraquinone



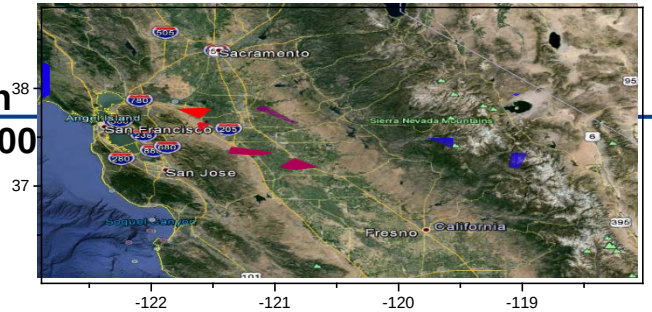
Scale: 0 – 1 ng PQ / μg $PM_{2.5}$

Fresno - Temporal Variation (Phenanthraquinone)

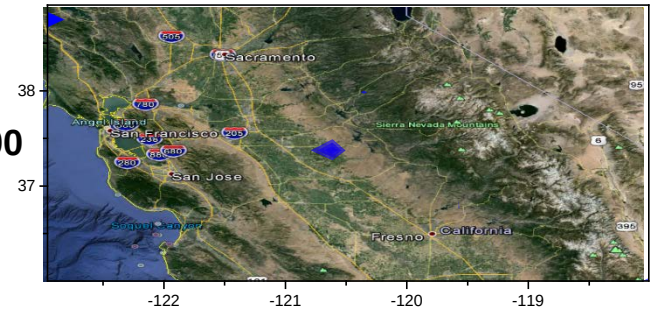
Morning 7:00-13:00

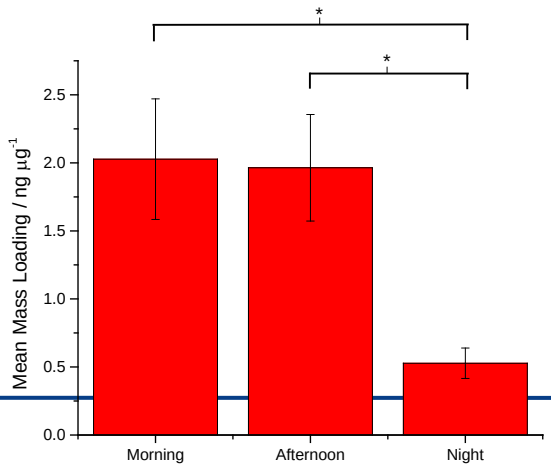


Afternoon 13:00-19:00



Night 19:00-7:00





Scale: 0 – 1 ng PQ / µg $PM_{2.5}$

Summary

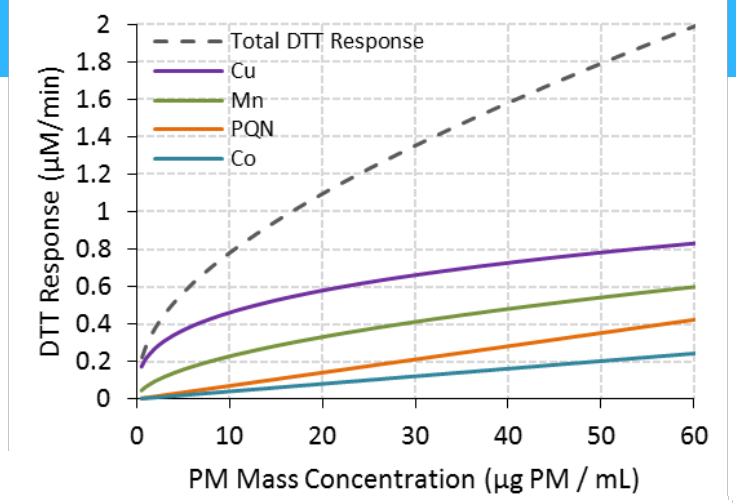
- Mass loadings of quinones are higher in Fresno than Claremont; more quinones are detectable in Claremont.
- Measurements at both sites consistent with photochemical production of quinones from anthropogenic sources.
- In Fresno – no change in redox-active quinone levels since 2004, but total quinone mass loadings decreased.

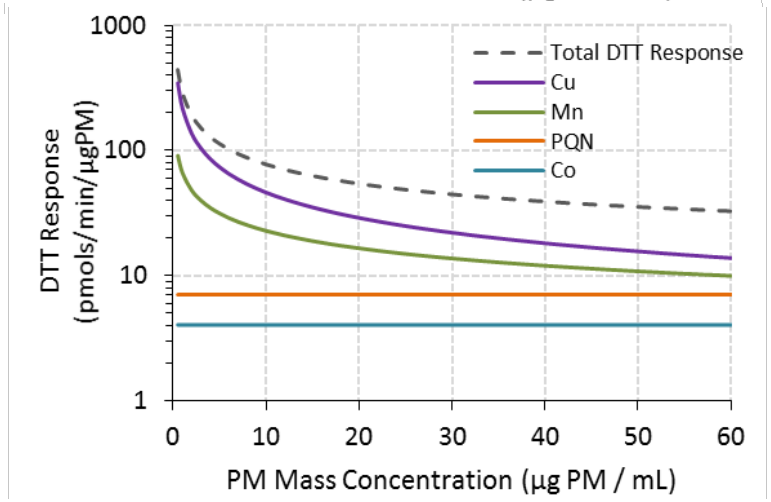
DTT

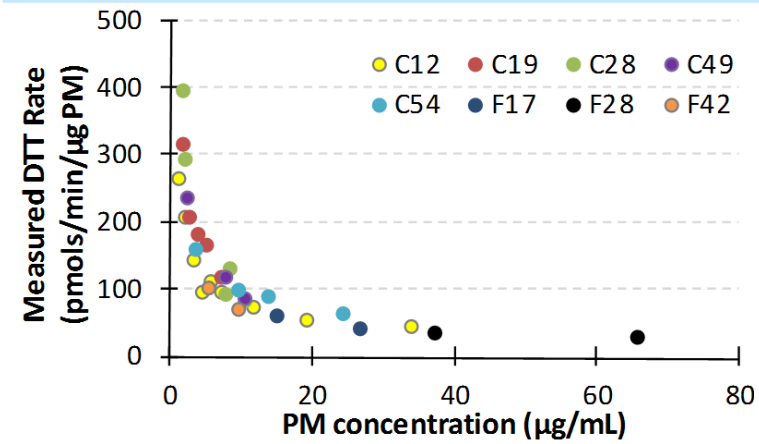
DTT Mass Bias

Charrier et al., Atm Env, In Press

- DTT mass normalization
  - Rates of DTT loss are generally normalized by mass of PM in extract (i.e., rate/mass)
  - Implicitly assumes linear concentration-response curves; not true for Cu and Mn
- Hypothetical PM sample
  - Calc'd DTT responses for single PM sample over a range of PM mass conc's in extract
  - Top panel shows extract results
  - 2nd panel shows mass-normalized results
- Actual samples (bottom figure)
  - 8 Claremont (C) and Fresno (F) samples
    - Each sample meas'd at several concentrations
  - "Mass-normalized" result depends on mass
  - Need to correct for this bias in DTT results
    - We recommend normalizing results to a PM extract concentration of 10 µg/mL



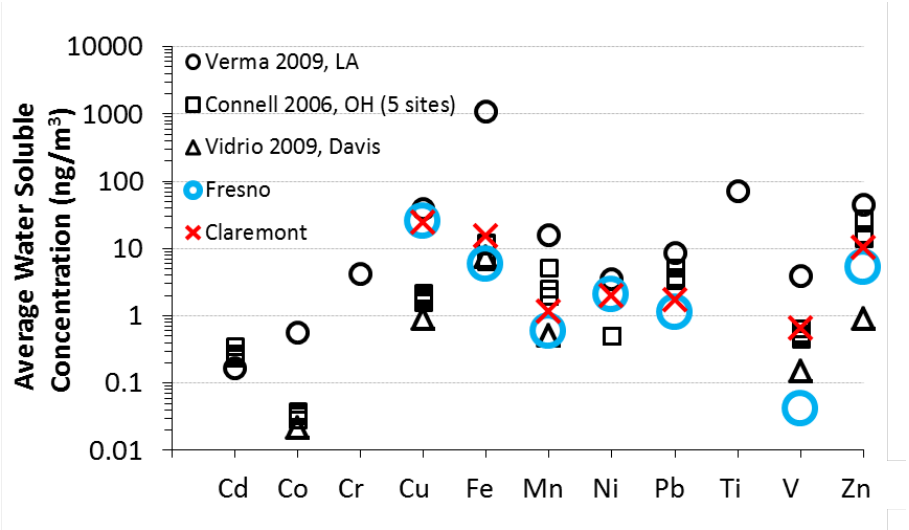


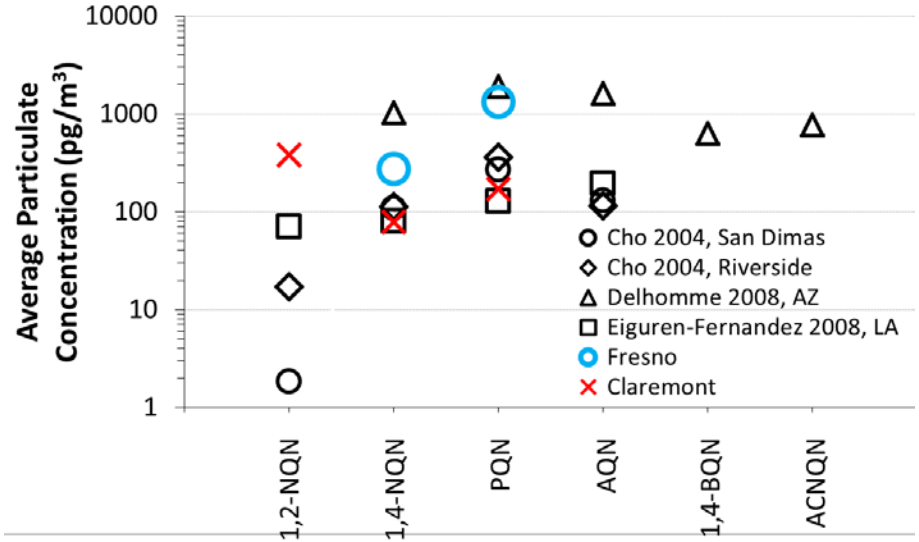


Metals and Quinones at Claremont & Fresno

Anastasio et al., In Prep

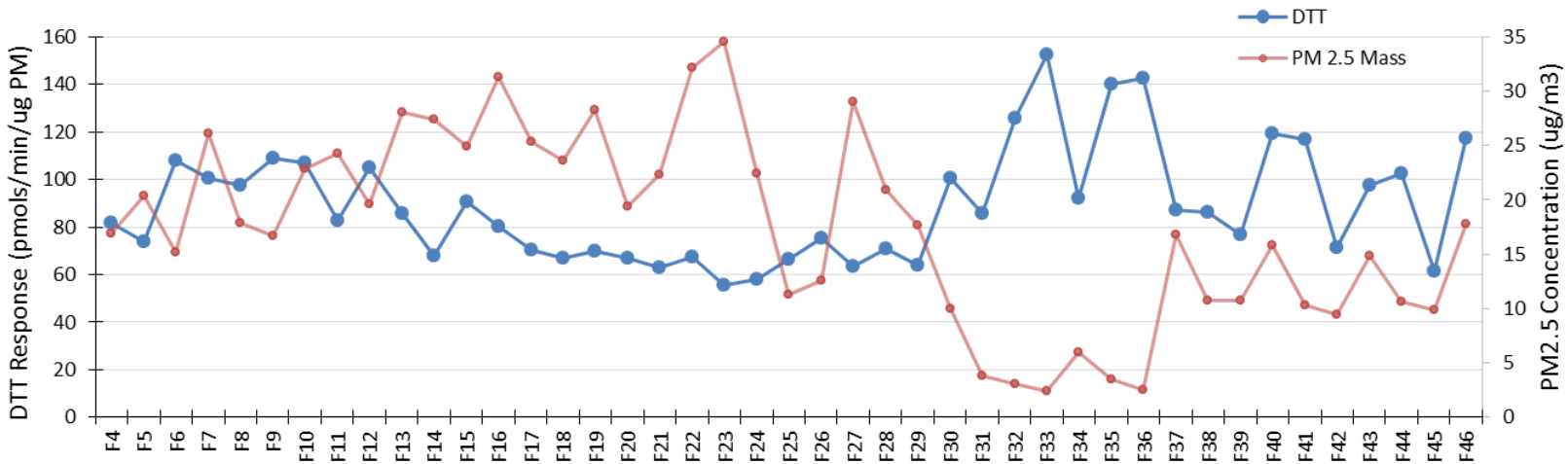
- Examine concentrations since these species likely drive DTT activity
- Average metal concentrations
  - Aqueous-soluble component only
  - Broadly similar to previous results
  - Cu is high (~ 25 ng/m^3), though lower than previous LA average
- Average quinone concentrations
  - SLF-soluble quinones for current work; only some of species shown
  - Results similar to previous work for 1,4-NQN and PQN
  - 1,2-NQN high for Claremont but Fresno was generally below LOD





