REGIONAL WATER QUALITY NEWSLETTER

DATE: Report for May 2008 Sampling conducted May 5 & 6 2008

From the Phoenix, Tempe, Glendale, CAP, SRP – ASU Regional Water Quality Partnership <u>http://enpub.fulton.asu.edu/pwest/tasteandodor.htm</u>

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SUMMARY: EVALUATION AND RECOMMENDATIONS

- 1. MIB & geosmin levels are < 10 ng/L (the odor threshold) throughout the system. MIB above 2 ng/L (reporting level) is only present in the upper few meters of the water column in Saguaro Lake.
- 2. Raw water TOC is dominated by water released from the Salt River and averages 5 to 5.5 mg/L in the SRP system and only 2.9 mg/L in the CAP system which is mostly Colorado River Water (very little Lake Pleasant Water).
- 3. CAP plans to start releasing Lake Pleasant Water into the CAP canal in June 2008. Depending upon T&O levels later this summer CAP will cease release of Lake Pleasant water as appropriate.
- 4. News Channel 15 plans a story on DBPs tonight (Monday)
- 5. A newspaper writeup on Pharmaceuticals in Phoenix water (or lack thereof) is attached
- 6. ASU sampling of the upper Verde River for EDC/PPCPs is included.
- 7. A detailed description of some organic matter characterization in our watershed is attached along with DBP formation.

Table 1 Summary of WTP Operations

	Verde WTP	Union Hills	24 th Street WTP	N.Tempe J.G. Martinez	Deer Valley	Glendale Cholla WTP ³	Val Vista	South Tempe	Chandler WTP
Location	Verde River	CAP	Arizona	Canal S		South Canal System			
PAC Type and Dose		None	14.4 ppm Calgon	None	28.8 ⁴ ppm Calgon	None	9 ppm	None	
Copper Sulfate		None	10 ppm	None	None	None	0.9 ppm	None	
PreOxidation		(1 ppm pretreatme nt)	None	None	None	6 ppm 8 hr/week	None	None	
Alum Dose Alkalinity pH		6.9 ¹ 126 7.9	67 130/101 6.7	28 ppm 132 7.03	60.4 135/103 6.7	50 129 7.1	57 136 6.9	30 108 7.6	
Finished water DOC DOC removal ²		2.36 18%	2.90 46%	4.03 25%	1.77 66%	2.97 42%	3.2 42%	4.44 19%	
Average turbidity over last 7 days			25	18	25	17	12	6.9	
Recommendations					High PAC usage demonst rates ability to remove TOC 4				

¹ Ferric chloride instead of alum; plus 2.25 ppm polymer (308) ² Calculated based upon influent and filtered water DOC (note that DOC – not TOC – is used in this calculation)

³ Sample from finished water includes a blend of surface and ground water sources

⁴ The carbon vendor has requested we schedule deliveries at least two weeks out. Various short term plant shut downs for construction as well as other factors has lead to us having to feed heavy to make room for the next delivery.

Turbidity in CAP Water:

The Alamo Dam is presently going through a planned release. The maximum flowrate of 2,000 cfs will occur today, Monday, March 31 between 11:00AM and 7:00PM with a gradual decrease back to the normal 40 cfs by 10:00PM. To contact the Army Corps of Engineers regarding this event please contact their Reservoir Operation Center (ROC) at (213) 452-3623.

We contacted CAP to determine if there was to be any reaction from them with respect to mitigating the potential turbidity event. CAP indicated there are no plans to change the operations at the Mark Wilmer Pump Station (Havasu Intake). We recommend that the Union Hills Water Treatment Plant staff be aware of the potential increase in turbidity along the CAP canal as a result of the Alamo Dam release. The data logger developed as a part of this project is currently running and we recommend that you should monitor the online reporting website (http://www.cap-

az.com/WaterQualityData/CanalWaterQuality.cfm) for any significant changes reported therein. Currently, the estimated canal travel time between the Havasu Intake and the UHWTP is 64-78 hours (2.5-3.25 days) and it is predicted that the Alamo release could take between 3-5 days to travel down the Bill Williams to Lake Havasu, meaning a total delay of 6-9 days.

Best Regards - Herb

System	SRP	CAP		
	Diversions			
Arizona Canal	728	78		
South Canal	603	0		
Pumping	87	0		
Total	1418	78		

SRP/CAP OPERATIONS Values in cfs. for May 5, 2008

SRP is releasing water from both Verde and Salt River Systems. Salt River release from Saguaro Lake: 779 cfs; Verde River release from Bartlett Lake: 500 cfs.

Lake Roosevelt is 98% full and the Verde River system is 96% full. Flow over Granite Reef dam into the Salt River Channel = 0 cfs.

CAP plans to start releasing Lake Pleasant Water into the CAP canal in June 2008. Depending upon T&O levels later this summer CAP will cease release of Lake Pleasant water as appropriate.

Sample Description	MIB (ng/L)	Geosmin (ng/L)	Cyclocitral (ng/L)
24 th Street WTP Inlet	<2.0	<2.0	2.0
24 th Street WTP Treated	<2.0	<2.0	<2.0
Deer Valley Inlet	<2.0	<2.0	<2.0
Deer Valley WTP Treated	<2.0	<2.0	<2.0
Val Vista Inlet	<2.0	<2.0	<2.0
Val Vista WTP Treated –East	<2.0	<2.0	<2.0
Val Vista WTP Treated -West	<2.0	<2.0	<2.0
Union Hills Inlet	<2.0	<2.0	<2.0
Union Hills Treated	<2.0	<2.0	<2.0
Tempe North Inlet	<2.0	<2.0	2.5
Tempe North Plant Treated	<2.0	<2.0	<2.0
Tempe South WTP	<2.0	<2.0	<2.0
Tempe South Plant Treated	<2.0	2.7	<2.0
Tempe South Plant Treated (Lab)			
Glendale WTP Inlet	<2.0	<2.0	<2.0
Glendale WTP Treated	<2.0	<2.0	<2.0
Glendale WTP Treated (Lab)]		

