

## Supplemental Information

# Adsorption of domoic acid to marine sediments and clays

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## SUPPORTING INFORMATION.

**Supp. Table 1.** Typical concentration of Instant Ocean™ Salt solution and natural seawater at 35 ppt salinity.

Ion	Concentration in Instant Ocean™ (ppm) <sup>a</sup>	Concentration in Seawater (ppm) <sup>b</sup>
Chloride	19,290	19,353
Sodium	10,780	10,781
Sulfate	2,660	2,712
Magnesium	1,320	1,284
Potassium	420	399
Calcium	400	412
Carbonate/Bicarbonate	200	126
Bromide	56	67
Strontium	8.8	7.9
Boron	5.6	4.5
Fluoride	1.0	1.28
Lithium	0.3	0.173
Iodide	0.24	0.06
Barium	Less than 0.04	0.014
Iron	Less than 0.04	Less than 0.001
Manganese	Less than 0.025	Less than 0.001
Chromium	Less than 0.015	Less than 0.001
Cobalt	Less than 0.015	Less than 0.001
Copper	Less than 0.015	Less than 0.001
Nickel	Less than 0.015	Less than 0.001
Selenium	Less than 0.015	Less than 0.001
Vanadium	Less than 0.015	Less than 0.002
Zinc	Less than 0.015	Less than 0.001
Molybdenum	Less than 0.01	0.01
Aluminum	Less than 0.006	Less than 0.001
Lead	Less than 0.005	Less than 0.001
Arsenic	Less than 0.004	0.002
Cadmium	Less than 0.002	Less than 0.001
Nitrate	None	1.8
Phosphate	None	0.2

<sup>a</sup>Concentrations received from Aquarium Systems, Inc. on November 30, 2004 (phone 1-800-822-1100) <sup>b</sup>Concentrations taken from *An Introduction to the Chemistry of the Sea*. 1998. M.E.Q. Pilson, provided for comparison.

**Supp. Table 2.** Elemental Composition for Suwannee River Natural Organic Matter (Received from the IHSS)

Sample	Cat. No.	H <sub>2</sub> O	Ash	C	H	O	N	S	P	δ <sup>13</sup> C	δ <sup>15</sup> N
Suwannee River	1R101N	8.15	7.0	52.47	4.19	42.69	1.10	0.65	0.02	nd	nd

Source: Elemental analyses by Huffman Laboratories, Wheat Ridge, CO, USA; Isotopic analyses by Soil Biochemistry Laboratory, Dept. of Soil, Water, and Climate, University of Minnesota, St. Paul, MN, USA H<sub>2</sub>O content is the %(w/w) of H<sub>2</sub>O in the air-equilibrated sample (a function of relative humidity). Ash is the %(w/w) of inorganic residue in a dry sample. C, H, O, N, S, and P are the elemental composition in %(w/w) of a dry, ash-free sample. δ<sup>13</sup>C and δ<sup>15</sup>N are the abundances of the respective stable isotopes in parts per thousand. The data for bulk source materials are reported on an as-stored basis, except that %H and %O are corrected for water content. An \* means that a sample is no longer available, and nd means that an item was not determined.

**Supp. Table 3.** Acidic functional groups for Suwannee River Natural Organic Matter (Received from the IHSS)

Sample	Cat. No.	Carboxyl	Phenolic	Q <sub>1</sub>	Log K <sub>1</sub>	n <sub>1</sub>	Q <sub>2</sub>	Log K <sub>2</sub>	n <sub>2</sub>	N	RMSE
Suwannee River	1R101N	9.85	3.94	10.57	3.94	3.60	2.61	9.74	1.19	112	0.0725

Source: School of Earth & Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, U.S.A.

Reference: J. D. Ritchie and E. M. Perdue, *Geochim. Cosmochim. Acta*, 67, 85-96 (2003).

Ten-ml solutions containing  $0.36 \pm 0.01 \text{ g L}^{-1}$  (on a dry ash-free basis) of an IHSS sample in 0.1 M NaCl were titrated from their initial pH values of 3.0-3.3 to final pH values of 10.5-10.7 in 25-35 minutes, using 6-8 μL increments of carbonate-free 0.100 M NaOH. All titrations were conducted under a N<sub>2</sub> atmosphere in a thermostated(sic) water bath at  $25.00 \pm 0.02$  Celsius. At least three titrations were obtained for each sample.