DTT Loss in PM Extracts

- DTT results (normalized to extract concentration of 10 µg/mL)
  - Time course is modestly interesting for Fresno (below); rather stable at Claremont
  - DTT rates are in the (wide) range of values reported previously
  - No difference in average values for morning, afternoon, overnight samples

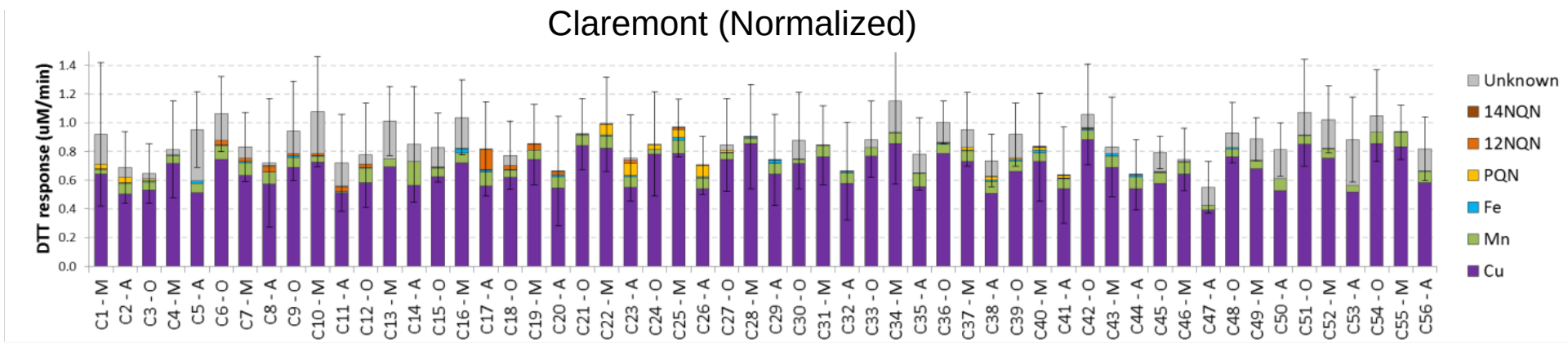


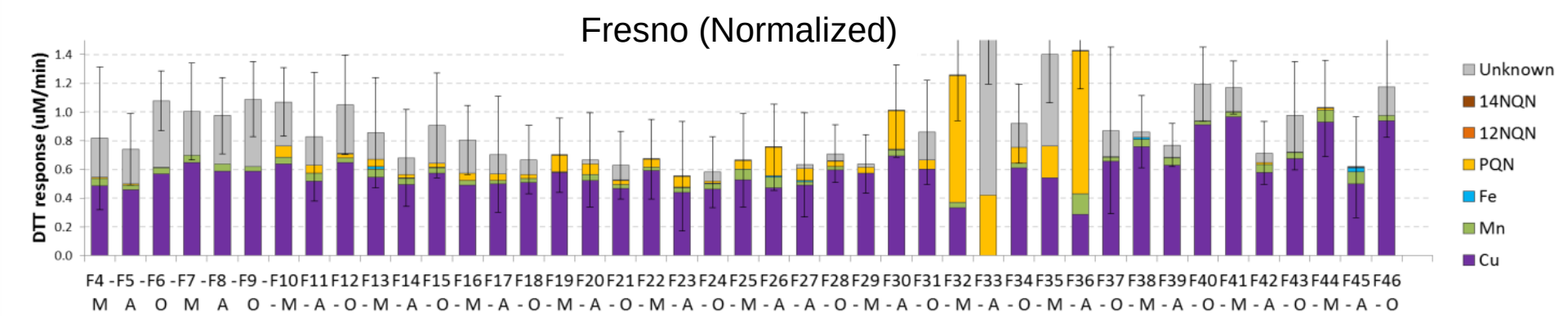
- Impacts of mass normalization
  - Results shown for Fresno
  - Big impacts on range & RSD
  - Smaller effect on mean
  - ~½ of variability in "standard" rates due to extract mass diff's

DTT Rates (pmol min^{-1} µg^{-1})	Standard Mass Normalization	Normalized to 10 µg/mL
Range	23 - 238	55 - 153
Mean ± σ	79 ± 42	89 ± 24
RSD	53%	27%

Species Responsible for DTT Loss

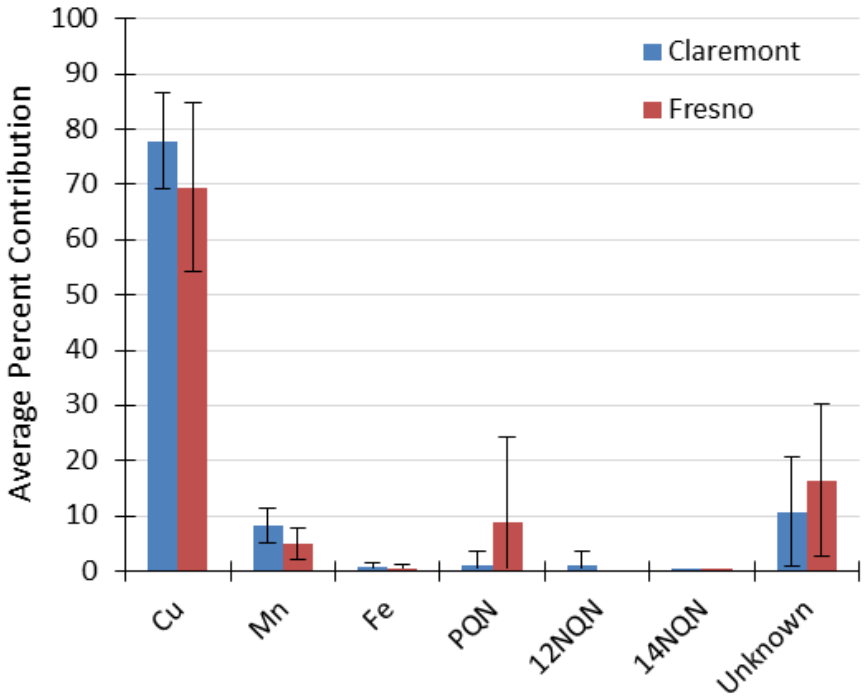
- Applied mechanistic approach using measured PM metals and quinones
- Results
  - Copper (purple) dominates DTT loss at both sites
  - Unknown species (gray) are small, but significant, and variable
  - PQN (yellow) generally small contribution, but dominates in a few Fresno PM





Species Responsible, Part 2

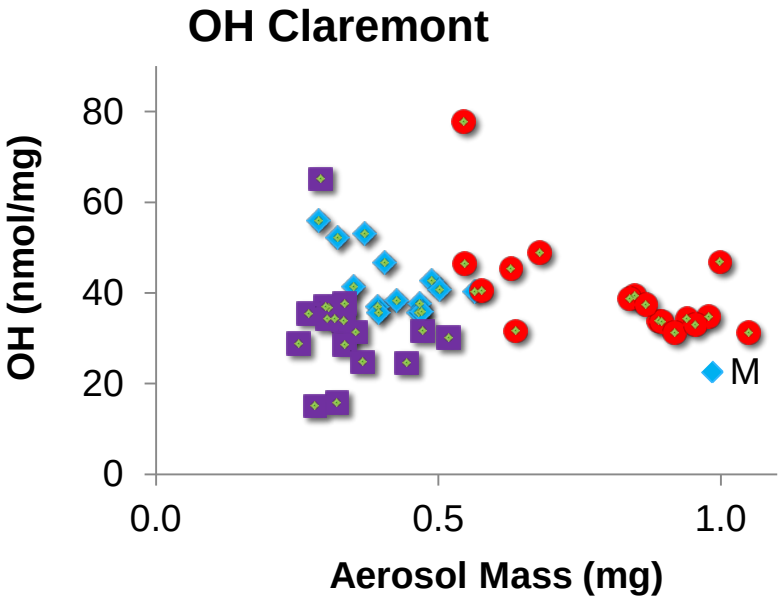
- Mechanistic approach
  - Graph show average percent contribution to DTT loss from each species
  - Copper dominates
  - Unknown species are significant
  - Manganese minor
  - PQN important at Fresno
  - Fe: < 1% at both sites

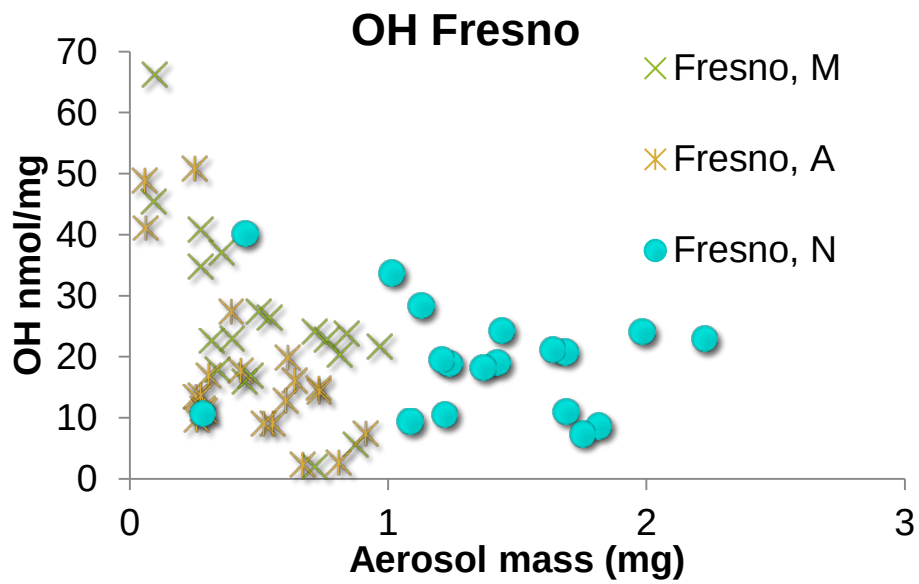


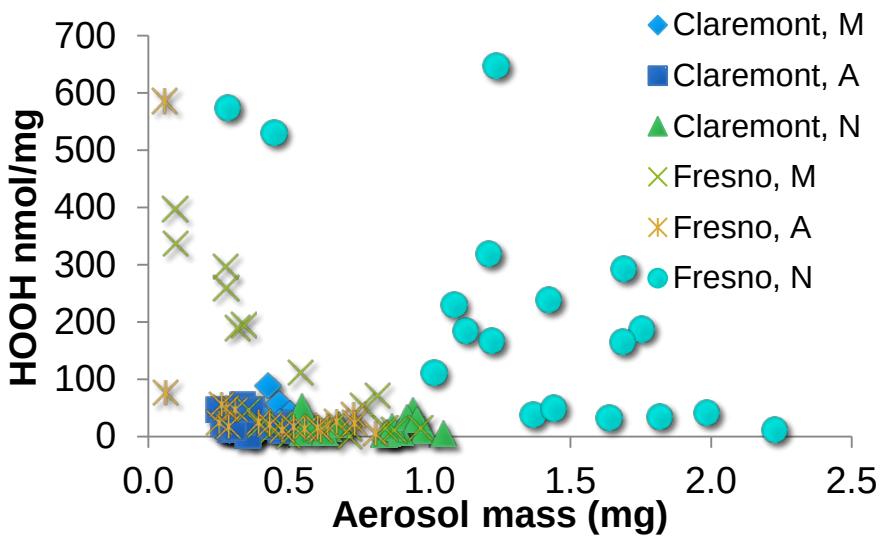
- Regression approach
  - There are strong correlations between DTT rates and
    - Fe, Cu, and Mn at Claremont
    - HULIS (especially), Fe, and Cu at Fresno
  - HULIS correlation might indicate that it is driving the “unknown” contribution
  - Fe correlations are more difficult to understand
    - Fe has very weak activity in the DTT assay and modest water-soluble amounts

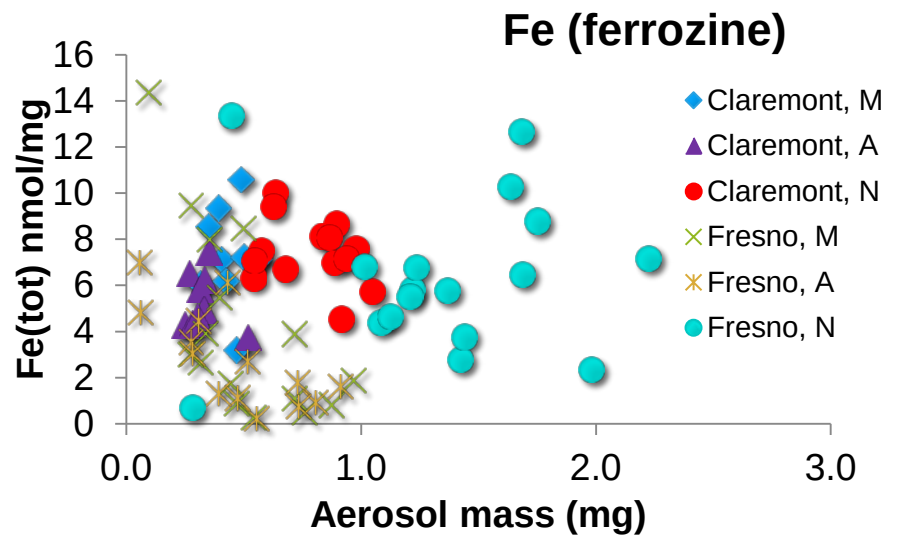
OH and H_2O_2, and comparisons to DTT etc.