Table 2 - Water Treatment Plants – May 5, 2008

System	Sample Description	MIB (ng/L)	Geosmin	Cyclocitral
			(ng/L)	(ng/L)
CAP	Waddell Canal	<2.0	<2.0	<2.0
	Union Hills Inlet	<2.0	<2.0	<2.0
	CAP Canal at Cross-connect	<2.0	<2.0	11.3
	Salt River @ Blue Pt Bridge	<2.0	<2.0	<2.0
	Verde River @ Beeline	<2.0	<2.0	3.0
AZ	AZ Canal above CAP Cross-connect	<2.0	<2.0	<2.0
Canal	AZ Canal below CAP Cross-connect	<2.0	<2.0	<2.0
	AZ Canal at Highway 87	<2.0	<2.0	<2.0
	AZ Canal at Pima Rd.	<2.0	<2.0	<2.0
	AZ Canal at 56th St.	<2.0	<2.0	<2.0
	AZ Canal - Inlet to 24 th Street WTP	<2.0	<2.0	2
	AZ Canal - Central Avenue	<2.0	<2.0	<2.0
	AZ Canal - Inlet to Deer Valley WTP	<2.0	<2.0	<2.0
	AZ Canal - Inlet to Glendale WTP	<2.0	<2.0	<2.0
South	South Canal below CAP Cross-connect	<2.0	<2.0	9.7
and	South Canal at Val Vista WTP	<2.0	<2.0	<2.0
Tempe	Head of the Tempe Canal	<2.0	<2.0	<2.0
Canals	Tempe Canal - Inlet to Tempe's South			
	Plant	<2.0	<2.0	<2.0
	Chandler WTP – Inlet			

Table 3 - Canal Sampling – May 5, 2008

Sample Description	Location	MIB (ng/L)	Geosmin (ng/L)	Cyclocitral (ng/L)
Lake Pleasant	Eplimnion			
Lake Pleasant	Hypolimnion			
Verde River @ Beeline		<2.0	<2.0	3.0
Bartlett Reservoir	Epilimnion	2.0	<2.0	<2.0
Bartlett Reservoir	Epi-near dock	<2.0	<2.0	<2.0
Bartlett Reservoir	Hypolimnion	<2.0	<2.0	<2.0
Salt River @ BluePt Bridge		<2.0	<2.0	<2.0
Saguaro Lake	Epilimnion	5.0	<2.0	16.4
Saguaro Lake	Epi - Duplicate	7.4	<2.0	17.5
Saguaro Lake	Epi-near doc	<2.0	<2.0	4.9
Saguaro Lake	Hypolimnion	<2.0	<2.0	<2.0
Verde River at Tangle Creek				
Havasu				

Table 4 - Reservoir Samples – May 6, 2008

Sample Description	DOC (mg/L)	UV254 (1/cm)	SUVA (L/mg-m)	TDN		DOC removal (%)
24 th Street WTP Inlet	5.36	0.142	2.6	0.964		
24 th Street WTP Treated	2.90	0.046	1.6	0.920		46
Deer Valley Inlet	5.20	0.140	2.7	0.970		
Deer Valley WTP Treated	1.77	0.025	1.4	0.901		66
Val Vista Inlet	5.66	0.151	2.67	0.959	1	
Val Vista WTP Treated –East	3.20	0.057	1.80	0.850		43
Val Vista WTP Treated -West	3.26	0.060	1.83	0.878		42
Union Hills Inlet	2.86	0.044	1.55	0.659	1	
Union Hills Treated	2.36	0.024	1.03	0.634		18
Tempe North Inlet	5.37	0.143	2.66	1.040	1	
Tempe North Plant Treated	4.03	0.078	1.92	0.925		25
Tempe South WTP	5.46	0.148	2.72	0.862	1	
Tempe South Plant Treated	4.44	0.101	2.27	0.826		19
Chandler WTP Inlet					1	
Chandler WTP Treated						
Glendale WTP Inlet	5.13	0.140	2.7	1.030	1	
Glendale WTP Treated	2.97	0.056	1.9	1.484		42

Table 5 - Water Treatment Plants - May 05, 2008

DOC = Dissolved organic carbon

UV254 = ultraviolet absorbance at 254 nm (an indicator of aromatic carbon content) SUVA = UV254/DOC

TDN = Total dissolved nitrogen (mgN/L)

System	Sample Description	DOC	UV254	SUVA	TDN
		(mg/L)	(1/cm)	(L/mg-m)	IDN
CAP	Waddell Canal	3.20	0.059	1.86	0.634
	Union Hills Inlet	2.86	0.044	1.55	0.659
	CAP Canal at Cross-connect	2.81	0.044	1.55	0.655
	Salt River @ Blue Pt Bridge	5.95	0.139	2.33	1.160
	Verde River @ Beeline	5.34	0.177	3.32	0.840
AZ	AZ Canal above CAP Cross-connect	5.63	0.150	2.67	0.940
Canal	AZ Canal below CAP Cross-connect	4.66	0.110	2.36	0.859
	AZ Canal at Highway 87	5.26	0.139	2.63	0.910
	AZ Canal at Pima Rd.	5.50	0.140	2.55	1.026
	AZ Canal at 56th St.	5.29	0.138	2.61	1.004
	AZ Canal - Inlet to 24 th Street WTP	5.36	0.142	2.64	0.964
	AZ Canal - Central Avenue	5.22	0.139	2.67	1.022
	AZ Canal - Inlet to Deer Valley WTP	5.20	0.140	2.69	0.970
	AZ Canal - Inlet to Glendale WTP	5.13	0.140	2.74	1.030
South	South Canal below CAP Cross-connect	5.49	0.151	2.75	0.950
and	South Canal at Val Vista WTP	5.66	0.151	2.67	0.959
Tempe	Head of the Tempe Canal	5.74	0.155	2.70	0.886
Canals	Tempe Canal - Inlet to Tempe's South Plant	5.46	0.148	2.72	0.862
	Chandler WTP – Inlet				

Table 6 - Canal Sampling - May 5, 2008

Table 7 - Reservoir Samples - May 05, 2008

Sample Description	Location	DOC (mg/L)	UV254 (1/cm)	SUVA (L/mg-m)	TDN
Lake Pleasant	Eplimnion				
Lake Pleasant	Hypolimnio				
Verde River @ Beeline		5.34	0.177	3.32	0.840
Bartlett Reservoir	Epilimnion	5.87	0.159	2.72	0.430
Bartlett Reservoir	Epi-near dock				
Bartlett Reservoir	Hypolimnio	6.45	0.209	3.24	1.152
Salt River @ BluePt Bridge		5.95	0.139	2.33	1.160
Saguaro Lake	Epilimnion	6.94	0.138	1.99	1.169
Saguaro Lake	Epi - Duplicate	7.45	0.139	1.87	1.035
Saguaro Lake	Epi-near doc				
Saguaro Lake	Hypolimnio	6.79	0.142	2.09	1.514
Verde River at Tangle					
Havasu (April 2008)		2.75	0.039	1.41	0.739

EDC/PPCP Newspaper article

Phoenix drinking water received a clean bill of health Monday after extensive testing found no pharmaceuticals in the water supply.

