

The distribution of biogenic thiols in surface waters of Galveston Bay

Degui Tang,¹ Chin-Chang Hung, Kent W. Warnken, and Peter H. Santschi

Texas A&M University, Department of Oceanography, 5007 Avenue U, Galveston, Texas 77551

Abstract

Thiolic compounds are important metal-complexing ligands as well as important components of the global sulfur biogeochemical cycle. A lack of information on the concentration and distribution of thiols in natural waters, especially in the dissolved fraction, is still a major impediment to a complete understanding of the role of thiols in these biogeochemical processes. The concentrations of dissolved, colloidal, and particulate thiols were measured along a salinity gradient in estuarine waters off of Galveston Bay, Texas. The majority of thiols were present in the dissolved fraction, although more thiolic species were detected in the particulate phase. Dissolved glutathione was present at higher concentrations (0.23 to 6.23 nM) than was the particulate glutathione (0.094 to 0.72 nM). Most γ -glutamylcysteine was present in the particulate phase, with concentrations as high as 2.24 nM in the middle of the estuary. Phytochelatin-2 was ubiquitous in surface waters, with chlorophyll *a*-normalized concentrations of up to 6.3 $\mu\text{mol g Chl } a^{-1}$. A major thiol peak was present in Lower Galveston Bay and a minor peak in Upper Galveston Bay, and in both regions, 5–6 mol of γ -glutamylcysteine were produced per mole of glutathione. This bimodal distribution indicates *in situ* production of thiols from two different phytoplankton communities in Galveston Bay during this period.

Sulfur is an essential element and is required for protein synthesis by all organisms. The metabolic studies of sulfur-containing species in natural waters have branched into two major areas, which are concerned with the following: (1) the global biogeochemical cycle of volatile sulfur species, such as dimethyl sulfide (DMS), carbonyl sulfide (OCS), and related compounds (Watts 2000) and (2) the detoxification properties of thiolic compounds, such as glutathione (GSH), cysteine (Cys), and sulfide, for ameliorating oxidative stress caused by trace metals, radicals, and other xenobiotic compounds (Grill et al. 1985; Giovanelli 1987). These two research areas are related at the molecular level in organisms during the synthesis and transformation of amino acids. Production of DMS is closely related to methylation reactions during methionine synthesis (Grone and Kirst 1992) and to the detoxification processes involved in GSH transformation (Meister and Anderson 1983). In water, organic matter-mediated photoreactions of dissolved organic sulfur compounds lead to the formation of OCS and related compounds (Zepp and Andreae 1995).

GSH, as one of the most abundant low-molecular weight (LMW) thiols in animals, plants, and bacteria, has been shown to play an important role in protecting cells against oxidative stress, radiation damage, and elevated levels of heavy metals (Giovanelli 1987). The use of GSH (rather than Cys) as the major active reduced sulfur species is thought to have evolved when organisms adapted to the oxic atmo-

sphere of the Earth (Fahey and Sundquist 1991). The synthesis and transformation of GSH, a tripeptide γ -glutamylcysteinylglycine, is catalyzed by various enzymes *in vivo* (Meister and Anderson 1983). Both GSH and Cys are important precursors of carbonylsulfide in natural waters, via photochemical and nonphotochemical pathways (Flock et al. 1997).

Compounds with sulfhydryl groups are known to be strong complexing ligands for some transition metals and for soft, B-type metals (Krezel and Bal 1999). Plants, algae, and fungi readily synthesize phytochelatins (PCs)[$(\gamma\text{GluCys})_n\text{Gly}$ and analogues; $n = 2\text{--}11$] as detoxifying agents in response to increasing metal stress, using GSH as a substrate (Grill et al. 1985). At present, culture experiments are the main source of information linking trace-metal concentrations to thiol production in living cells (Grill et al. 1988; Ahner and Morel 1995). However, there is no simple relationship between metal stress and thiol production, because the latter is also related to nutrient supply and light availability in the culture media (Matrai and Vetter 1988; Rijstenbil et al. 1998). In addition, this complexity in available data could also be related to the observation that strong metal-thiolates are not reactive to the fluorescence tag when fluorometric techniques are applied (Tang et al. 2000). Nonetheless, particulate thiols, including GSH and PCs, have been successfully detected in natural samples from estuarine and coastal ocean waters (Matrai and Vetter 1988; Ahner et al. 1997; Tang et al. 2000) as well as in open ocean water (Ahner et al. 1998).