OH & H_2O_2: no clear dependence on mass concentration in the assay solution



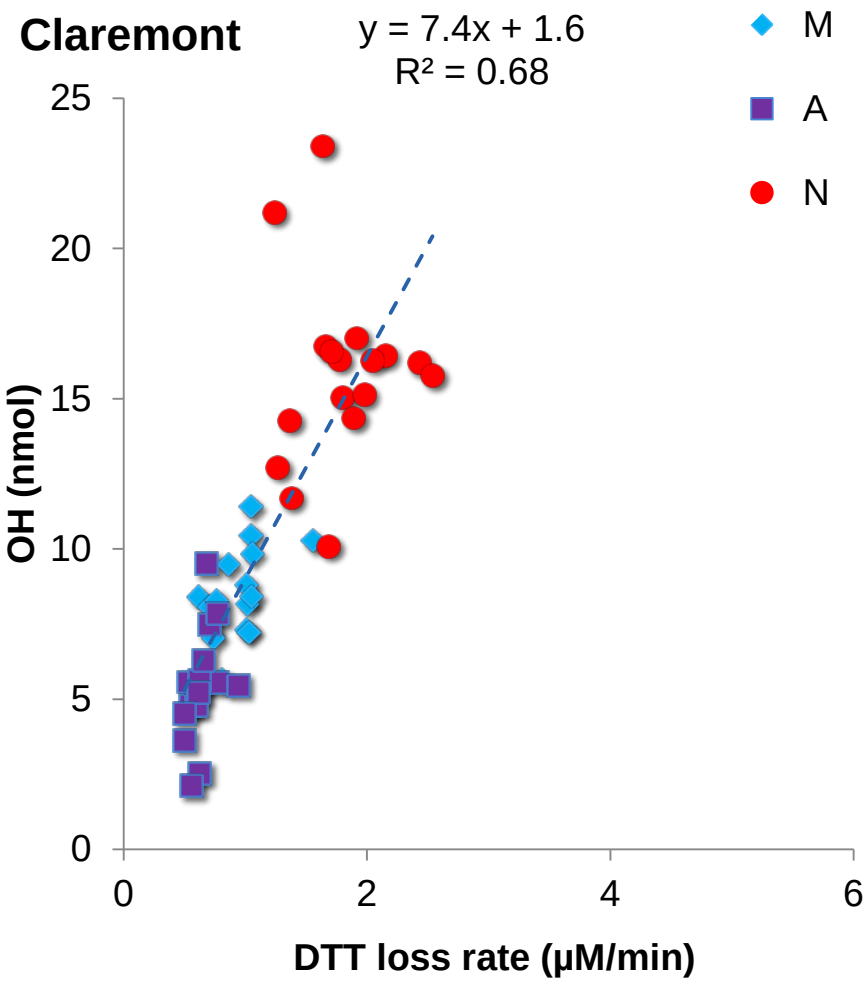


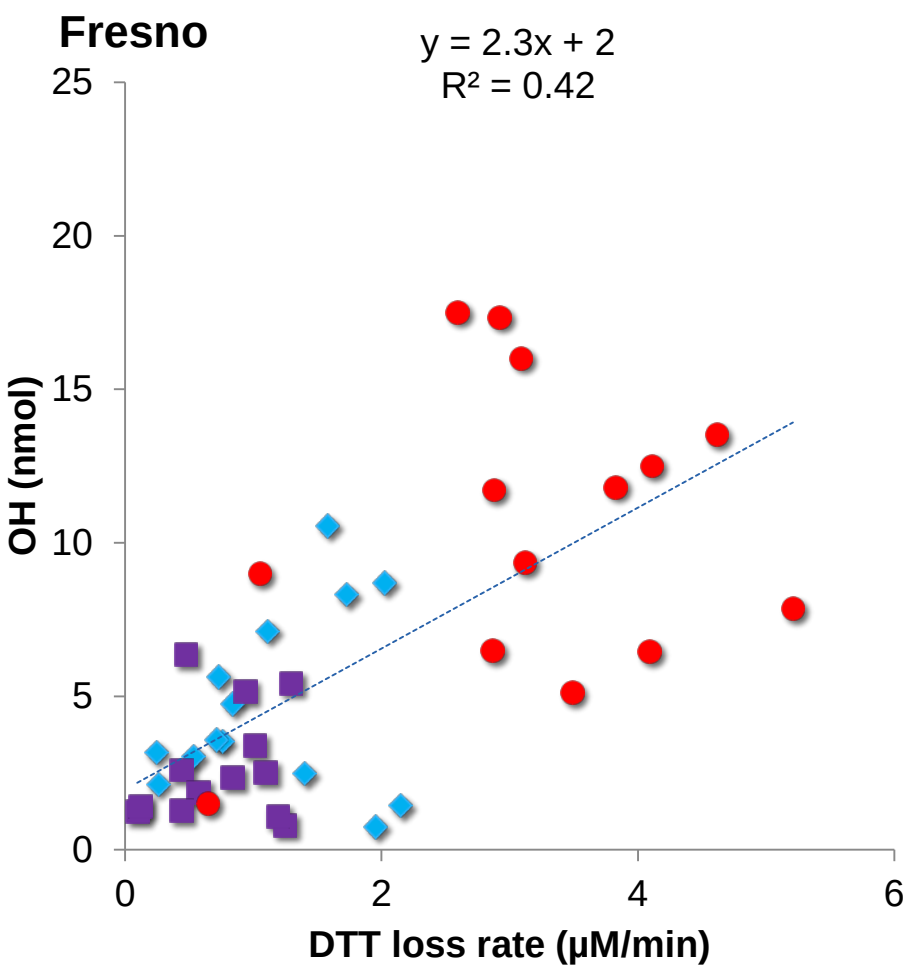




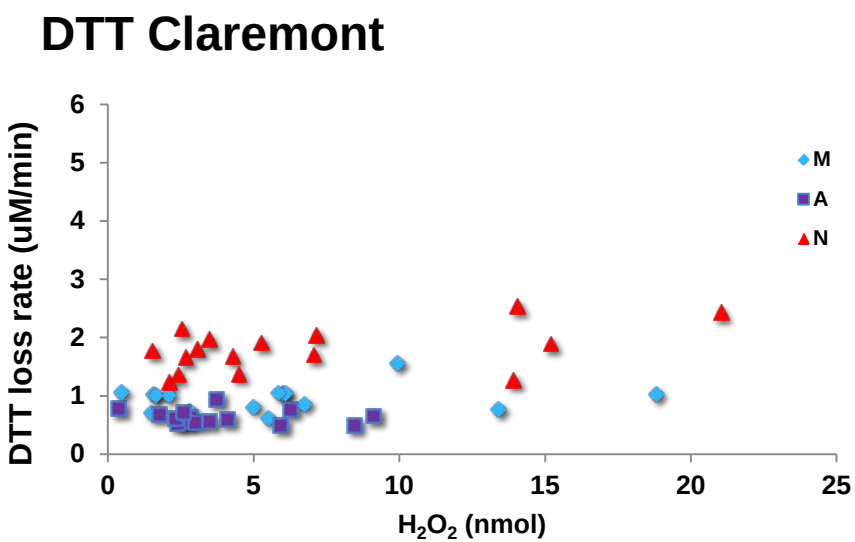
ROS assays in relation to one another

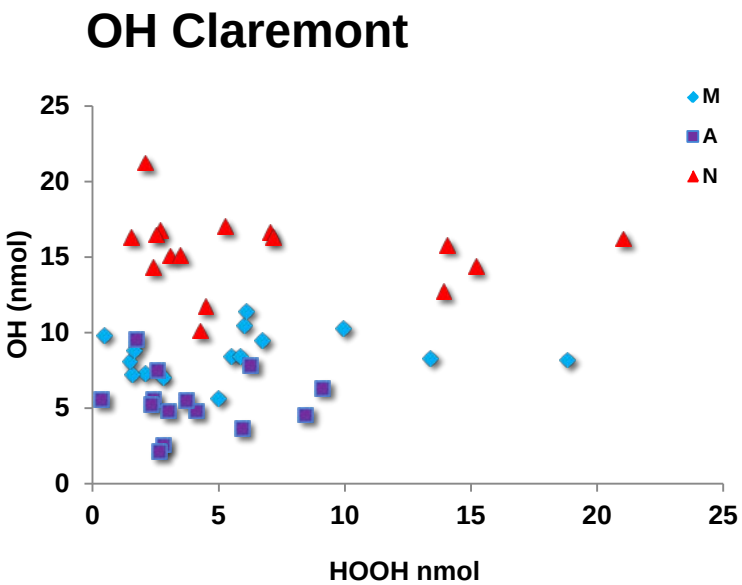
OH vs. DTT: correlated but different slopes