Tests conducted by an independent laboratory at each of Phoenix's six water treatment plants showed no traces of drugs and met all standards of the Environmental Protection Agency, officials said at an afternoon press conference.

"I'm pleased today to report that Phoenix tap water meets all EPA requirements . . . and that no trace of pharmaceuticals were found in any of the tests," Mayor Phil Gordon said. "Providing safe and healthy water to our residents (is something that) we do very well."

Officials decided to test the water after an article in the *Las Vegas Review-Journal* indicated that at least nine trace pharmaceuticals have been identified in Lake Mead's water, some of which eventually reaches Phoenix via the Central Arizona Project. But the chemicals were in tiny quantities and posed on health threat, the article said.

Drugs are thought to enter the water supply after being passed through the digestive tract. Other drugs get flushed into the system.

No one knows for sure whether exposure to small quantities of these drugs can cause problems. Exposure to higher concentrations would pose a threat, officials say.

Phoenix gets most of its water from the Salt River Project and the Central Arizona Project. Neither one tests for pharmaceuticals, spokesmen said. Tucson, which reported finding three pharmaceuticals in its water, also gets a portion of its supply from the CAP, but it is unknown whether the drugs came from CAP water.

Phoenix has no current plans to test the water again but is working with research organizations that are working to develop testing standards for pharmaceuticals, said Ken Kroski, a water department spokesman.

"That's something we have got to figure out," Kroski said. "This is something that is in its infancy. It's going to take a little while for the EPA to come up with standards."

EDC/PPCP sampling

(See article at end of newsletter for the Associate Press article – or one of them)

ASU is sampling and monitoring for EDC/PPCPs in the SRP watershed and other selected locations. Below is data from a March 2008 sampling campaign. Samples from the upper Verde River area are labled as "Sedona ..." and their locations are shown on the attached map. Data is on the next page.

Most EDC/PPCP concentrations are very low (< 10 ng/L). We hope to study the effects of increased recreational use in the watershed this summer, including the popular lower Salt River and Slide Rock recreational areas.

No.	Sample name	Location	Elevation (ft)	Temp. (C)	рН	Conductivity (ms)	NPOC (mg/L)	TDN (mg/L)	UV254
1	Sedona A	N. 34.86270 W. 111.76164	4215	4.1	7.75	0.181	6.288	0.458716	0.27200
2	Sedona B	N. 34.49013 W. 111.50240	3844	4.7	7.69				
3	Sedona C	N. 34.46000 W. 111.53473	3499	6.4	7.79	0.207	5.554	0.492901	0.29250
4	Sedona D	N. 34.71886 W. 111.91654	3270	7.7	7.71				
5	Sedona E	N. 34.72333 W. 111.99127	3246	8.4	8.01	0.403	5.844	0.653029	0.19900
6	Sedona F	N. 34.76558 W. 112.03638	3318	1					
7	Sedona G	N. 34.67126 W. 111.94742	3200	8.4	7.79	0.393	5.844	0.662805	0.21400
8	Sedona H	N. 34.60937 W. 111.89640	3141	8.3	7.86				
9	Sedona I	N. 34.54998 W. 111.84981	3069	7.7	7.76	0.303	7.036	0.702422	0.29500
10	WTP #1 Inlet				8.27	1.035	2.807	0.645936	0.03910
11	WTP#2 pre-filtration				7.89	1.049	2.503	0.576671	0.02245
12	WTP #2 inlet				7.83	0.420	6.189	3.030856	0.02790
13	WTP #2 pre-filtration				8.08	0.653	2.900	1.883891	0.05840
14	Metro area WWTP influent								
15	WWTP effluent (before UV)								
16	WWTP effluent (post UV)								

Sample sites 14-16 are being monitored Feburary – April 2008

Sample Name	Ibuprofen	Naproxen	Warfarin	Dilantin	Triclosan	Diclofenac	Tetrabromobishpenol A	Sucralose
Blank-Lab	0	0	0	0	2	0	0	2
Blank-field	0	0	0	1	2	1	0	1
Sedona A	1	2	1	1	2	0	0	18
Sedona B	1	2	1	1	2	0	1	28
Sedona C	1	2	1	1	2	0	0	16
Sedona D	1	1	1	0	2	0	1	19
Sedona E	0	1	0	1	2	0	1	4
Sedona F	0	1	0	0	2	0	2	13
Sedona G	0	1	0	0	2	0	0	5
Sedona H	1	1	0	1	2	0	2	4
Sedona I	1	1	0	0	3	0	2	4
WTP #1 inffluent	1	1	0	2	2	0	2	10
WTP#1 effluent	1	0	0	3	2	0	1	9
WTP#2 inffluent	1	1	0	1	4	0	3	4
WTP #2 effluent	1	1	0	1	2	0	2	6
WWTP inffluent	3830	1220	0	43	241	0	0	22
WWTP eff before UV	10	6	0	94	63	4	2	560
WWTP eff after UV	9	6	0	99	49	14	1	574
25 ppt QC	19	29	30	24	31	28	28	38

Sample Name	Acetaminophen	Caffeine	Carbamazepine	Cotinine	Diazepam	Fluoxetine	Hydrocodone	Meprobamate	Pentoxifylline	Oxybenzone	Sulfamethoxazole	DEET	Erythromycin -H2O	Trimethoprim	Primidone
Blank-Lab	0	2	0	0	0	1	0	0	0	3	0	1	0	0	0
Blank-field	1	3	0	0	0	1	0	0	0	3	0	1	0	1	0
Sedona A	3	13	1	1	0	1	1	1	0	4	1	1	0	1	0
Sedona B	4	12	1	1	0	1	0	1	0	2	1	0	0	1	0
Sedona C	4	12	1	1	0	1	1	1	1	3	1	0	0	1	1
Sedona D	2	10	1	1	0	1	1	1	1	3	1	0	0	0	1
Sedona E	1	12	0	1	0	1	0	0	1	3	0	0	0	0	0
Sedona F	1	9	0	1	0	1	0	0	1	3	0	0	0	0	0
Sedona G	1	5	0	1	0	1	0	0	1	2	0	0	0	0	0
Sedona H	1	6	0	1	0	1	0	0	1	2	1	0	0	0	0
Sedona I	1	4	0	1	0	1	0	0	1	2	1	0	0	0	0
WTP #1 inffluent	1	11	3	3	0	1	0	7	1	2	11	3	0	1	5
WTP#1 effluent	0	4	2	3	0	1	0	7	1	1	0	3	0	0	6
WTP#2 inffluent	1	23	1	1	0	1	0	1	1	22	3	3	0	1	0
WTP #2 effluent	1	6	1	1	0	1	0	1	1	2	3	2	0	0	0
WWTP inffluent	1020	4840	51	250	1	0	24	191	3	182	633	79	107	133	148
WWTP eff before UV	1	0	76	7	1	7	27	259	1	9	987	26	0	70	161
WWTP eff after UV	2	0	76	6	2	6	26	262	1	2	945	27	0	86	136
25 ppt QC	21	24	28	15	33	21	23	34	27	15	60	20	42	23	44