Theoretical calculations indicate that the sulfhydryl group is the primary complexing group of GSH for many trace metals (e.g., copper [Cu], lead [Pb], cadmium [Cd], mercury [Hg], and zinc [Zn]) in neutral or basic conditions (Krezel and Bal 1999). Recent studies have shown that thiols can account for almost all of the Cu-complexing ligands in phytoplankton media (Leal et al. 1999). The conditional stability constants derived from some Cu titration experiments in these media (Brand et al. 1986) and from field samples (Tang

¹ Corresponding author (tangd@tamug.tamu.edu).

Acknowledgments

This manuscript greatly benefited from discussions with J. Pinckney about the spatiotemporal distribution of phytoplankton species in Galveston Bay. The insightful suggestions of L.-S. Wen during the early stages of this work are also acknowledged. The TAMUG small boat operation kindly provided personnel and funds for collecting samples from Galveston Bay. The suggestions of two anonymous reviewers are greatly appreciated. This work was funded in part by the Office of Naval Research (N00014-99-1-0037) and the Texas Institute of Oceanography.

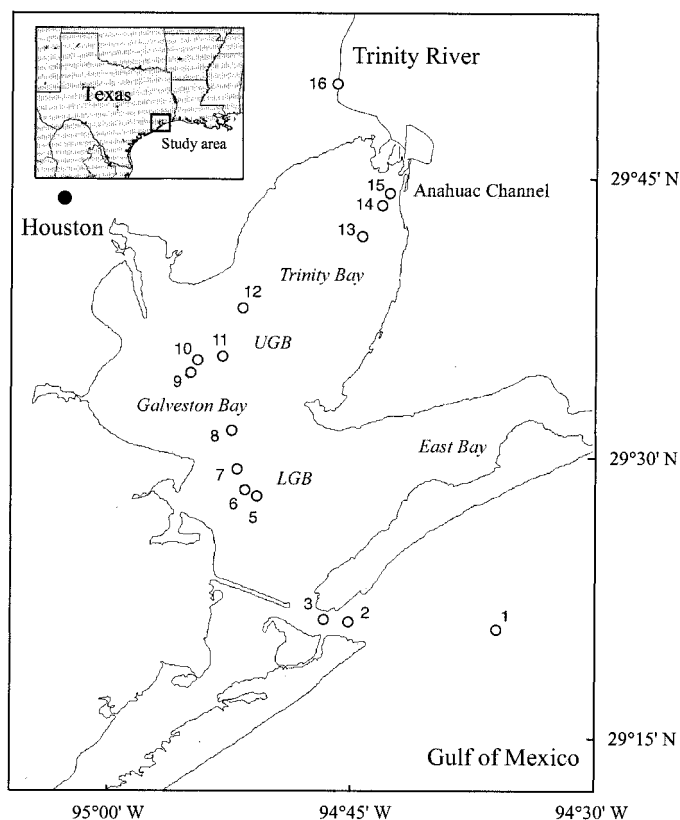


Fig. 1. Sampling locations in Galveston Bay Estuary and Trinity River on 16–19 August 1999. UGB, Upper Galveston Bay; LGB, Lower Galveston Bay.

2000) are close to those associated with GSH- and Cys-Cu complexes (Leal et al. 1999). However, only a few studies have been conducted to measure the concentrations of thiolic compounds by voltammetric methods (Luther and Tsamakis 1989; Le Gall and van den Berg 1993, 1998). To date, few studies have been conducted to detect thiols in the operationally defined “dissolved” phase ($<0.45 \mu\text{m}$), using low-level techniques that take advantage of online separation followed by sensitive detection (e.g., high-performance liquid chromatography [HPLC]). The lack of reliable data limits our understanding of the role and fate of thiols in natural waters and of their importance in trace-metal speciation.

We have previously developed a method (Tang et al. 2000) using reverse-phase HPLC with fluorescence detection optimized to determine LMW thiols using ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F). Here, we apply this method to determine the concentrations of dissolved and colloidal thiols in estuarine waters collected from Galveston Bay (Texas), and we provide the first estuarine distribution of GSH. The production of various thiolic species in the different regions of Galveston Bay will be discussed.

Methods

Sample collection—Estuarine water was collected along a salinity gradient during 16–19 August 1999 (Fig. 1) using

ultraclean sampling procedures (Wen et al. 1996). Briefly, water was pumped from about 20 cm below the surface through acid-cleaned FEP-Teflon tubing positioned upstream of the boat, filtered in-line using acid-cleaned prefilters and filters ($0.45 \mu\text{m}$) (MSI), and collected in acid-cleaned low-density polyethylene bottles (500 ml) or Teflon bottles (1 liter). The filtered samples were stored in a cooler in double plastic bags (the inner bag had been acid-cleaned) during transport and were transferred to a refrigerator (4°C) once in the laboratory. For dissolved ($<0.45 \mu\text{m}$) thiol analysis, water samples were acidified in situ by adding 1.0 ml of methanesulfonic acid to a 500-ml sample in the field immediately after collection. Particulate thiols were collected in the laboratory within 8 h of sampling via gentle vacuum filtration ($<5 \text{ psi}$) of 150–250-ml unfiltered water samples through $0.4 \mu\text{m}$ polycarbonate filters (Poretics), which were then stored at -20°C until analysis.