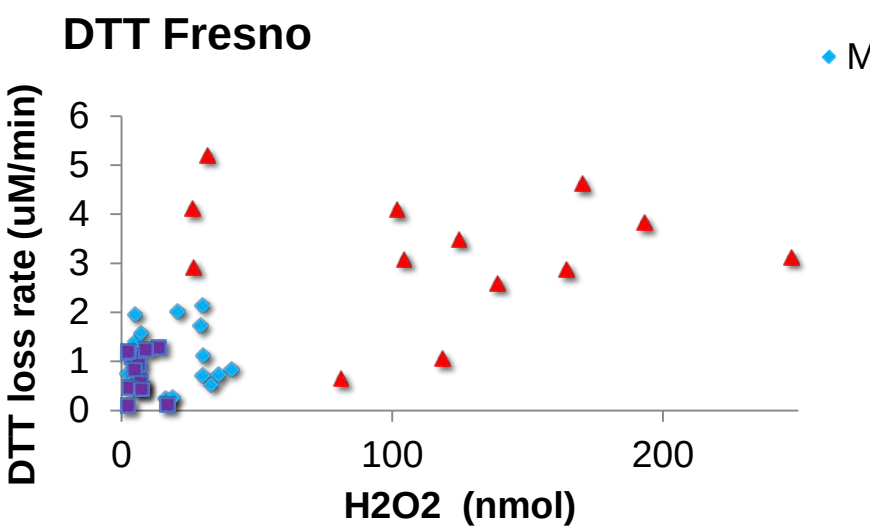


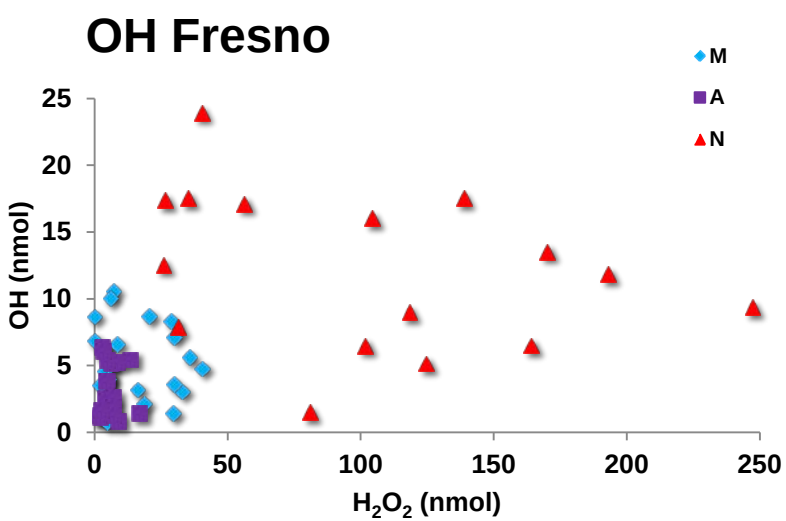


H_2O_2 behaves differently from OH and DTT. M



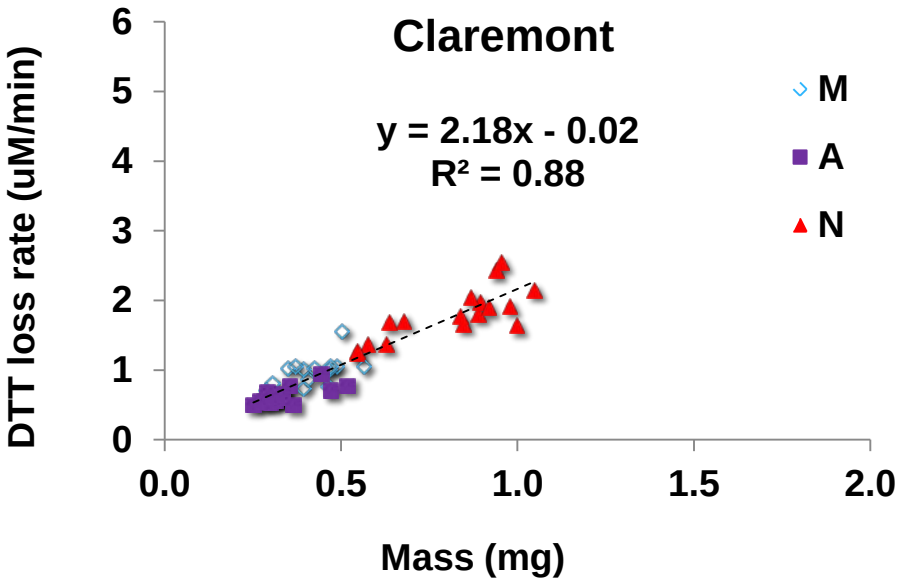


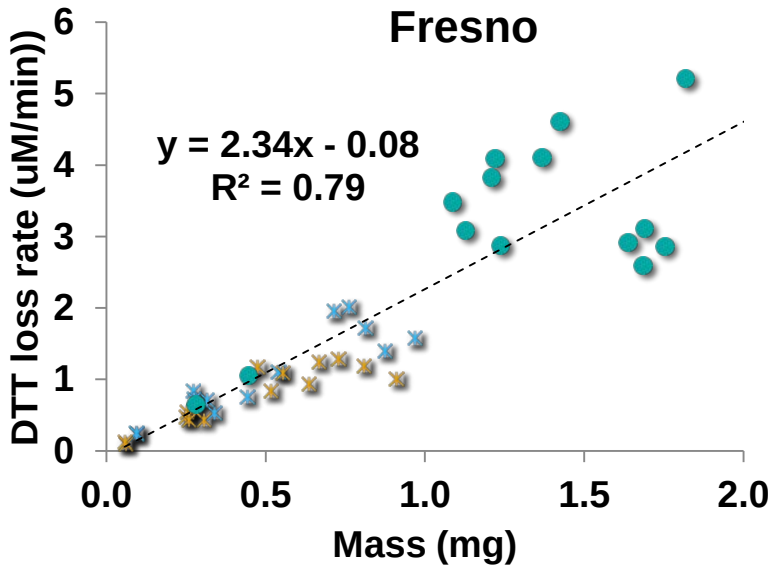


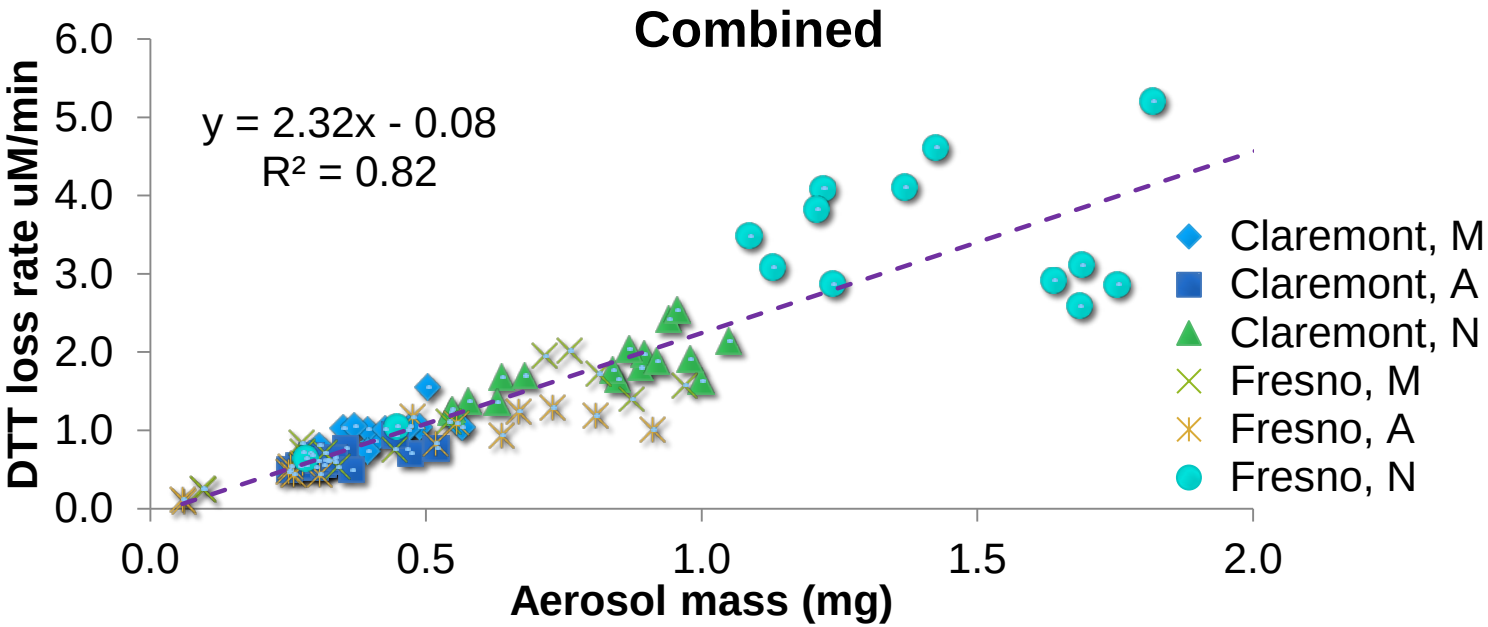


Do chemical components do a better job than mass at predicting ROS?

DTT correlates strongly with mass

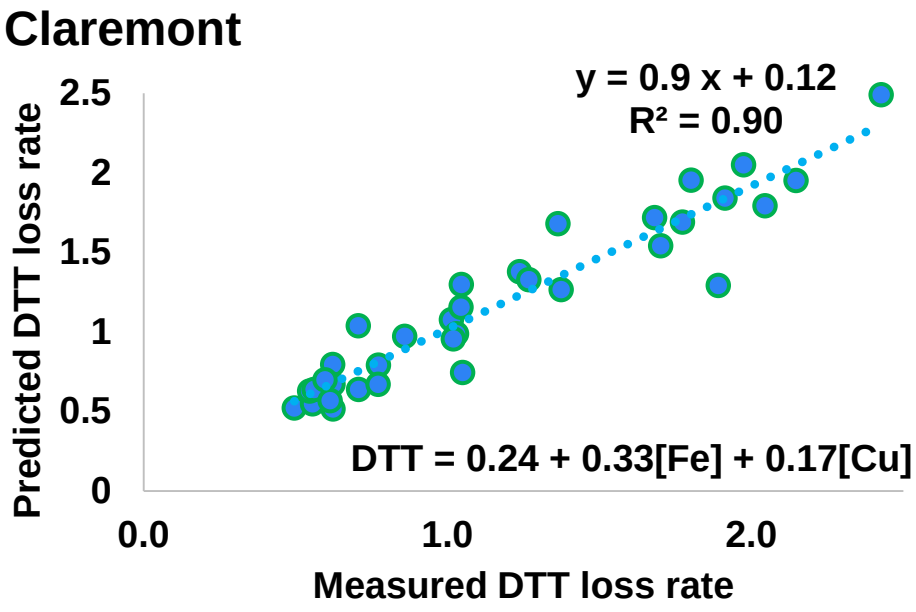


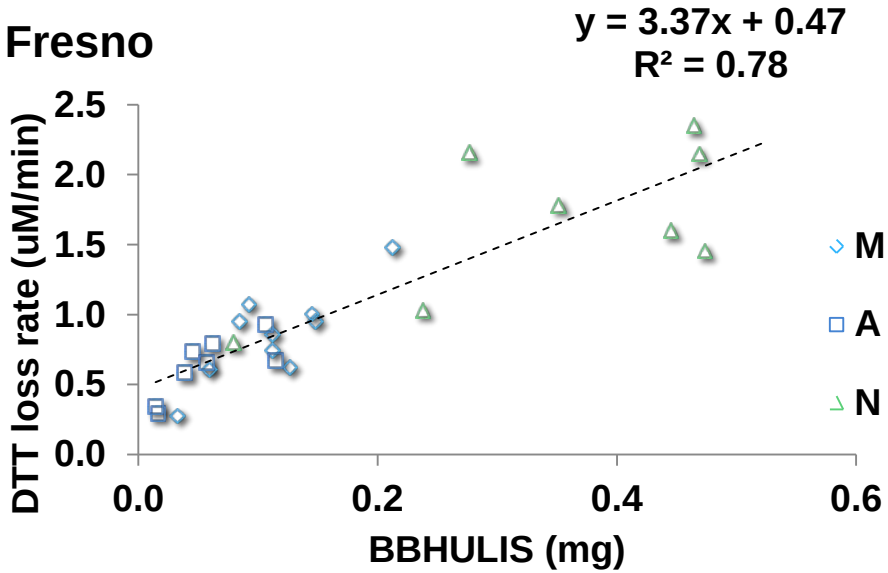




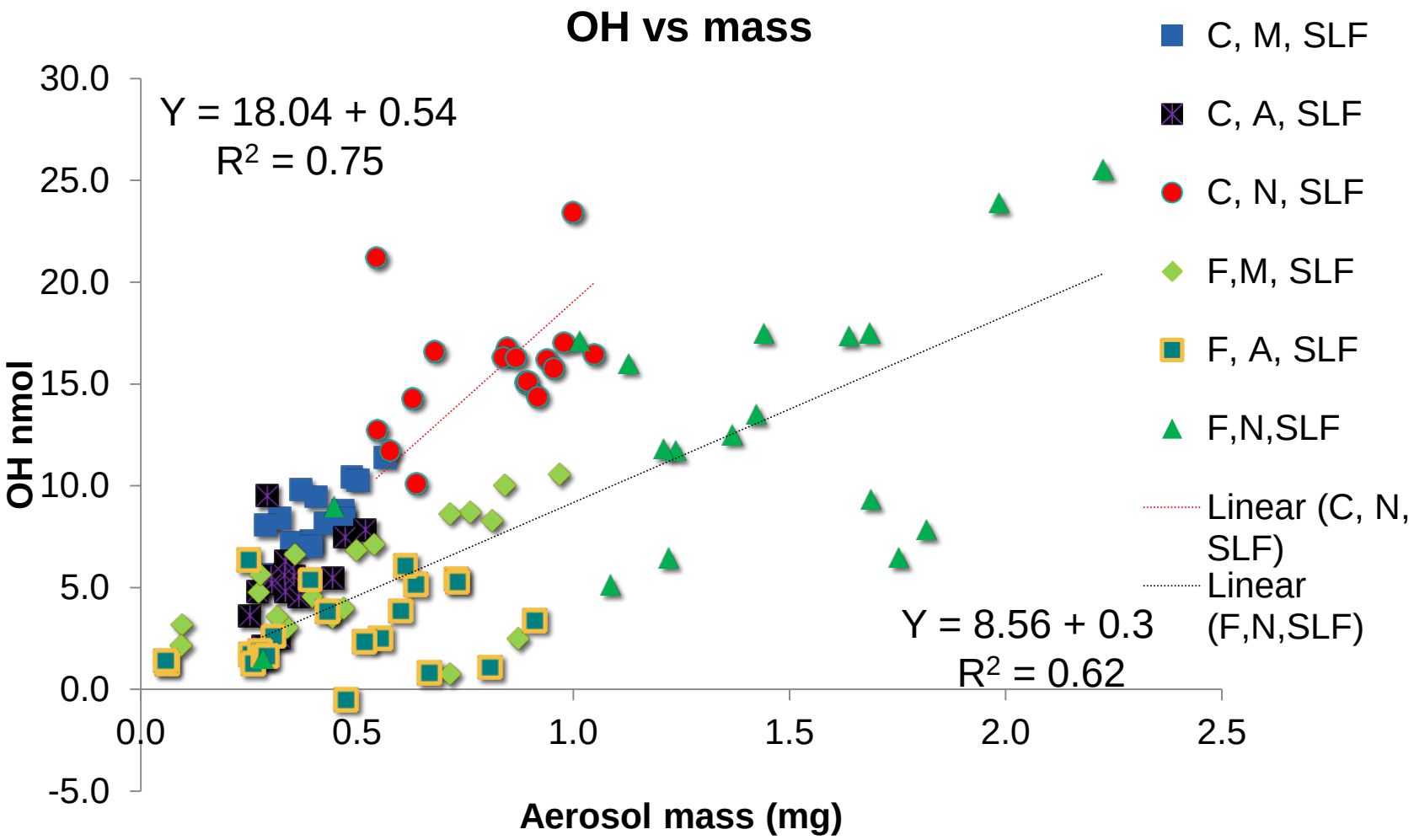
DTT is more strongly predicted by Fe and Cu in the Claremont summer samples,