May 6, 2008

From: Paul Westerhoff, Seong-Nam Nams, Aaron Dotson, Chao-An Chiu

Subject: NOM Characterization / City of Phoenix

ASU conducted NOM characterization on samples provided to ASU by Malcolm Pirnie Inc / NCS Engineering that were collected from various watershed sites and delivered in 55-gallon plastic drums or 5 to 10 gallon plastic containers. This memo summarizes the preliminary results and the current status of data analysis for the following:

Tier 1 – Raw Water Characterization:

- Organic carbon and nitrogen measurements
- Fluorescence spectroscopy
- Size exclusion chromatographs of organics
- Chlorine reactivity (S1 and S2)

Tier 2 – NOM Fractionation and DBP Reactivity Tests

- Colloid and resin fractionation to obtain NOM isolates
- DBP formation tests on NOM isolates

Samples were provided to ASU between December 2007 and March 2008.

Date taken	Water Samples	UV254 (cm⁻¹)	DOC (mg/L)	SUVA ₂₅₄ (L/mg-m)	TDN (mg/L as N)	DIN (mg/L as N)	DON (mg/L as N)
	Lake Pleasant	0.084	4.561	1.832	0.646	0.391	0.255
3-14- 2008	Bartlett Lake	0.176	6.019	2.918	0.331	0.067	0.264
	Saguaro Lake	0.124	5.85	2.121	1.081	0.655	0.426

 Table 1.2. Summary of water quality of three *climatological* lake samples

2.0 Tier 2 – DOM Fractionation and DBP Reactivity of Isolates

2.1 Methods

2.1.1 DOM Isolation

The DOM isolation procedure is complex, and described in detail within Appendix A. Briefly, water was received and roto-evaporated to concentrate DOM. The concentrated DOM was placed in a 3500 dalton dialysis tube. Over time, as colloidal organics were retained within the dialysis tube, dissolved organic matter diffused through the dialysis tube into the surrounding water. The colloidal material was removed from the dialysis tube and freeze dried. The water outside the dialysis tube was repeatedly changed and collected; it turned to a deep yellow color due to the organic materials. This water was then applied to XAD 8 and XAD 4 resin packed columns, in series. Hydrophobic and amphilic material "stuck" onto the resins, respectively. Material was desorbed from the resins using, first, sodium hydroxide to elute off acids and, second, acetonitrile/water to elute off neutrals. These samples were further treated and then freeze-dried. Hydrophilic materials and all accompanying salts passed through both resins. DOM isolation yielded the following isolates from each water source:

- 1. Organic colloids
- 2. Hydrophobic acids (i.e., fulvic- and humic-like acids) (HPO-A)
- 3. Hydrophobic neutrals (HPO-N)
- 4. Amphilic acids (AMP-A)
- 5. Amphilic neutrals (AMP-N)
- 6. Hydrophilic material (acids and bases) (HPI)

2.1.2 DBP Formation Tests

The disinfection by-product formation potential (DBPFP) of natural organic matter (NOM) isolates was determined by chlorinating a solution containing a known concentration of a single NOM isolate. Each NOM isolate was dissolved in 1-L of deionized water (Nanopure Infinity by Barnstead International, MA) to a DOC concentration between 2 - 4 mg/L and buffered to a pH of 7.0 with 5 mM sodium phosphate buffer. The solution was filtered through a pre-muffled 0.7 µm glass fiber filter (Whatman GF/F) to remove any particulate NOM. The chlorine dose (mg-Cl₂/L) for each solution was calculated as five times the DOC concentration plus eight times the ammonia concentration (as N). The solutions were chlorinated using a stock solution of sodium hypochlorite of sufficient concentration to ensure no more than 1% change in solution volume. Upon chlorination, each solution was immediately transferred into two headspace free 500 mL bottles and placed in the dark at 20 °C. The first bottle was removed from the dark at 1 hour and decanted into bottles provided by the City of Phoenix for trihalomethane (THM) and haloacetic acid (HAA) analysis. The provided bottles contained sufficient amount of preservative to preserve the sample and quench the residual chlorine. The second bottle was removed from the dark after 7 days and decanted in to additional bottles provided by the city for THM and HAA analysis. The small amount of sample remaining in the 500 mL bottles after decanting into the THM and HAA bottles was analyzed for free chlorine residual using the Hach DPD method.

2.2 Preliminary Results

All NOM isolation is complete and elemental analysis has been performed. The first phase of DBPFP testing only included the climatological samples and we are planning on performing the DBPFP test on the others in the next week or two. We isolated a total of 7 samples (3 climatological, 3 lakes, 1 treated):

Climatological

- 1. Salt River, December 2007
- 2. Verde River, December 2007
- 3. Verde River, January 2008

Lakes

- 1. Lake Pleasant, March 2008
- 2. Bartlett Lake, March 2008
- 3. Saguaro Lake, March 2008

Treated

1. Saguaro Lake, March 2008 treated with alum

Results will be presented for each one showing percentage of DOM in each fraction and the reactivity of each fraction with free chlorine to form THMs or HAAs. Results will be presented for one climatic samples collected: Verde River, December 2008. Tabular forms are included as Tables 2.1 and 2.2. Figures 2.1 through 2.3 are for the Verde River (12/2007) climatic sample; data for other climatic samples are in subsequent figures. Organic colloids constitute nearly 30% of the DOC in the sample, and another 35% of the DOC is hydrophobic acids. Together these comprise nearly 2.8 mg/L of the total 4.3 mg/L of DOC isolated. Lesser percentages were obtained for the other DOM fractions. The hydrophilic material formed brominated DBPs, because all the bromide from the initial water sample is concentrated here. For this reason all DBPs are presented on a molar basis to remove interference of interpretations between chlorine and bromine molecular weights.