Carboxyl is the charge density (meq/g C) at pH 8.0; Phenolic is two times the change in charge density (meq/g C) between pH 8.0 and pH 10.0.

The overall charge density (in meq/g C) of a humic substance increases systematically with pH (or with the concentration of H<sup>+</sup>). The equation on the right, known historically as a modified Henderson-Hasselbalch equation for two classes of binding sites, was used to fit titration data and obtain the set of model fitting parameters in this

$$e_{tot} = \left( \frac{Q_1}{1 + [K_1[H^+]]^{n_1}} \right) + \left( \frac{Q_2}{1 + [K_2[H^+]]^{n_2}} \right)$$

table. The fitting parameters of the model were obtained by a nonlinear least-squares fit of the model to aggregated sets of replicate titration data. Q<sub>1</sub> and Q<sub>2</sub> are the maximum charge densities of the two classes of binding sites, Log K<sub>1</sub> and Log K<sub>2</sub> are the mean log K values for proton binding by the two classes of sites, and n<sub>1</sub> and n<sub>2</sub> are empirical parameters that control the width (in log K) of a class of proton binding sites. N is the number of fitted titration data points, and RMSE is the root mean-square error for fitting this model to the data (see Reference). This model, also known in more recent literature as a Langmuir-Freundlich equation, is the primary building block of the NICA class of models.

An \* means that a sample is no longer available, and nd means that an item was not determined.

**Supp. Table 4.**  $^{13}\text{C}$  NMR for carbon distribution in Suwannee River Natural Organic Matter (Received from the IHSS)

Sample	Cat. No.	Carbonyl 220-190 ppm	Carboxyl 190-165 ppm	Aromatic 165-110 ppm	Acetal 110-90 ppm	Heteroaliphatic 90-60 ppm	Aliphatic 60-0 ppm
Suwannee River	1R101N	8	20	23	7	15	27
Reference: K. A. Thorn, D. W. Folan, and P. MacCarthy (1989), <i>Characterization of the International Humic Substances Society Standard and Reference Fulvic and Humic Acids by Solution State Carbon-13 (<math>^{13}\text{C}</math>) and Hydrogen-1 (<math>^1\text{H}</math>) Nuclear Magnetic Resonance Spectrometry</i> , U.S. Geological Survey, Water-Resources Investigations Report 89-4196, Denver, CO, 93 pp.							
This table contains the electronically integrated peak area percentages for selected ranges of chemical shift.							

**Supp. Table 5.** Isolation of Suwannee River NOM by Reverse Osmosis (Received from the IHSS)

IHSS has recently used reverse osmosis to isolate more than 1000g of natural organic matter (NOM) from the Suwannee River. The sample was collected from the same site that was used originally to collect the standard Suwannee River humic and fulvic acids. The new NOM sample is now available from the IHSS for \$20 per 100mg. The elemental composition of dry Suwannee River NOM is: 48.8 %C; 3.9 %H; 39.7 %O; 1.02 %N; 0.60 %S; 0.02 %P; 7.0 %Ash (Total 101.0%). A brief description of the sampling trip and isolation procedure follows.

From May 1 through May 9, 1999, a group representing the International Humic Substances Society (IHSS) visited the Suwannee River in south Georgia, U.S.A. to collect a new reference sample of natural organic matter (NOM). This reference NOM sample will complement the standard and reference humic and fulvic acids that were previously collected from the Suwannee River. The members of this group included Dr. James Alberts and Dr. Monika Takács of the University of Georgia Marine Institute at Sapelo Island and Dr. Michael Perdue and Ms. Lili Ding of the School of Earth and Atmospheric Sciences at the Georgia Institute of Technology.

The sample was collected at the first dam on the Suwannee River sill, on the southwestern edge of the Okefenokee Swamp near Fargo, GA. The project was authorized by the Board of Directors of IHSS, and access to the sill was granted by the Okefenokee National Wildlife Refuge in Folkston, GA. During the nine-day project, 33 concentrated samples were collected. For each sample, a 120-gallon aliquot of Suwannee River water was prefiltered successively through 1  $\mu\text{m}$  and 0.4  $\mu\text{m}$  polypropylene filters and concentrated to about six gallons using a RealSoft PROS/2S portable reverse osmosis system. After a concentrated sample was obtained, its temperature was maintained at or below the temperature of the river. Overall, 3960 gallons of water (about 14,990 liters) were concentrated to a final volume of 199 gallons (about 753 liters).