and about equally well by biomass burning in the Fresno winter samples



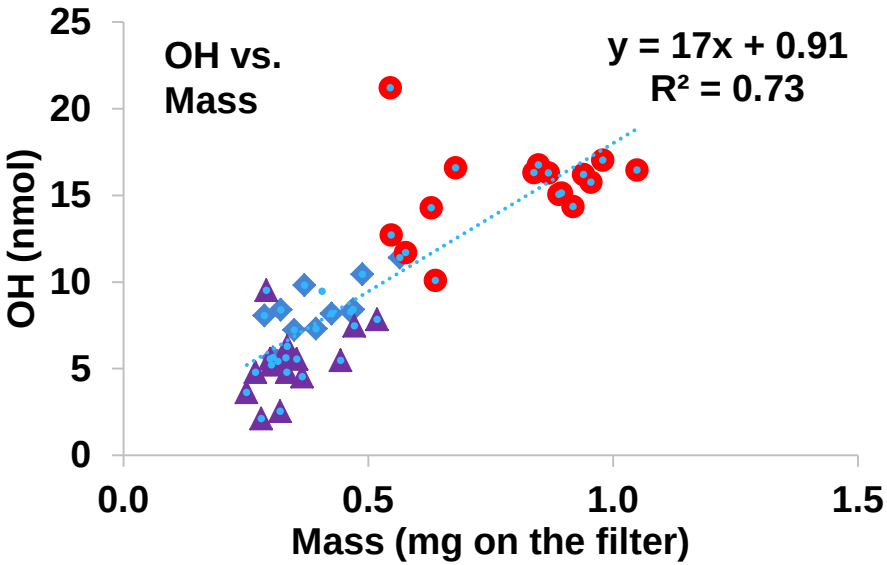


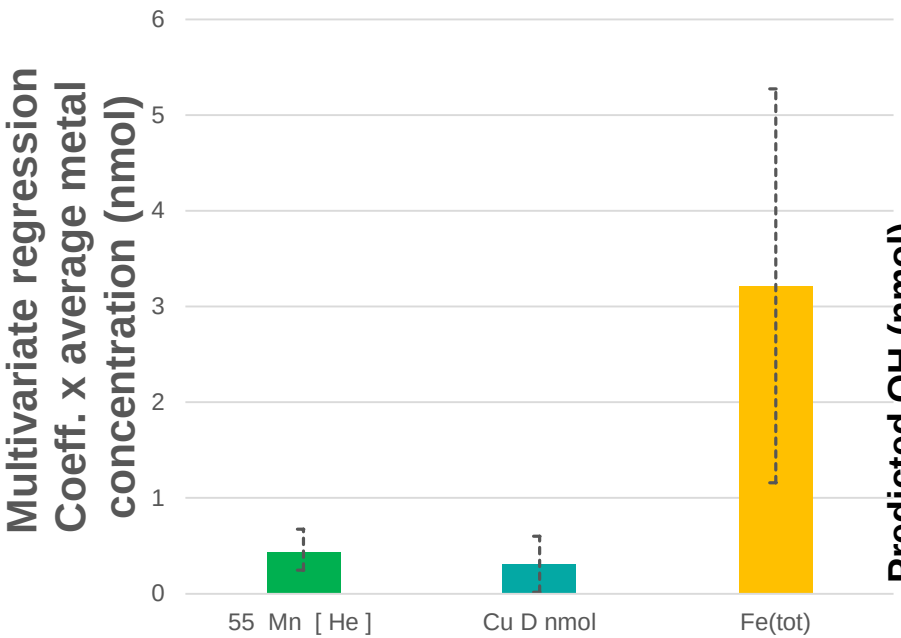
OH: is also strongly correlated with mass; slopes are very different between the two sites. Fresno afternoon samples have minimal activity.

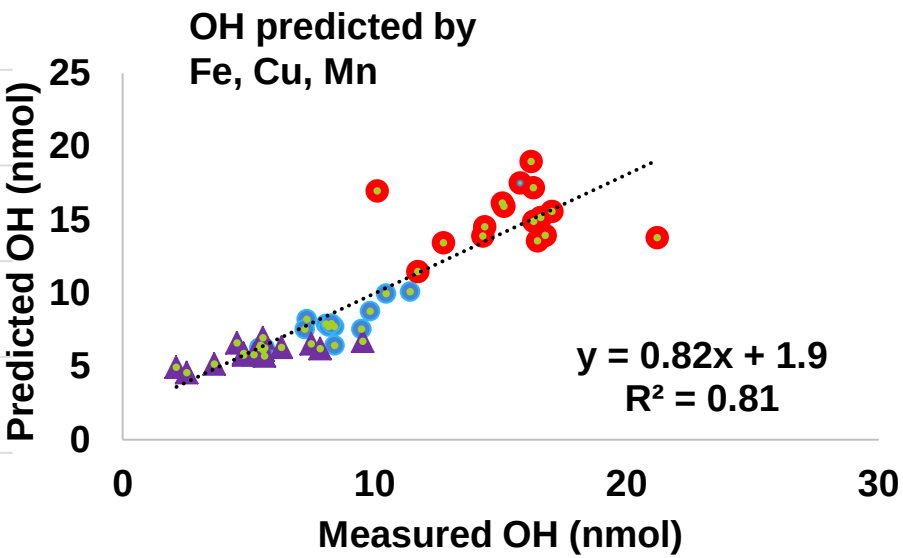


OH Claremont

OH = 3 + 0.65*Cu + 2.4*Mn + 1.8*Fe(ferrozine)

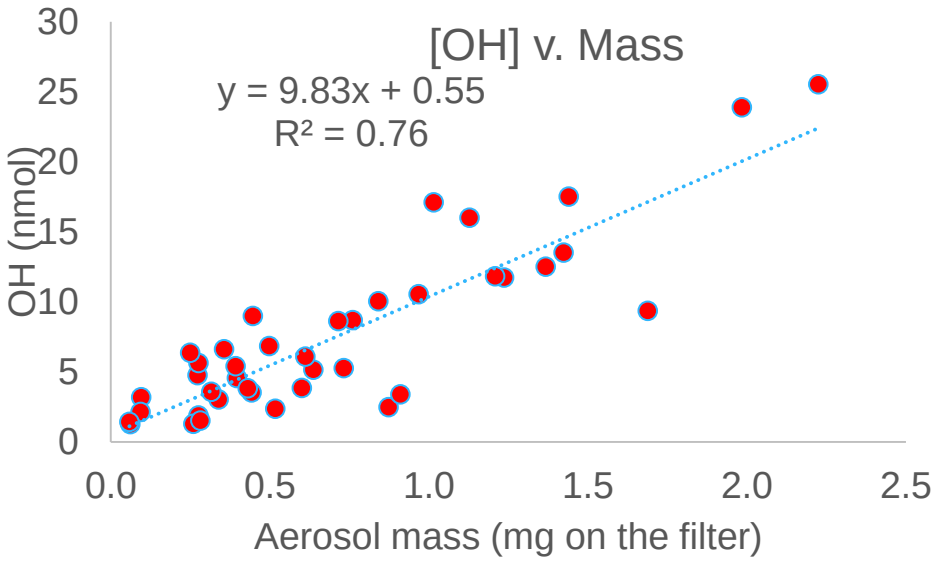


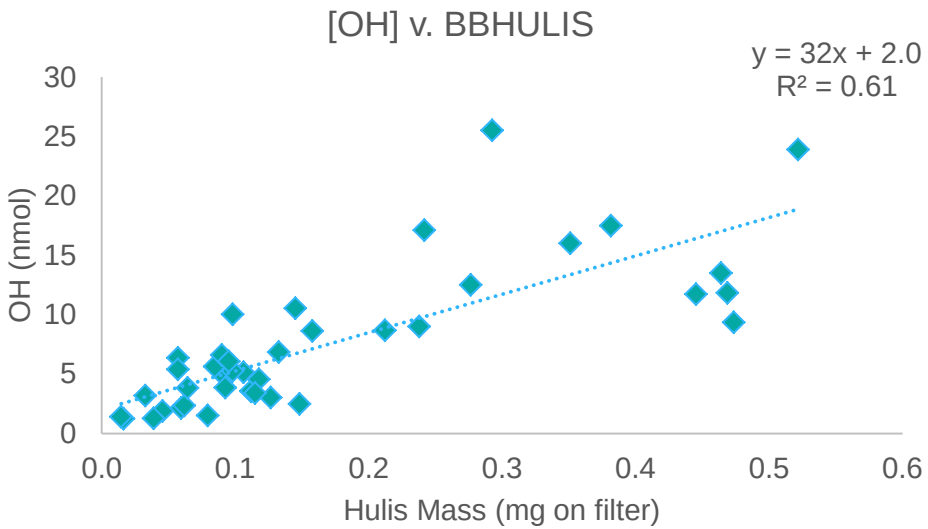




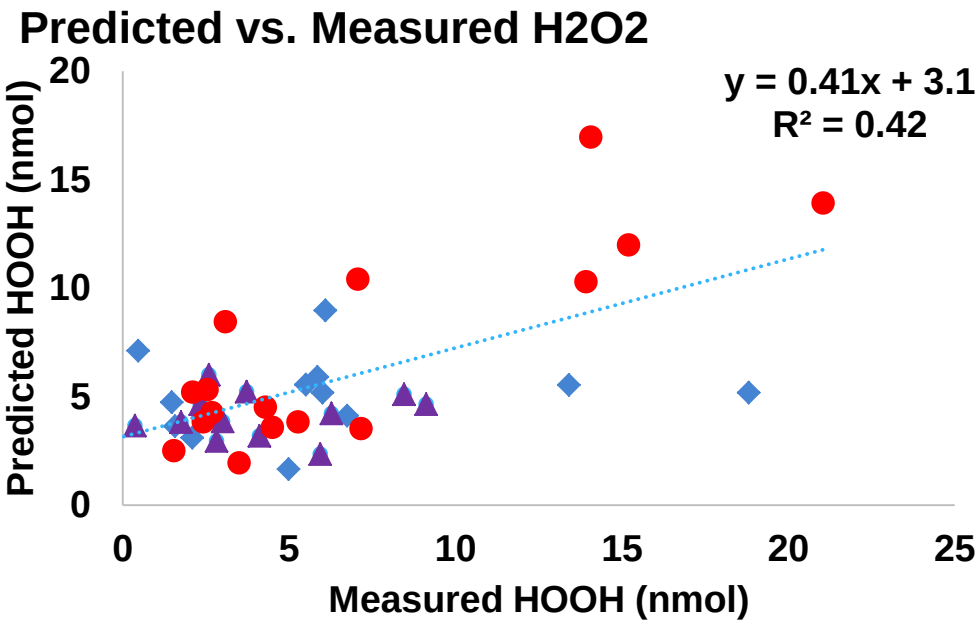
OH Fresno:
Mass is the best single predictor, followed by biomass burning aerosol (BBA), soluble iron (measured with ferrozine), and copper.

The data have too many gaps to perform a reliable multivariate regression on BBA, Fe, Cu and other metrics.