THM formation increased by nearly a factor of 4 between 24 hour and 7 days of incubation time with free chlorine. This suggests there are both slow and fast reacting NOM sites. All the NOM fractions exhibited THM and HAA formation. The least reactive fraction was the hydrophilic materials. The acid fractions (HPO-A and AMP-A) were among the most reactive, followed very closely by the colloids and neutral fractions. When the reactivity of each fraction is considered along with the amount of DOC that the isolate accounted for in the initial bulk water sample, Figure 2.2 shows the relative importance of each fraction to the overall DBP formation. The colloidal and HPO-A fractions account for over 50% of the THM formation potential and nearly 90% of the HAA9 formation. The percentage of DBP associated with each fraction to the total DBP level formed was almost independent of the contact time. The NOM appears to have

both slower and faster reacting DBP sites, with perhaps the hydrophilic materials accounting for more of the faster reacting sites. This is probably due to the bromide in the hydrophilic fraction, since bromination reactions occur more rapidly than chlorination reactions.

Results from other NOM isolates are tabulated and will be discussed in future memos. Tables 2.3 and 2.4 summarize the main part of this work.



Figure 2.1 Verde River DOM isolation from 12/2007. Larger plot shows the amount of organic carbon or nitrogen, based upon elemental analysis of each isolate. The percentage represents the amount of carbon or nitrogen recovered based upon initial DOC and DON and the volume of water processed. Inset figure illustrates the percentage distribution of carbon and nitrogen among the different DOM isolates.



Figure 2.2 THM formation in each isolate after 24 hr and 7 day incubation periods (Verde River 12/2007).



Figure 2.3 HAA9 formation in each isolate after 24 hr and 7 day incubation periods (Verde River 12/2007).

		1 h	our	7 day								
NOM	CHCl ₃	CHBrCl ₂	CHBr ₂ Cl	CHBr₃	CHCl₃	CHBrCl ₂	CHBr ₂ CI	CHBr₃				
Isolate	nM/mg-DOC											
	Salt River (Climatological), December 2007											
Colloids	77.2	2.4	0.5	BDL	348.9	3.3	BDL	0.5				
HPO-A	101.5	2.8	0.5	BDL	487.0	4.9	BDL	0.0				
HPO-N	47.6	1.9	0.5	BDL	339.0	3.0	BDL	0.3				
AMP-A	86.9	3.7	0.9	BDL	400.8	7.0	BDL	BDL				
AMP-N	56.4	2.3	0.5	BDL	305.6	3.6	BDL	0.4				
HPI	19.7	24.5	43.6	3.6	83.5	81.2	103.2	12.9				
Verde River (Climatological), December 2007												
Colloids	132.8	2.7	1.4	2.7	536.6	5.3	BDL	1.8				
HPO-A	97.1	2.4	1.4	0.7	464.0	6.0	BDL	0.8				
HPO-N	47.0	1.3	1.1	1.0	385.1	3.4	BDL	0.7				
AMP-A	96.3	3.6	BDL	0.5	686.0	6.2	BDL	0.6				
AMP-N	38.4	1.3	1.1	0.7	554.0	3.1	BDL	0.7				
HPI	13.9	25.7	45.4	12.0	56.1	74.7	109.9	22.4				
		Verde R	iver (Clima	tological)	, January	/ 2008						
Colloids	132.1	2.7	2.0	2.0	637.2	5.1	BDL	0.7				
HPO-A	103.3	1.6	1.0	0.6	432.3	3.6	BDL	BDL				
HPO-N	44.3	0.7	0.8	1.1	327.0	2.0	BDL	0.3				
AMP-A	88.6	2.0	1.4	BDL	466.1	5.0	BDL	BDL				
AMP-N	47.1	1.1	0.9	0.7	319.0	2.4	BDL	0.5				
HPI	33.0	32.1	34.4	4.6	153.5	91.5	66.9	6.1				

Table 2.1. Summary of DBPFP THMs

Table 2.2	Summary	of DBPFP	HAAs
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	1 hour								7 day									
	MCAA	MBAA	DCAA	DBAA	BCAA	TCAA	TBAA	CDBAA	BDCAA	MCAA	MBAA	DCAA	DBAA	BCAA	TCAA	TBAA	CDBAA	BDCAA
NOM Isolate									nM/mg	J-DOC								
Salt River (Climatological), December 2007																		
Colloids	BDL	NA	47.4	BDL	BDL	81.6	BDL	BDL	BDL	27.9	BDL	211.1	BDL	2.6	390.9	BDL	BDL	4.3
HPO-A	BDL	NA	49.9	BDL	BDL	81.9	BDL	BDL	1.4	23.7	BDL	259.5	BDL	2.5	377.9	BDL	BDL	4.7
HPO-N	BDL	NA	22.6	BDL	BDL	29.1	BDL	BDL	BDL	18.5	BDL	143.6	BDL	BDL	102.0	BDL	BDL	1.5
AMP-A	4.7	NA	44.5	BDL	BDL	71.6	BDL	BDL	2.0	31.5	BDL	330.0	BDL	4.3	312.4	BDL	BDL	7.1
AMP-N	BDL	NA	28.3	BDL	BDL	34.4	BDL	BDL	BDL	18.7	BDL	158.9	BDL	BDL	113.4	BDL	BDL	2.0
HPI	BDL	NA	29.0	11.7	45.1	20.8	2.5	31.0	44.2	28.1	46.5	131.9	23.4	62.8	45.8	4.0	44.5	73.6
Verde River (Climatological), December 2007																		
Colloids	BDL	NA	96.5	BDL	BDL	172.0	BDL	BDL	2.9	28.3	BDL	393.3	BDL	6.1	761.5	BDL	BDL	6.4
HPO-A	BDL	NA	56.7	BDL	BDL	105.8	BDL	BDL	2.2	19.7	BDL	326.6	BDL	4.0	474.6	BDL	BDL	7.8
HPO-N	BDL	NA	26.2	BDL	BDL	41.3	BDL	BDL	BDL	21.1	BDL	149.8	BDL	BDL	148.2	BDL	BDL	2.7
AMP-A	4.8	NA	54.1	BDL	1.3	88.0	BDL	BDL	2.7	27.7	BDL	354.9	BDL	5.3	373.2	BDL	BDL	8.7
AMP-N	BDL	NA	29.0	BDL	BDL	35.9	BDL	BDL	BDL	20.6	BDL	165.5	BDL	1.5	119.1	BDL	BDL	2.2
HPI	30.8	NA	41.7	21.6	63.3	23.3	7.5	44.4	61.4	120.8	12.2	243.2	44.2	129.1	54.8	12.1	68.3	81.9
							Verde Ri	ver (Clima	tological),	January 2	2008							
Colloids	BDL	NA	120.6	BDL	BDL	193.7	BDL	BDL	4.5	47.0	BDL	396.3	BDL	7.4	883.5	BDL	BDL	8.3
HPO-A	BDL	NA	60.2	BDL	BDL	106.2	BDL	BDL	1.4	24.2	BDL	301.2	BDL	2.4	475.2	BDL	BDL	4.9
HPO-N	BDL	NA	24.2	BDL	BDL	31.9	BDL	BDL	BDL	17.9	BDL	98.9	BDL	BDL	111.5	BDL	BDL	1.5
AMP-A	BDL	NA	53.5	BDL	BDL	90.1	BDL	BDL	1.8	26.8	BDL	321.1	BDL	3.2	394.0	BDL	BDL	5.4
AMP-N	BDL	NA	29.2	BDL	BDL	37.4	BDL	BDL	BDL	19.9	BDL	200.6	BDL	1.6	187.0	BDL	BDL	2.7
HPI	23.6	NA	40.2	9.4	45.4	42.7	1.9	27.7	52.0	28.6	8.6	279.4	26.9	106.5	137.8	4.3	55.3	106.1