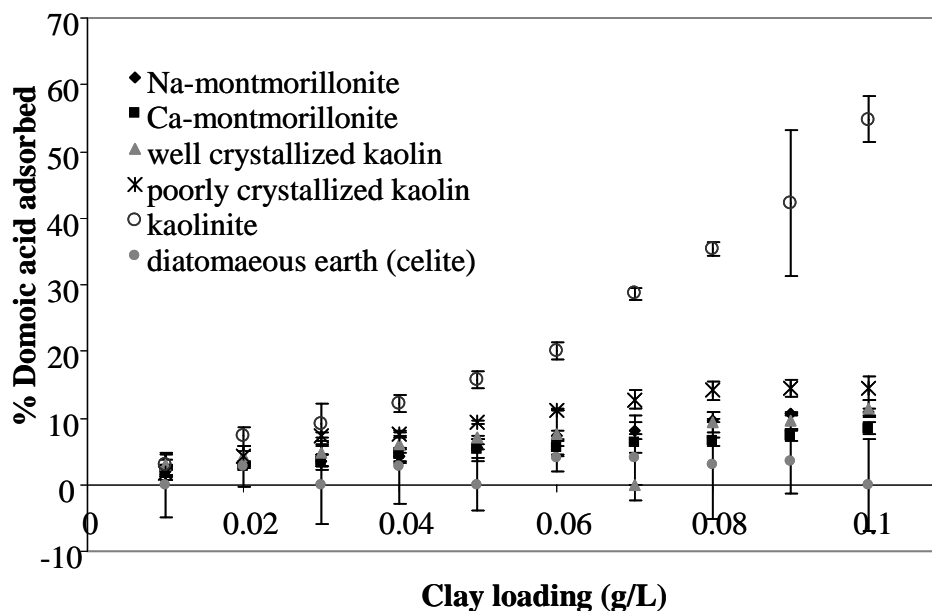
Because the chemical composition of the Suwannee River was expected to vary during the time required to collect the entire sample, a volume-weighted composite sample of the prefiltered Suwannee River water was prepared by collecting and combining 10-mL aliquots at periodic intervals (every 40 gallons). The composite sample, which was stored on ice at all times, had a pH of 3.93 and a TOC concentration of 39.2 mg/L (3.27 mmol/L).

While a sample was being concentrated, it was circulated continuously through a cation exchange resin ( $\text{H}^+$ -form) to remove most major cations. The "field-desalted" concentrated samples had pH values of  $2.8 \pm 0.1$ , well below the average pH of 3.93 for the prefiltered water. During the operation of the reverse osmosis system, the permeate flow rate gradually decreased due to adsorption of organic matter on the reverse osmosis membranes. At the low pH values of the concentrated samples, the adsorbed organic matter was probably enriched in humic acids. To recover this organic matter and to increase the permeate flow rate, a single 12-liter solution of 0.01 M NaOH was used on three occasions (about once every three days) to rinse the reverse osmosis membranes. Its final pH was about 6.5.