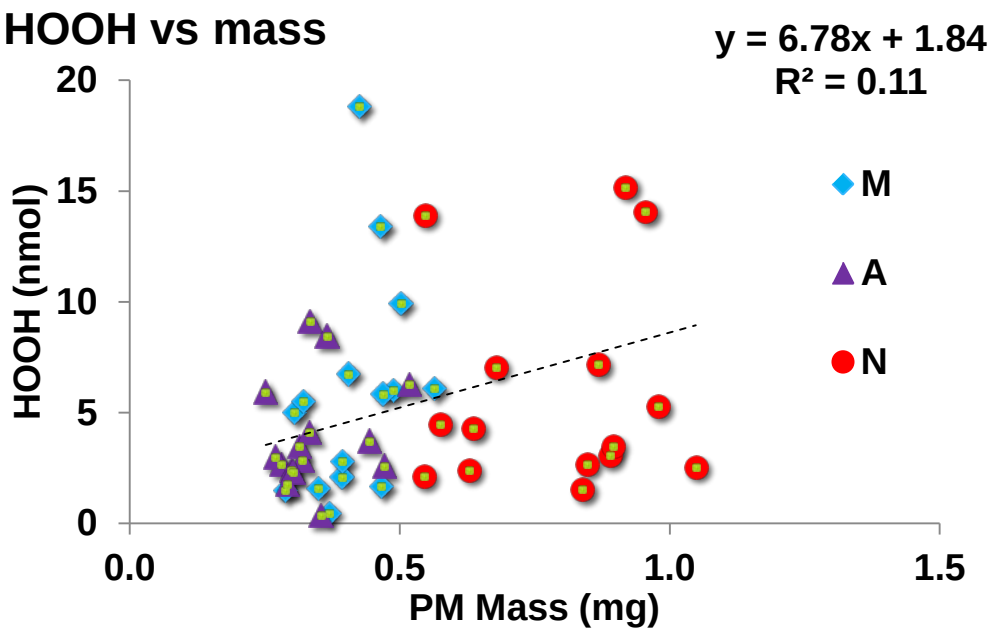


H_2O_2 =2.27 + 8.31×Mn + 0.97×Cu - 0.51×Fe

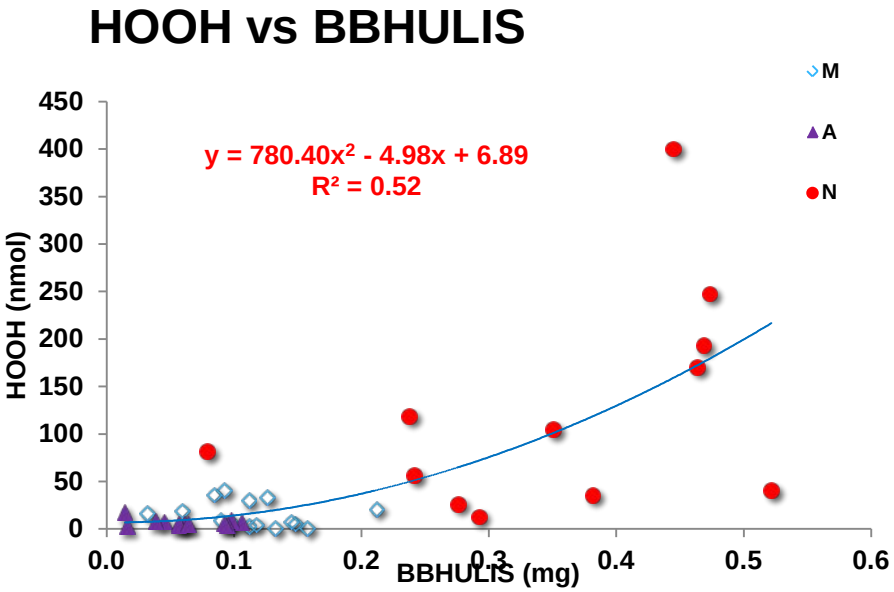


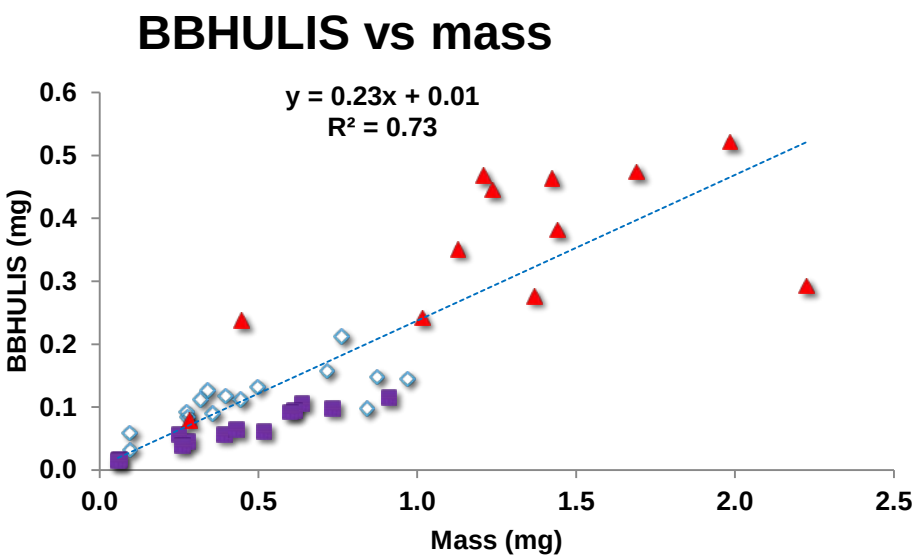
H_2O_2 Claremont:

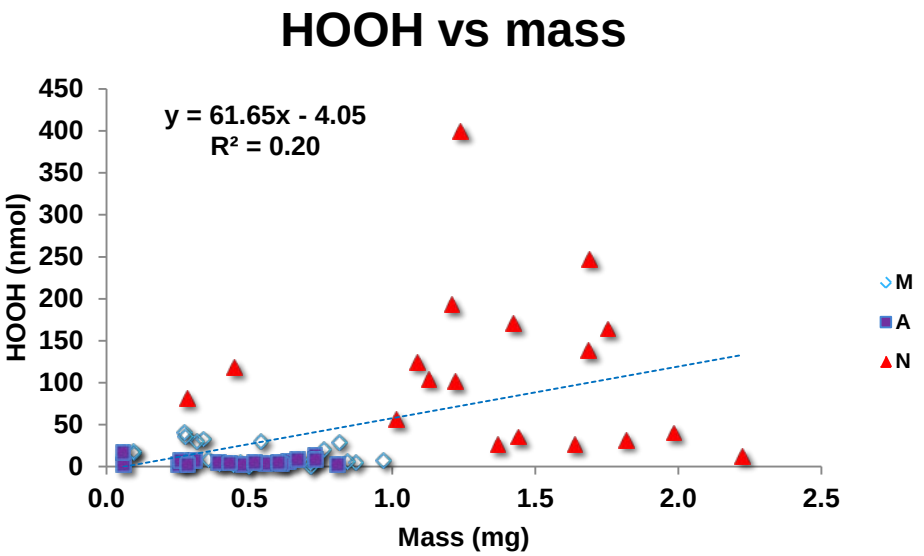
as has been observed before, H_2O_2 is not well correlated with mass, or components.



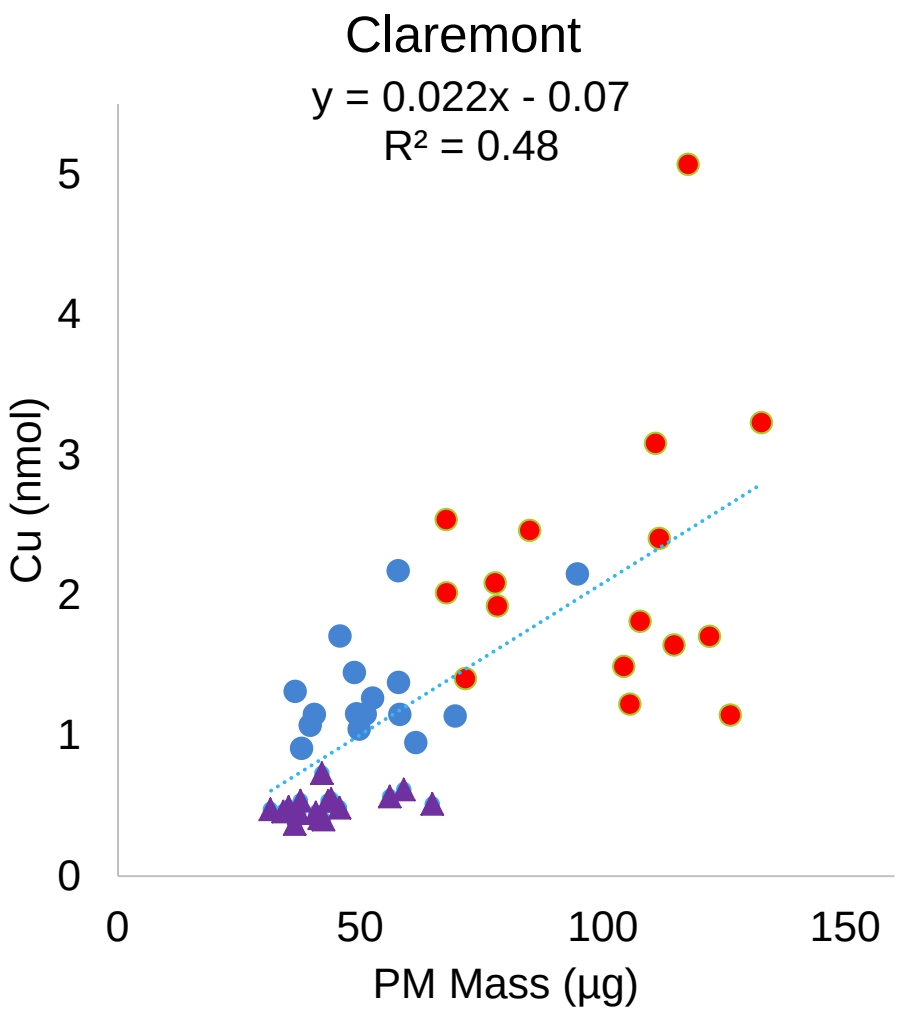
H_2O_2 Fresno

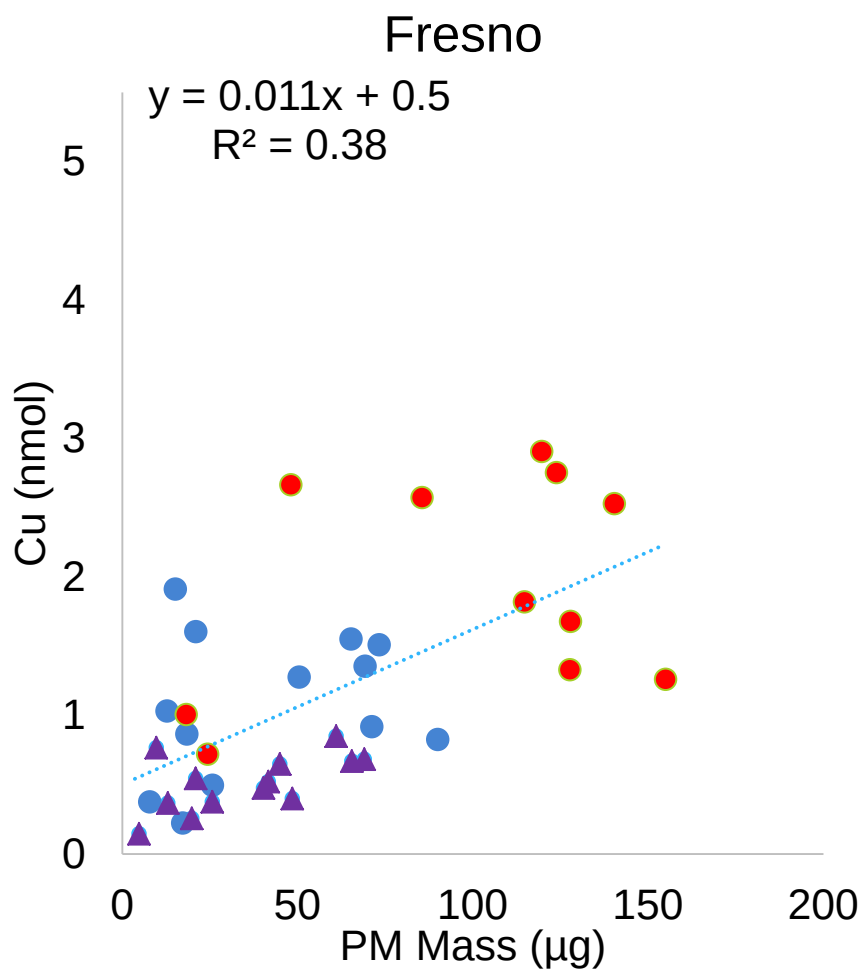

HOOH vs BBHULIS
y = 780.40x² - 4.98x + 6.89
R² = 0.52
HOOH (nmol)
BBHULIS (mg)
M
A
N
0
50
100
150
200
250
300
350
400
450
0.0
0.1
0.2
0.3
0.4
0.5
0.6


BBHULIS vs mass
y = 0.23x + 0.01
R² = 0.73
BBHULIS (mg)
Mass (mg)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.0
0.5
1.0
1.5
2.0
2.5

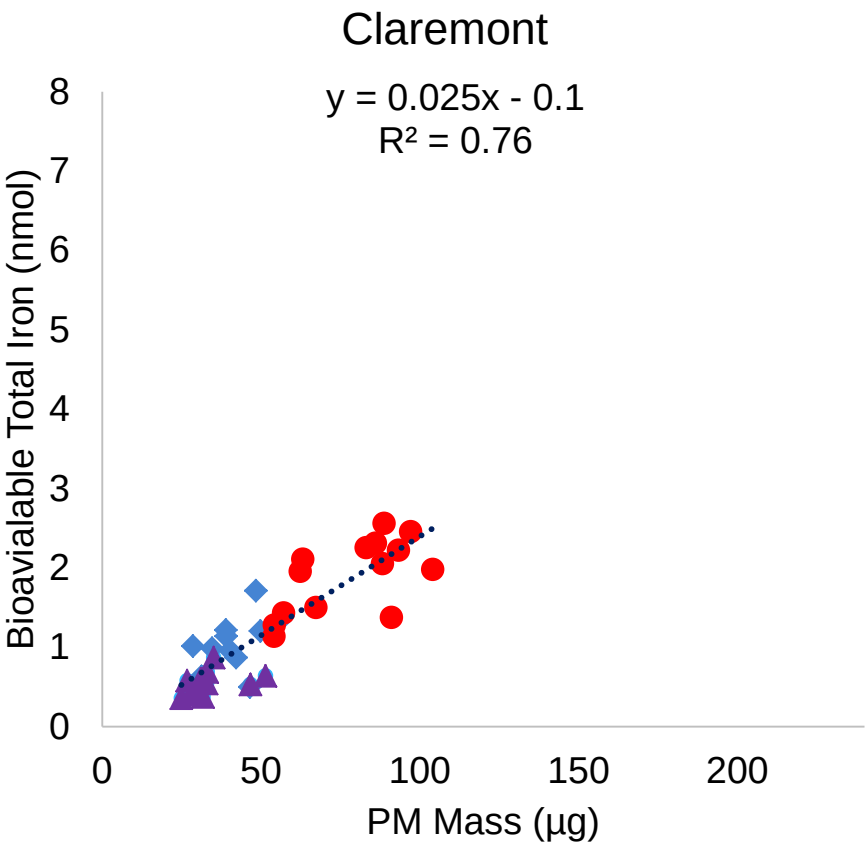

HOOH vs mass
y = 61.65x - 4.05
R² = 0.20
HOOH (nmol)
Mass (mg)
M
A
N
0
50
100
150
200
250
300
350
400
450
0.0
0.5
1.0
1.5
2.0
2.5