Figure 2.4 Salt River DOM isolation from 12/2007. Larger plot shows the amount of organic carbon or nitrogen, based upon elemental analysis of each isolate. The percentage represents the amount of carbon or nitrogen recovered based upon initial DOC and DON and the volume of water processed. Inset figure illustrates the percentage distribution of carbon and nitrogen among the different DOM isolates.



Figure 2.5 THM formation in each isolate after 24 hr and 7 day incubation periods (Salt River 12/2007).



Figure 2.6 HAA9 formation in each isolate after 24 hr and 7 day incubation periods (Salt River 12/2007).



Figure 2.7 Verde River DOM isolation from 1/2008. Larger plot shows the amount of organic carbon or nitrogen, based upon elemental analysis of each isolate. The percentage represents the amount of carbon or nitrogen recovered based upon initial DOC and DON and the volume of water processed. Inset figure illustrates the percentage distribution of carbon and nitrogen among the different DOM isolates.



Figure 2.8 THM formation in each isolate after 24 hr and 7 day incubation periods (Verde River 1/2008).



Figure 2.9 HAA9 formation in each isolate after 24 hr and 7 day incubation periods (Verde River 1/2008).

Source	Sampling Date	Volume (L)	рН	TOC	DOC	TDN	DON
Salt River - Climatological	12/14/2007	77.7	8.00	3.54	3.64	0.672	0.476
Verde River - Climatological (DEC)	12/14/2007	76.2	8.15	5.54	5.49	1.978	0.720
Verde River - Climatological (JAN)	1/1/2008	76.8	8.18		4.82	1.220	0.640
Saguaro Lake	3/4/2008	77.2	8.75	6.10	5.85	1.081	0.426
Bartlett Lake	3/15/2008	77.4	9.22	6.28	6.02	0.614	0.388
Lake Pleasant	3/15/2008	76.1	7.65	4.79	4.56	1.070	0.593
Saguaro Lake - Coagulated	3/30/2008	36.8	7.05	NA - Filtered	3.92	0.924	0.362
Source	Isolate	Mass (mg)	Volume (mL)	С	Н	Ν	C:N
Salt River - Climatological	Colloids	159.5		34.8	4.486	3.646	9.5
Salt River - Climatological	HPO-A	1006.3		15.743	1.345	0.506	31.1
Salt River - Climatological	HPO-N	255.65		20.12	2.159	5.75	3.5
Salt River - Climatological	AMP-A	1537.9		3.55	0.189	0.352	10.1
Salt River - Climatological	AMP-N	194.04		18.06	1.785	1.166	15.5
Salt River - Climatological	HPI		1130	60.09	NA	64.1	1.09
Verde River - Climatological (DEC)	Colloids	1496.6		7.13	0.946	0.838	8.5
Verde River - Climatological (DEC)	HPO-A	275.8		37.101	3.164	1.166	31.8
Verde River - Climatological (DEC)	HPO-N	175.25		15.132	1.462	0.732	20.7
Verde River - Climatological (DEC)	AMP-A	181.01		14.738	1.235	0.987	14.9
Verde River - Climatological (DEC)	AMP-N	166.2		9.734	0.899	1.05	9.3
Verde River - Climatological (DEC)	HPI		4000	10.44	NA	16.6	0.73
Verde River - Climatological (JAN)	Colloids	566.3		11.31	1.721	1.089	10.4
Verde River - Climatological (JAN)	HPO-A	269.4		48.955	4.462	1.196	40.9
Verde River - Climatological (JAN)	HPO-N	43.6		54.218	6.063	1.378	39.3
Verde River - Climatological (JAN)	AMP-A	116.4		42.059	4.117	2.117	19.9
Verde River - Climatological (JAN)	AMP-N	41.7		36.218	4.356	3.943	9.2
Verde River - Climatological (JAN)	HPI		1000	58.79	NA	68.3	1.00
Saguaro Lake	Colloids	182.8		42.17	6.17	5.07	8.3
Saguaro Lake	HPO-A	305.1		49.55	5.08	1.58	31.4
Saguaro Lake	HPO-N	80.4		37.84	4.26	2.65	14.3
Saguaro Lake	AMP-A	111.6		43.67	4.46	2.72	16.0
Saguaro Lake	AMP-N	41.1		31.44	3.92	2.98	10.5
Saguaro Lake	HPI		1000	91.5	NA	59.34	1.5
Bartlett Lake	Colloids	248.4		45.22	5.65	4.89	9.3
Bartlett Lake	HPO-A	377.7		51.32	4.97	1.28	40.1
Bartlett Lake	HPO-N	61.8		50.80	6.02	1.00	50.9
Bartlett Lake	AMP-A	106.4		45.96	4.58	2.13	21.6
Bartlett Lake	AMP-N	41.8		34.15	4.43	3.37	10.1
Bartlett Lake	HPI		1000	76.12	NA	16.488	4.6
Lake Pleasant	Colloids	291.9		29.93	4.48	3.25	9.2
Lake Pleasant	HPO-A	218.8		51.02	5.05	1.39	36.8
Lake Pleasant	HPO-N	43.6		48.00	5.65	2.39	20.1
Lake Pleasant	AMP-A	86.5		44.92	4.72	2.75	16.3
Lake Pleasant	AMP-N	35.6		38.32	4.88	5.11	7.5
Lake Pleasant	HPI		2000	38.28	NA	25.28	1.5
Saguaro Lake Coagulated	Colloids	48.2		44.42	6.57	5.18	8.6
Saguaro Lake Coagulated	HPO-A	112		50.93	5.44	1.52	33.4
Saguaro Lake Coagulated	HPO-N	45.1		31.64	3.47	2.13	14.8
Saguaro Lake Coagulated	AMP-A	35.3		43.44	4.67	3.19	13.6
Saguaro Lake Coagulated	AMP-N	27.6		30.96	3.59	4.44	7.0
Saguaro Lake Coagulated	HPI		1000	30.6	NA	26.04	1.2