Stirred-cell ultrafiltration—Ultrafiltration, using an Amicon stirred cell (No. 8200) assembled with a 1-kDa regenerated cellulose membrane (YM1), was carried out to separate the colloidal fraction ($1 \text{ nm}–0.45 \mu\text{m}$) from the dissolved fraction ($<0.45 \mu\text{m}$). The pressure in the cell was maintained at 60 psi throughout the experiment using ultrapure nitrogen; this resulted in a constant permeation flow rate of 1.3 ml min^{-1} . Prior to use, the new membrane was first soaked in Nanopure water ($>18.3 \text{ M}\Omega$, Barnstead) at least three times and was then stored in a detergent solution (0.01% Micro). The membrane was then rinsed with water and soaked in a NaOH solution (0.1 N) for at least 30 min before assembly. A 10-ml HCl solution (0.006 M) and 20 ml of water were filtered through the assembled cell under working conditions, and the cell was rinsed again with sample water three times before we actually processed a sample. Samples without acidification were used for ultrafiltration experiments, which were conducted in a Class-100 clean bench within 1 month postcollection. Generally, 130 ml of sample water was concentrated to about 20 ml, thereby yielding concentration factors of between 6 and 9.3.

Other ancillary parameters—For nutrient determinations, samples were collected in 30-ml acid-cleaned High Density Polyethylene (HDPE) vials after in-line filtration and were analyzed by a flow-injection spectrophotometric method (Grasshoff et al. 1983). Particulate organic carbon (POC) samples were collected on precombusted (5 h at 450°C) glass-fiber filters, and concentrations were determined using a Perkin-Elmer CHN analyzer (PE CHNS/O 2400). Dissolved organic carbon (DOC) samples were collected in precombusted amber glass bottles, and measurements were conducted using a catalytic high-temperature DOC analyzer (Shimadzu TOC 5000). Samples for chlorophyll *a* (Chl *a*) analysis were collected on $0.45 \mu\text{m}$ polycarbonate filters and were determined by HPLC following acetone extraction (Murray et al. 1986).

Thiol determination—Thiols in the dissolved, colloidal, and particulate fractions were determined using reversed-phase HPLC with fluorescence detection of derivatives of SBD-F (Tang et al. 2000). Briefly, a Waters HPLC system

with a C18 column (Waters Symmetry; 250 × 4.6 mm; particle size, 5 μm) was used for separation and determination during a gradient elution of acetonitrile, complementary to the aqueous trifluoroacetic acid solution (0.1%). The aqueous samples or supernatants of the particulate extraction were buffered to a pH value of close to 9.0 with a borate solution (0.1 M) and NaOH (1 M). The reducing agent, tri-*n*-butylphosphine, was used in the derivatization, and results are reported as the total concentration of a particular thiol, which includes the free, disulfide, and metal-bound forms in each size fraction.

Results

The presence of thiols in the three size fractions of Galveston Bay waters—The optimized SBD-F method is very sensitive for all LMW thiols except cysteine. Typical chromatograms for the HPLC fluorescence detection are shown in Fig. 2 for (a) the injection of 2 pmol of each thiol, including Cys, GSH, γ -glutamylcysteine (γ EC), *n*-acetyl-L-cysteine (NAC), and phytochelatin-2 (PC₂) and for the thiol determinations in the (b) colloidal (1 nm–0.45 μm), (c) dissolved (<0.45 μm), and (d) particulate (≥0.45 μm) fractions from a natural sample collected at Station 9 (salinity = 16.2). The presence of Cys could not be verified at these low concentrations because of its low derivatization efficiency and proximity to the injection peak. The detection limit of GSH, which was calculated using three times the standard deviation of the standard with the lowest concentration, is 0.01 pmol for a 150-μl injection, a value that is equivalent to 0.09 nM of GSH in the original water or supernatant of the particulate extract. The detection limits for PC₂, γ EC, and NAC are 0.08, 0.03, and 0.06 pmol, respectively.