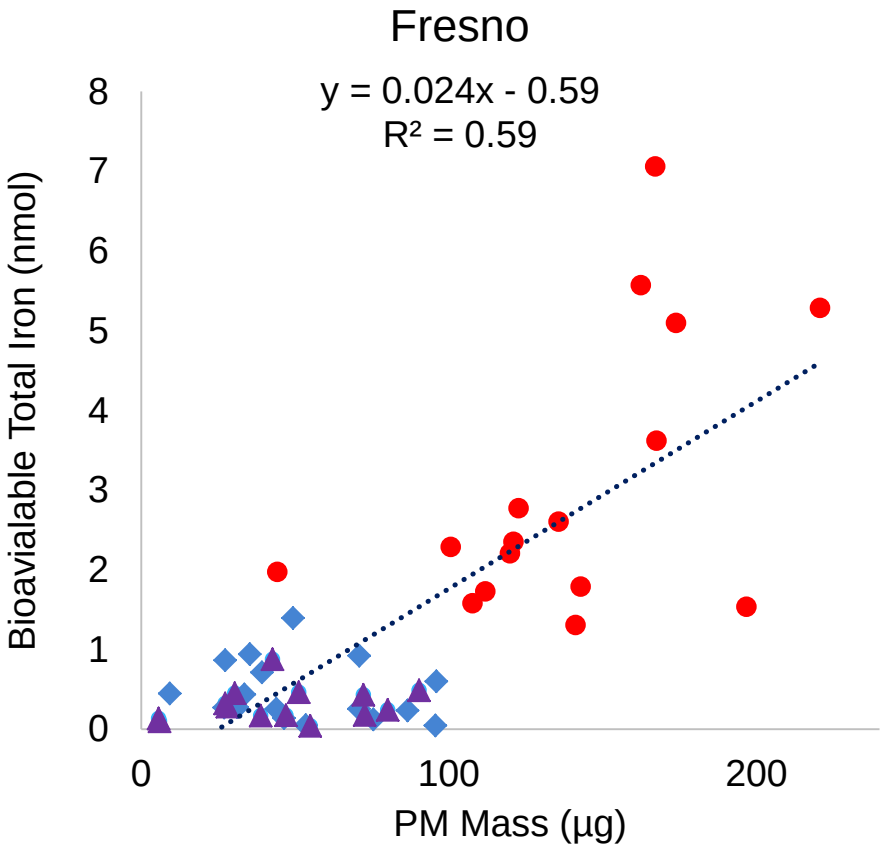
Copper: similar range for both sites; Very low & constant in afternoon especially in Claremont



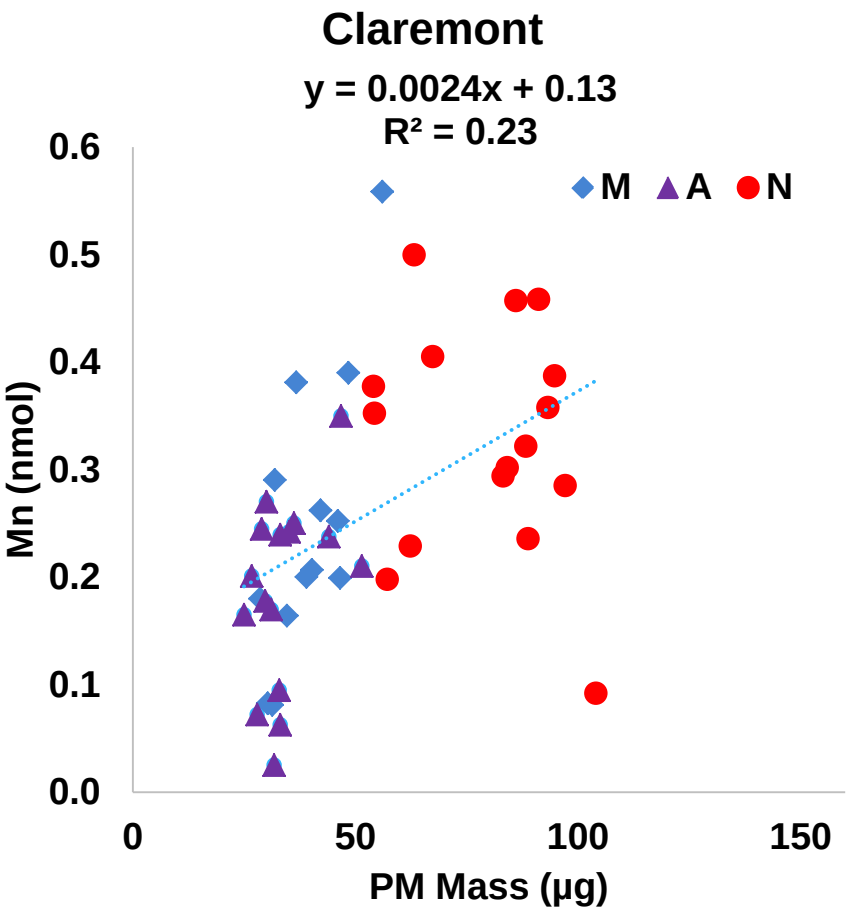


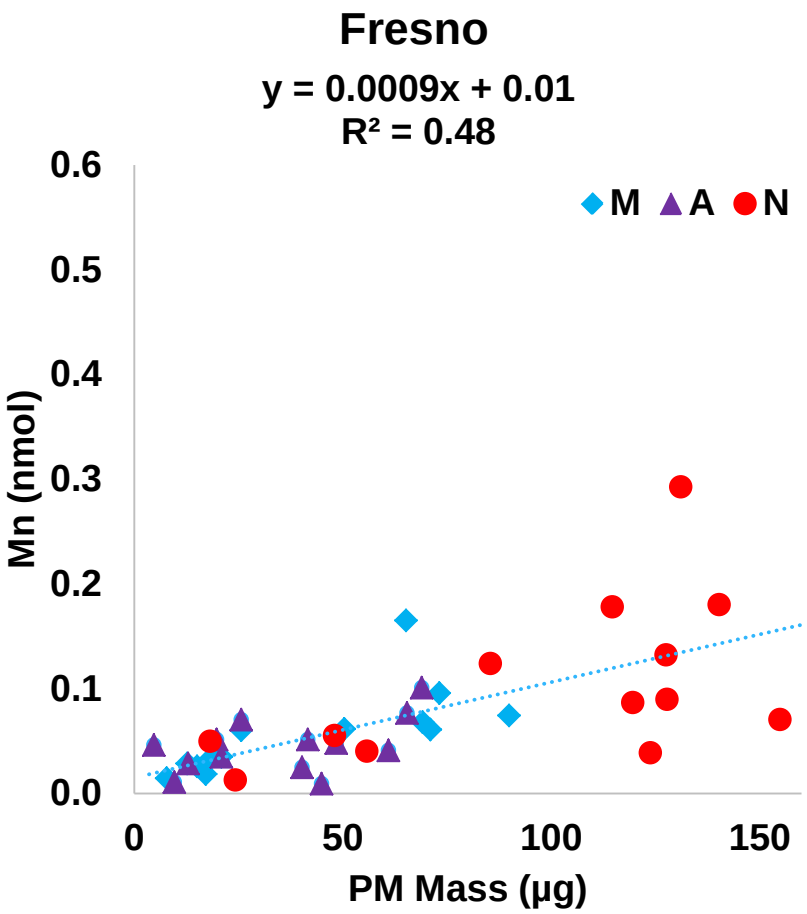
Soluble Iron: same range for both sites except Fresno Overnight samples. Much better correlated with mass in Claremont than Fresno. Very low in afternoon samples, especially in Fresno.





Soluble Mn: Much higher in Claremont than Fresno; evenly distributed by time of day





BBHULIS interactions with iron & copper

Effect of Fulvic Acid on Key Reactions

Gonzalez et al., 2016 submitted — Units of $M^{-1}\ s^{-1}$

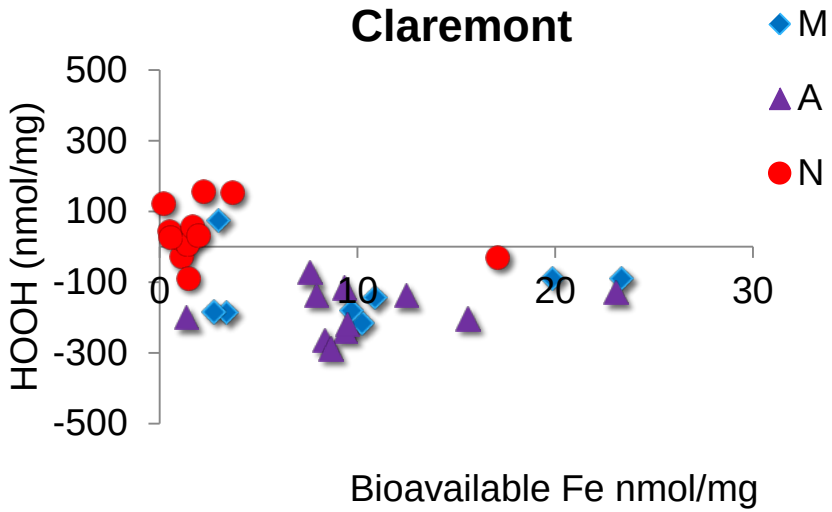
- $Fe(II) + O_2 \rightarrow Fe(III) + O_2^{\cdot -}$ — $k = 0.98$
- $FA\text{-}Fe(II) + O_2 \rightarrow FA\text{-}Fe(III) + O_2^{\cdot -}$ — $k = 2.8$

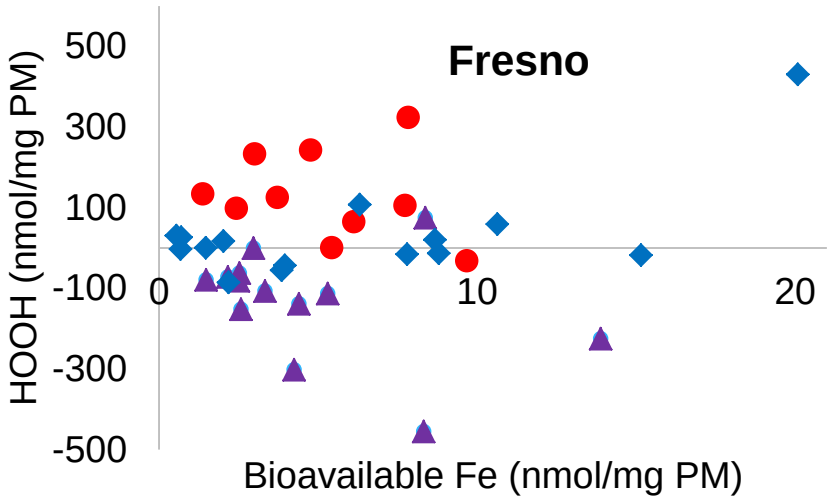
- $Fe(II) + O_2^{\cdot -} + 2\ H^+ \rightarrow Fe(III) + H_2O_2$ — $k = 200$
- $FA\text{-}Fe(II) + O_2^{\cdot -} + 2\ H^+ \rightarrow FA\text{-}Fe(III) + H_2O_2$ — $k = 2\times10^7$

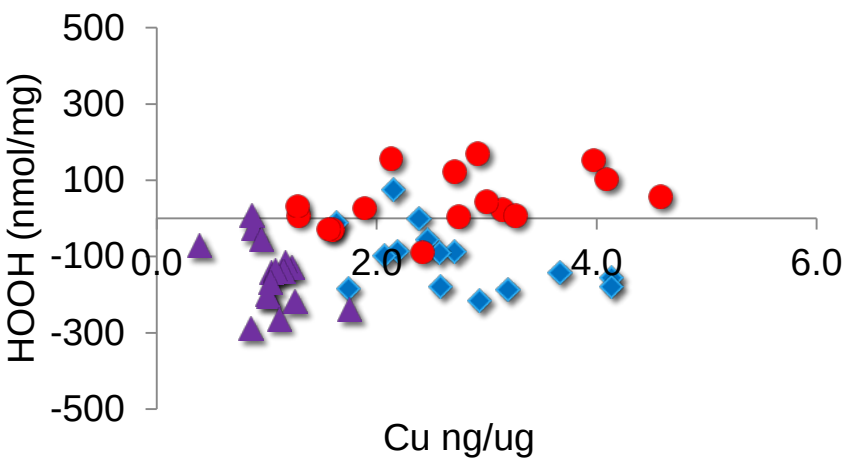
- $Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH\cdot$ — $k = 55$
- $FA\text{-}Fe(II) + H_2O_2 \rightarrow FA\text{-}Fe(III) + OH^- + OH\cdot$ — $k = 2\times10^7$

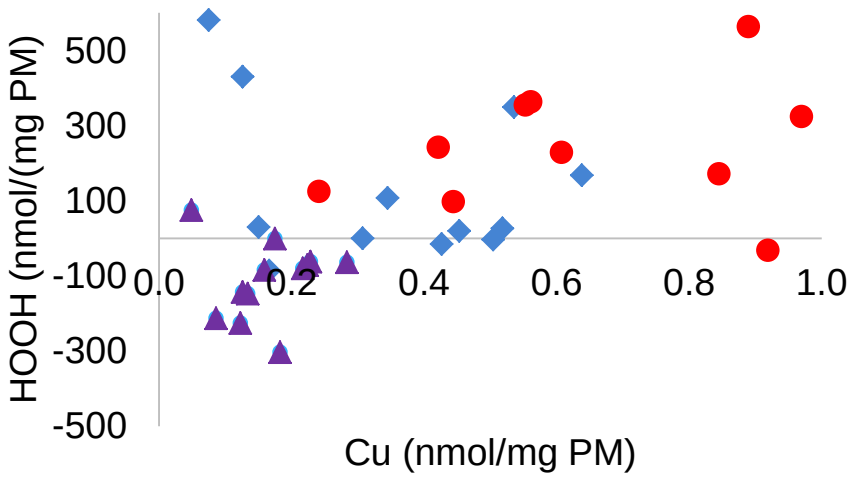
- $Fe(III) + HAsc^- \rightarrow Fe(II) + H^+ + HAsc^{\cdot -}$ — $k = 1\times10^2$
- $FA\text{-}Fe(III) + HAsc^- \rightarrow FA\text{-}Fe(II) + H^+ + HAsc^{\cdot -}$ — $k = 1\times10^2$