Table 2.3 – summary of isolates and carbon (C), hydrogen (H) and Nitrogen (N) elemental analysis

		C (mg/L)	N (mg/L)	%C	DON (mg/L)	% DON	DIN/TDN
Salt River - Climatological	Colloids	0.71	0.07	13%	0.070	9%	0.06
Salt River - Climatological	HPO-A	2.04	0.07	37%	0.062	8%	0.06
Salt River - Climatological	HPO-N	0.66	0.19	12%	0.181	23%	0.04
Salt River - Climatological	AMP-A	0.70	0.07	13%	0.068	9%	0.03
Salt River - Climatological	AMP-N	0.45	0.03	8%	0.028	4%	0.03
Salt River - Climatological	HPI	0.87	0.93	16%	0.385	49%	0.59
Not	Recovered (mg/L)	-1.80	-0.69	100%	-0.318	100%	
Total	Recovered (mg/L)	5.44	1.360		0.794		
D	OC/TDN Recovery	149%	202%		167%		
		•					
]	C (mg/L)	N (mg/L)	%C	DON (mg/L)	% DON	DIN/TDN
Verde River - Climatological (DEC)	Colloids	1 40	0.16	33%	0 147	26%	0.11
Verde River - Climatological (DEC)	HPO-A	1.34	0.04	32%	0.041	7%	0.04
Verde River - Climatological (DEC)	HPO-N	0.35	0.02	8%	0.016	3%	0.03
Verde River - Climatological (DEC)	AMP-A	0.35	0.02	8%	0.023	4%	0.02
Verde River - Climatological (DEC)	AMP-N	0.21	0.02	5%	0.023	4%	0.01
Verde River - Climatological (DEC)	HPI	0.55	0.87	13%	0.306	55%	0.65
Not	Recovered (ma/L)	1.29	0.84	100%	0.165	100%	
	Total (mg/L)	4.20	1,142		0.555		
D	OC/TDN Recovery	77%	58%		77%		
				•			
]	C (mg/L)	N (mg/L)	%C	DON (mg/L)	% DON	DIN/TDN
Verde River - Climatological (JAN)	Colloids	0.83	0.08	19%	0.053	11%	0.35
Verde River - Climatological (JAN)	HPO-A	1 72	0.00	39%	0.040	9%	0.00
Verde River - Climatological (JAN)	HPO-N	0.31	0.01	7%	0.007	2%	0.06
Verde River - Climatological (JAN)	AMP-A	0.64	0.03	14%	0.031	7%	0.03
Verde River - Climatological (JAN)	AMP-N	0.20	0.02	4%	0.021	5%	0.01
Verde River - Climatological (JAN)	HPI	0.77	0.89	17%	0.313	67%	0.65
Not	Recovered (ma/L)	0.36	0.15	100%	0.173	100%	
	Total (mg/L)	4.46	1.075		0.467		
D	OC/TDN Recovery	92%	88%		73%		
	1	C (mg/L)	N (mg/L)	%C	DON (mg/L)	% DON	DIN/TDN
Saguaro Lake	Colloids	1.00	0.12	19%	0.099	17%	0.17
Saguaro Lake	HPO-A	1.96	0.06	37%	0.059	10%	0.05
Saguaro Lake	HPO-N	0.39	0.03	7%	0.026	5%	0.04
Saguaro Lake	AMP-A	0.63	0.04	12%	0.038	7%	0.02
Saguaro Lake	AMP-N	0.17	0.02	3%	0.016	3%	0.01
Saguaro Lake	HPI	1.19	0.77	22%	0.341	59%	0.56
	Total (mg/L)	5.34	1.03	100%	0.580	100%	
	Recovery	91%	96%		1.362		
				-		-	
		C (mg/L)	N (mg/L)	%C	DON (mg/L)	% DON	DIN/TDN
Bartlett Lake	Colloids	1.45	0.16	24%	0.130	34%	0.17
Bartlett Lake	HPO-A	2.50	0.06	41%	0.060	15%	0.05
Bartlett Lake	HPO-N	0.41	0.01	7%	0.008	2%	0.04
Bartlett Lake	AMP-A	0.63	0.03	10%	0.029	7%	0.02
Bartlett Lake	AMP-N	0.18	0.02	3%	0.018	5%	0.01
Bartlett Lake	HPI	0.98	0.21	16%	0.142	37%	0.33
	Total (mg/L)	6.16	0.49	100%	0.385	100%	
	Recovery	102%	80%]	99%		
		C (mg/L)	N (mg/L)	%C	DON (mg/L)	% DON	DIN/TDN
Lake Pleasant	Colloids	1.15	0.12	25%	0.103	21%	0.17
Lake Pleasant	HPO-A	1.47	0.04	32%	0.038	8%	0.05
Lake Pleasant	HPO-N	0.27	0.01	6%	0.013	3%	0.04
Lake Pleasant	AMP-A	0.51	0.03	11%	0.031	6%	0.02
Lake Pleasant	AMP-N	0.18	0.02	4%	0.024	5%	0.01
Lake Pleasant	HPI	1.01	0.66	22%	0.281	57%	0.58
L	Total (mg/L)	4.58	0.90	100%	0.489	100%	
L	Recovery	100%	84%	I	82%		
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	O ella i de	C (mg/L)	N (mg/L)	%C	DON (mg/L)	% DON	DIN/IDN
Saguaro Lake Coagulated		0.58	0.07	15%	0.056	13%	0.17
Saguaro Lake Coagulated	HPU-A	1.55	0.05	39%	0.044	10%	0.05
Saguaro Lake Coagulated	HPU-N	0.39	0.03	10%	0.025	6%	0.04
Saguaro Lake Coagulated	AIVIP-A	0.42	0.03	10%	0.030	1%	0.02
Saguaro Lake Coagulated	AIVIP-N	0.23	0.03	6%	0.033	8%	0.01
Saguaro Lake Coagulated	HPI Tetal (m. m/l.)	0.83	0.71	21%	0.248	5/%	0.65
	i otal (mg/L)	4.00	0.91	100%	0.435	100%	
	Recovery	102%	99%		120%		