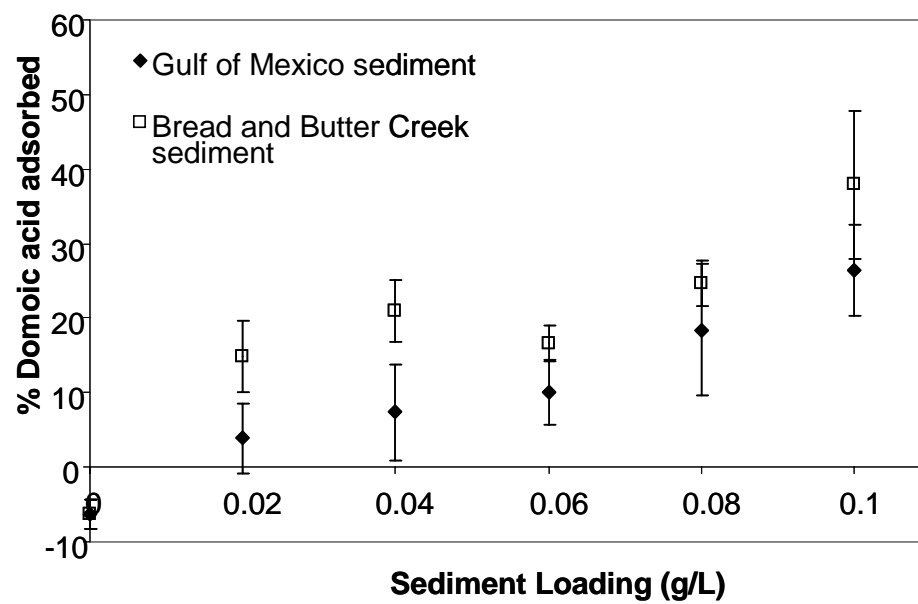
The samples were transported to Dr. Perdue's laboratory at Georgia Tech on May 10, 1999. In the laboratory, the reverse osmosis membranes were rinsed a final time with 10 liters of 0.01 M NaOH to recover some additional adsorbed organic matter. The two NaOH rinse solutions were desalted using a cation exchange resin ( $\text{H}^+$ -form) and then mixed into the other 33 concentrated samples. The concentrated samples were then desalted for a final time, using 250-ml columns of cation exchange resin ( $\text{H}^+$ -form) at a flow rate of about 100 ml/min. A volume-weighted composite sample was prepared from the desalted, concentrated samples. Its pH was 2.54 and its TOC concentration was 725 mg/L (60.4 mmol/L). From the volumes and TOC concentrations of the prefiltered water and the final concentrated samples, the percent recovery of organic carbon was calculated to be 92.9%.

During the three weeks that the samples were being processed in Dr. Perdue's laboratory at Georgia Tech, they were stored in a cold room at 4-6 °C. For two days, while the cold room was not operational, the samples were cooled with ice.

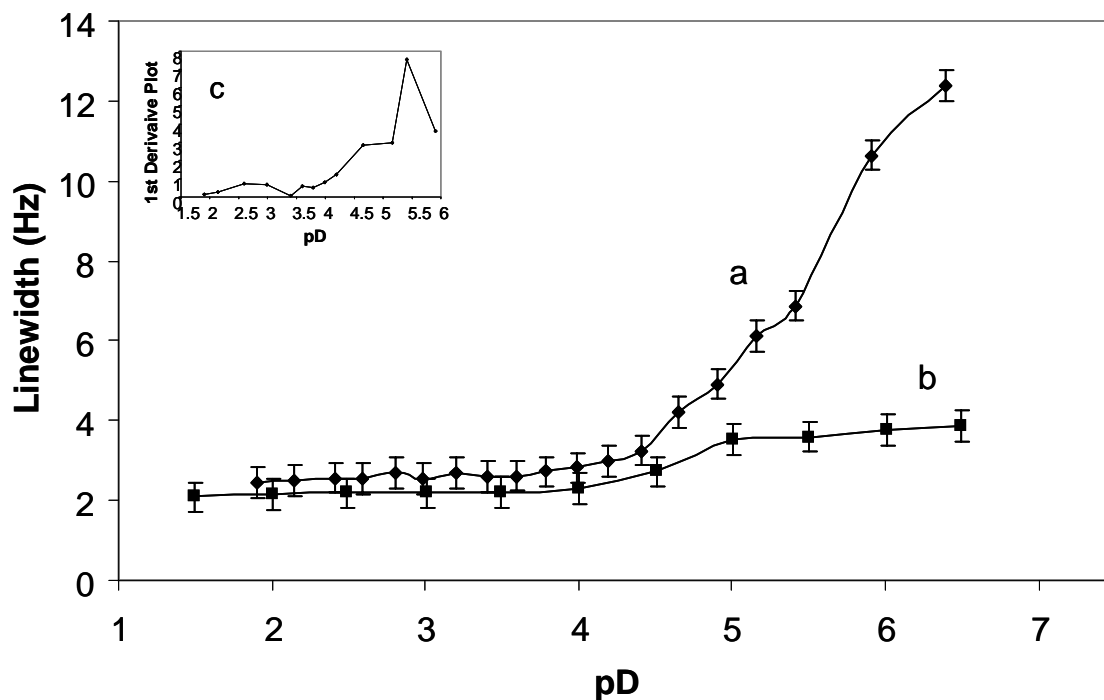
On May 31, the 31 carboys of desalted, concentrated samples were shipped by truck to Van Drunen Farms in Momence, IL, where they were stored in a cold room until an IHSS representative could arrive to supervise the freeze-drying process. Finally, on June 7-9, the samples were freeze-dried under the supervision of Dr. Perdue. Approximately 1060 g of freeze-dried natural organic matter was obtained. The freeze-dried product was transferred back to Georgia Tech for pulverization, homogenization, and final drying.