H_2O_2 @ 24 hours vs. Fe and Cu: What a difference BBA makes

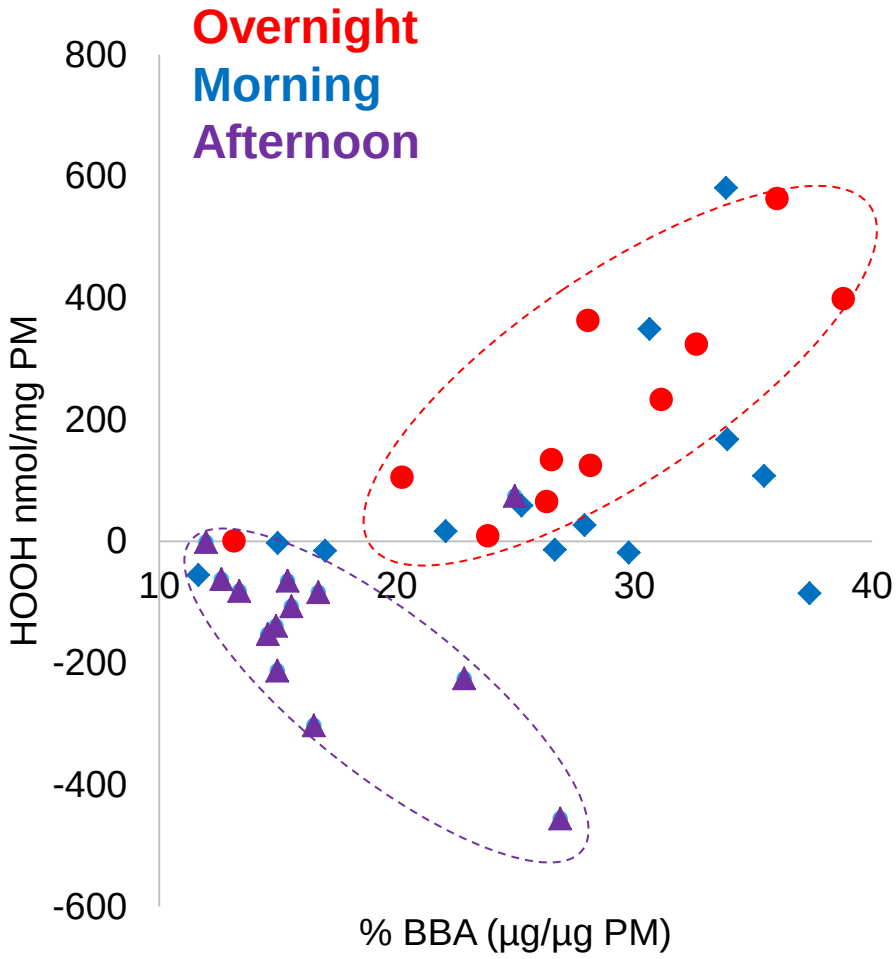


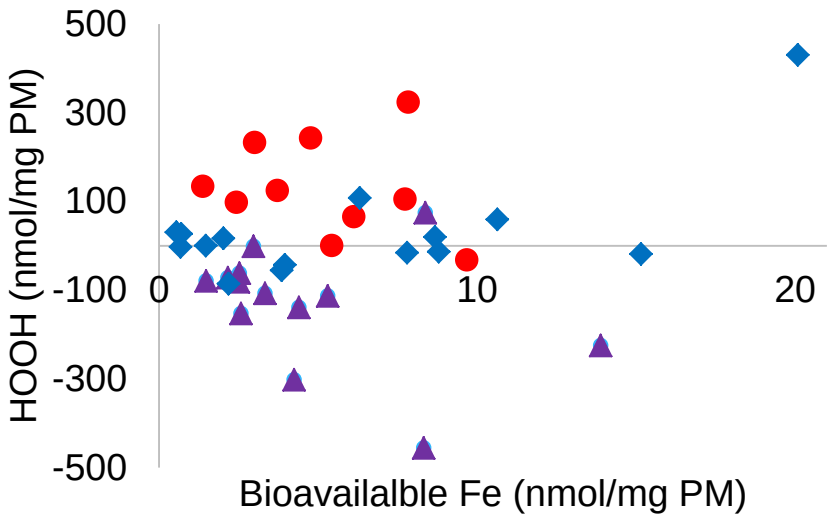


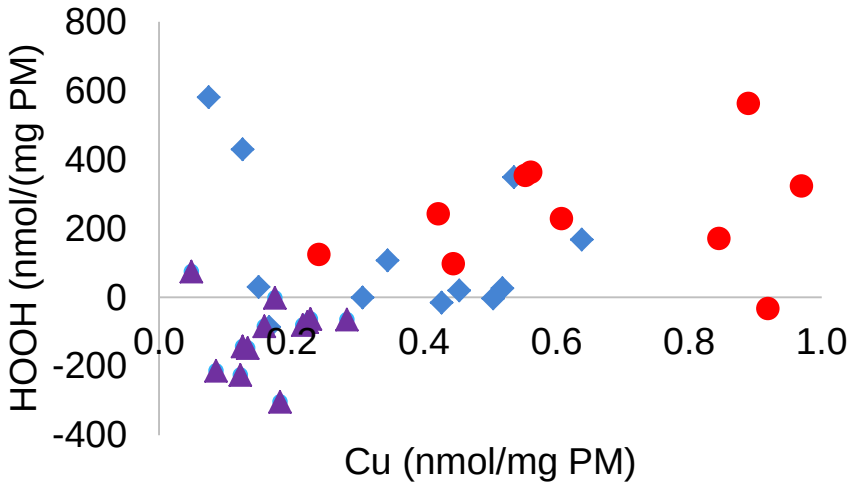




H_2O_2 after 24 hours extraction v. Biomass Burning Aerosol mass







ROS Summary

- DTT is the same at the two sites, once aerosol mass concentration is considered.
- OH is higher in the Claremont samples. It was very low in the Fresno samples unless they contained biomass burning aerosols (BBA).
- H_2O_2 is much higher in the Fresno samples, where significant biomass burning aerosol was present, and this is associated with strong interactions between BBA with Fe and Cu.

ROS Summary

- The largest differences between Fresno and Claremont are:

  -Significant biomass burning aerosol in Fresno, absent in Claremont;

  -Somewhat higher Cu, Fe and Mn in Claremont compared to Fresno, except samples with high BBA in Fresno, which have high iron.

- The BBA activity could be due to
  - higher solubilization of available iron,
  - higher Fe content,
  - or increases in the rates of the oxidant producing reactions by complexed iron and other transition metals.
  - Since copper is fairly well solubilized already, BBA may not impact its activity as much.

Comparison of field and lab results

	OH summer	OH winter	OH lab*	DTT summer	DTT winter	DTT lab**	H_2O_2 summer	H_2O_2 winter	H_2O_2 Lab***
Cu ICP	③	Other evidence	~①	②	② (weak)	①	② weak	② weak	①
Fe (Ferrozine)	①	②	~②	①	--	④	② negative weak	② weak	Negative
Mn ICP	②	--	--		--	②	① weak	--	--
quinones	--	--	PQN, 1,4- &1,2- NQN	--	--	③ PQN	--	--	PQN, 1,4- &1,2- NQN
BB HULIS	--	①	Not tested	--	①	Not tested	--	①	Not tested
Mass	--	①	--	--	--	--	--	--	--

DTT correlates most strongly with iron, WSOC and biomass burning; AA with copper

(Fang et al. ACP 2016)

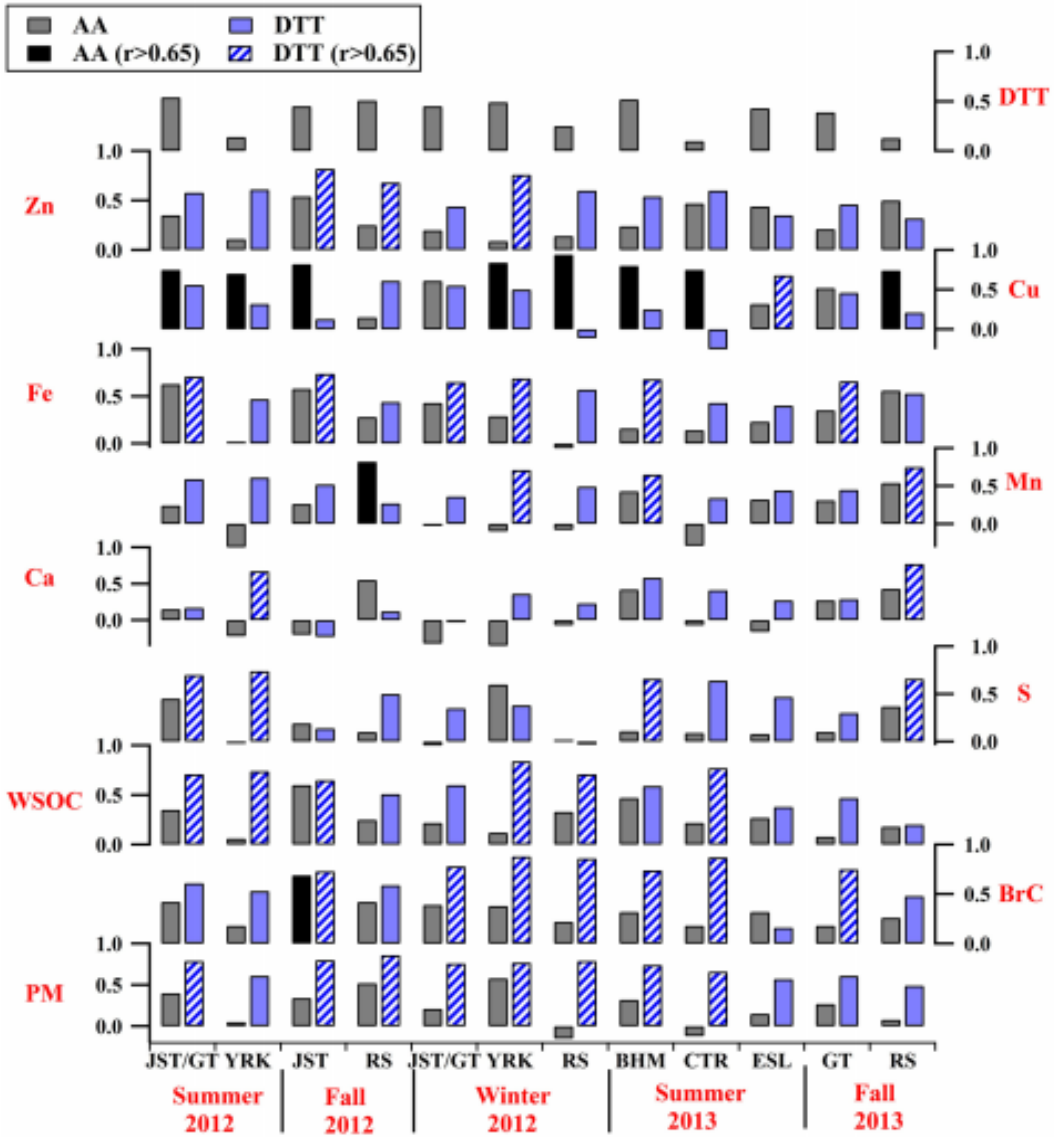


Figure 2. Correlation coefficient (Pearson's *r*) of fine particle water-soluble AA or DTT activities with $PM_{2.5}$ mass and selected chemical species at various sites in the southeastern US. A more detailed correlation table is provided in Table S2 in the Supplement.

Conclusions

- Lots of ROS assays and conditions; results depend strongly on the extraction solution and the assay.
- We were unable to find relationships between ROS quinones, although lab results support such relationships This may be due partly to limited amounts of quinone data.
- DTT and OH are generally well correlated with aerosol mass, but (clearly) mass doesn’t consume DTT or generate OH. DTT and OH are best predicted by:
  - Soluble iron, copper, and where present, biomass burning aerosol.
  - In most cases, iron measured with ferrozine is much more strongly correlated to ROS than iron measured by ICP.

Conclusions

- Organics in aerosols play a significant role in adjusting the activity of the redox active transition metals. This is most clearly seen in the hydrogen peroxide data.
- DTT consumption had the same mass-response in both cities, but OH did not. Fresno samples had lower OH production but much higher levels of hydrogen peroxide, pointing to a different distribution of reactive oxygen species, which may be related to the “even” response of the DTT samples.

Conclusions

- Some major unanswered questions:
  - What the assays are measuring. The chemistry is complicated, but it is not intractable.
  - Which assay is best.
  - Role of endogenous v. exogenous ROS.
  - The source and distribution of active iron.

Acknowledgements

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