Table 2.4 - calculation of mass recoveries

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Appendix A – Detailed Methodology for DOM Characterization

Resin Cleaning Procedure

References: Adapted from Aiken et al., 1992 and Leenheer, 1981

- 1. Remove large resin beads sieving through a $600 \ \mu m$ sieve.
- 2. Rinse thoroughly with Nanopure water and load into Soxhlet
- 3. Extract with methanol for 24 hours
- 4. Extract with acetonitrile for 24 hours
- 5. Rinse thoroughly with Nanopure water and pack into column.
- 6. Rinse with Nanopure water until effluent DOC is less than 0.5 mg-C/L
- 7. Immediately before using resin should be rinsed with 3 successive rinses of 1 L of 0.1 N NaOH followed by 1 L of 0.1 N HCl

Resin Isolation Procedure

References: Adapted from Aiken et al., 1992, Hwang, et al., 2001, Leenheer, et al., 2007



Resin Fractionation Sample Preparation and Colloid Isolation

- 1. Filter sample through a 1 μ m filter and adjust pH with HCl to 4.
- 2. Concentrate sample by rotary evaporation to a salt slurry.
- 3. Load salt slurry in a 3,500 Da dialysis tube and dialyze against 1-L of 0.1 N HCl. Note: Dialysis tube should be prepared by soaking in DI for 12 hours prior to use

- 4. Dialyze for 12 to 24 hour intervals replacing the permeate solution at each interval with 1-L of 0.1 N HCl and storing the permeate solution for later processing. Dialysis should be performed until all salts are visually dissolved and permeate solution is near colorless.
- 5. Dialyze against DI water until the permeate solution conductivity is less than 100 mS/cm in a similar manner to step 4. Store the permeate solution with 0.1 N HCl dialysis permeate for resin fractionation.
- 6. Dialyze against two aliquots of 4-L of 0.2 N HF for 24 hours each. Discard the 0.2 N HF.
- 7. Dialyze repeatedly against DI water until dialysis permeate is less than 10 mS/cm.
- 8. Freeze-dry the contents of the dialysis tube to isolate **COLLOIDS**.

Resin Fractionation

- 1. Rinse resins with 0.1 N NaOH at a flowrate of 100 mL/min (15 bedvolumes/hr) until effluent pH < 10.
- 2. Rinse resins with 0.1 N HCl at a flowrate of 100 mL/min (15 bedvolumes/hr) until effluent pH < 2.
- 3. Repeat Steps 1 and 2 twice for a total of three rinses of acid and base.
- 4. Rinse resins with distilled water until effluent conducitivity is $< 10 \,\mu$ S/cm
- 5. Adjust sample (dialysis permeate) to a pH of 2.0
- 6. Process sample through the resin at a flowrate of 15 bedvolumes/hr. The effluent of the resins should be directed to waste until breakthrough of conductivity.
- 7. Rinse resins with 2-L of 0.1 N HCl.
- 8. Column effluent contains **hydrophilic** (**Shpigel et al.**) organic material. Rotary evaporate to concentrate and bring to a pH of 2.0 with NaOH. Note: This fraction could be desalted but requires a significant amount of precipitation reactions.
- 9. Separate the DAX-8 and XAD-4 resin columns and close valves so columns remain filled.
- 10. Regenerate the MSC-1H column by passing 1-L of 4 N HCl through the column followed by DI water until effluent conductivity is $< 10 \,\mu$ S/cm.
- 11. Rinse DAX-8 column with one bedvolume of DI water.
- 12. Connect the DAX-8 column to the MSC-1H column.
- 13. Pump 1-L of 0.1 N NaOH followed by 1 bedvolume of DI water through the DAX-8 and MSC-1H columns begin and end sample collection upon conductivity breakthrough (or after pumping 0.5 bedvolumes of water through). Note: this elution produces a color front in the resin as the organic matter is eluted that should be easy to track.
- 14. Rotary evaporate sample to a volume suitable to freeze-dry. Freeze-dry multiple times until material is not sticky to isolate **hydrophobic acids** (**HPO-A**).
- 15. Disconnect the DAX-8 column from the MSC-1H column.
- 16. Rinse DAX-8 column with 1-L 0.01 N HCl followed by 1 bedvolume of DI rinse.
- 17. Pump 0.8-L of a solution of 75% acetonitrile/25% water followed by DI water through the DAX-8 column and begin sample collection after pumping 0.5 bedvolumes onto the column. Note: this elution does produce a heat front as the organic matter is eluted but it may be challenging to follow.
- 18. Rotary evaporate sample to a volume suitable to freeze-dry. Freeze-dry multiple times until material is not sticky to isolate **hydrophobic neutrals** (**HPO-N**).
- 19. Repeat Step 13.

- 20. Rinse XAD-4 column with 1 bedvolume of DI water.
- 21. Connect the XAD-4 column to the MSC-1H column.
- 22. Pump 1-L of 0.1 N NaOH followed by 1 bedvolume of DI water through the XAD-4 and MSC-1H columns begin sampling after processing 0.5 bedvolumes. Note: this elution produces a color front in the resin as the organic matter is eluted that should be easy to track.
- 23. Rotary evaporate sample to a volume suitable to freeze-dry. Freeze-dry multiple times until material is not sticky to isolate **amphiphilic acids** (**AMP-A**).
- 24. Disconnect the XAD-4 column from the MSC-1H column.
- 25. Rinse XAD-4 column with 1-L 0.01 N HCl followed by DI rinse until the effluent conductivity is less than $10 \,\mu$ S/cm.
- 26. Pump 0.8-L of a solution of 75% acetonitrile/25% water followed by DI water through the XAD-4 column and begin sample collection after pumping 0.5 bedvolumes onto the column. Note: this elution produces a significant amount of bubbles, once the column is filled with bubbles mix and continue elution.
- 27. Rotary evaporate sample to a volume suitable to freeze-dry. Freeze-dry multiple times until material is not sticky to isolate **amphiphilic neutrals** (**AMP-N**).