**Supp. Figure 1.** Plot of the fraction of domoic acid adsorbed to different clays. (pH 8.2, 25 °C, 32<sup>0/00</sup> salinity, [DA]<sub>0</sub> = 0.96 μM).



**Supp. Figure 2.** Plot of the fraction of domoic acid adsorbed to Gulf of Mexico sediment and Bread and Butter Creek sediment. (pH 8.2, 25 °C, 32 ‰ salinity,  $[DA]_0 = 0.96 \mu\text{M}$ ).



**Supp. Figure 3.** a) As pD increased, the linewidth of the singlet at 1.8 ppm increased in the presence of Cu(II), implying the formation of a complex. [Domoic acid] = 642  $\mu$ M; [Cu(II)] = 642  $\mu$ M; DCl and NaOD used for pD adjustment. b) As pD increases the linewidth of the singlet at 1.8 ppm remains the same for KA alone. c) First derivative plot for inflection point assignment.



**Supp. Calc. 1.** Calculations for the stability constant determination of the Cu(II)-DA complex.

Titration solution contains H<sub>2</sub>O, Cu(NO<sub>3</sub>)<sub>2</sub>•2.5H<sub>2</sub>O, and DCl. Therefore the amount of free copper able to bind to domoic acid at the equivalence point (half the initial concentration as opposed to the entire initial concentration where no signal would be present) is dependent upon the following equation:

$$[Cu]_{TOTAL} = [Cu(II)](1 + \sum \beta_{iOH} [OH^-]^i + \sum_{iCl} [Cl^-]^i + \sum_{iNO_3} [NO_3^-]^i)$$

**pD 5.41 = pH 5.81**

$[Cu]_{tot}$ (M)	$3.21 \times 10^{-4}$	$[OH^-]$ (M)	$1.02 \times 10^{-9}$	$[Cl^-]$ (M)	0.03	$[NO_3^-]$ (M)	$1.28 \times 10^{-3}$
		$k_1[OH^-]$	$1.02 \times 10^{-17}$	$k_1[Cl^-]$	0.003	$k_1[NO_3^-]$	$9.52 \times 10^{-4}$
		$k_2[OH^-]^2$	$6.61 \times 10^{-35}$	$k_2[Cl^-]^2$	$7.15 \times 10^{-4}$	$k_2[NO_3^-]^2$	$3.95 \times 10^{-7}$
<u>SUM</u>			$1.02 \times 10^{-17}$		$3.71 \times 10^{-3}$		$9.52 \times 10^{-4}$

$$[Cu(II)] = 3.20 \times 10^{-4}$$

Association constants, K, for the DA:Cu(II) complex were estimated based on the assumption that domoic acid, like other amino acids, complexes metals most effectively in the fully deprotonated (Y<sup>4-</sup>) form.

$$\alpha_4 = \frac{(K_1 K_2 K_3 K_4)}{([H^+]^4 + K_1 [H^+]^3 + K_1 K_2 [H^+]^2 + K_1 K_2 K_3 [H^+] + K_1 K_2 K_3 K_4)}$$

$pK_a$	1.85	4.47	4.75	10.60
$K$	0.01	$3.39 \times 10^{-5}$	$1.78 \times 10^{-5}$	$2.51 \times 10^{-11}$
$[H^+] (M)$	$1.55 \times 10^{-6}$			
$\alpha_4$	$7.88 \times 10^{-3}$			

$$K = \frac{[Cu(II) - DA]}{[Cu(II)][DA]}$$

$$[DA]_0 = [Cu]_0 (M) \quad 6.42 \times 10^{-4}$$

$$[Cu(II) - DA] (M) \quad 3.21 \times 10^{-4}$$

$$[DA] (M) \quad 9.54 \times 10^{-9}$$

$$[Cu(II)] (M) \quad 3.20 \times 10^{-4}$$

$$\mathbf{K} \quad \mathbf{1.05 \times 10^8}$$