Distribution of nutrients, organic carbon, and Chl a—The sampling stations, visited along a salinity gradient, can be separated into three regions (Wen et al. 1999): Lower Galveston Bay (LGB), Upper Galveston Bay (UGB), and Trinity River/Anahuac Channel (TR/AC). In LGB (Stations 1–8), samples were actually collected in the Houston ship channel, which is one of the busiest waterways in the United States. Since the water depth in the ship channel is much greater (average 13 m) than it is in the rest of Galveston Bay (average, 2 m), the longitudinal mixing is enhanced compared to that noted in other areas. The TR/AC (Stations 13–16) region is strongly influenced by the Trinity River, which is the major freshwater source to Galveston Bay, accounting for 83% of the annual freshwater input. In UGB, (Stations 9–12), waters are less dynamically mixed compared to those of LGB, and they are less turbid than those of TR/AC. Thus, planktonic abundance (e.g., biomass) is usually higher in this region because of the region's calmer water (Pinckney pers. comm.) and higher benthic nutrient-exchange rates (Warnken 1998). Differences in the hydrodynamic conditions of these three regions seem to have strong effects on the concentrations and distributions of chemical and biological species (see below).

Nutrients (with the exception of nitrate), organic carbon, and Chl *a* were linearly decreasing with salinity in LGB and

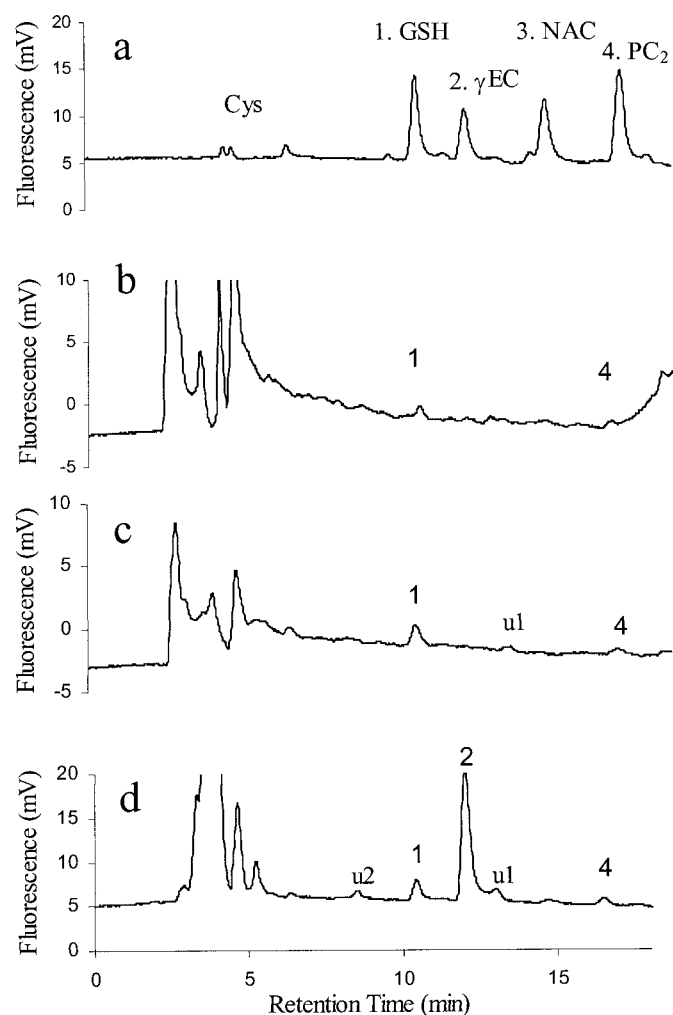


Fig. 2. Chromatograms of the fluorescence detected from thiol-SBD derivatives. (a) Thiol standard (2 pmol) of Cys, GSH (1), γ EC (2), NAC (3), and PC₂ (4); thiols in (b) the colloidal (1 nm–0.45 μm); (c) dissolved (<0.45 μm); and (d) particulate (≥0.45 μm) fraction at Sta. 9 (sal = 16.2). The u1 and u2 represent unknown thiol species.

were present at elevated concentrations in UGB (Fig. 3). Nitrate concentrations were low, potentially limiting the primary production. The phosphate and silicate concentrations were higher, with a maximum at salinity 10 (Sta. 12), which could be attributed to benthic exchange in this region (Santschi 1995; Warnken 1998; Wen et al. 1999). In the TR/AC region, POC, DOC, and Chl *a* concentrations decreased from the river out into the bay, whereas high concentrations of nitrate and ammonium at the Anahuac Channel (Sta. 14) may again be attributed to benthic exchange or to point-source input. Overall, the nonconservative distribution profiles of nutrients, organic carbon, and Chl *a* that were present during this sampling period are similar to those previously reported for nutrients, trace metals, and DOC (Santschi 1995; Wen et al. 1999).

Distribution of thiols in Galveston Bay—Dissolved (<0.45 μm) and colloidal (>1 kDa and <0.45 μm) thiol concentra-

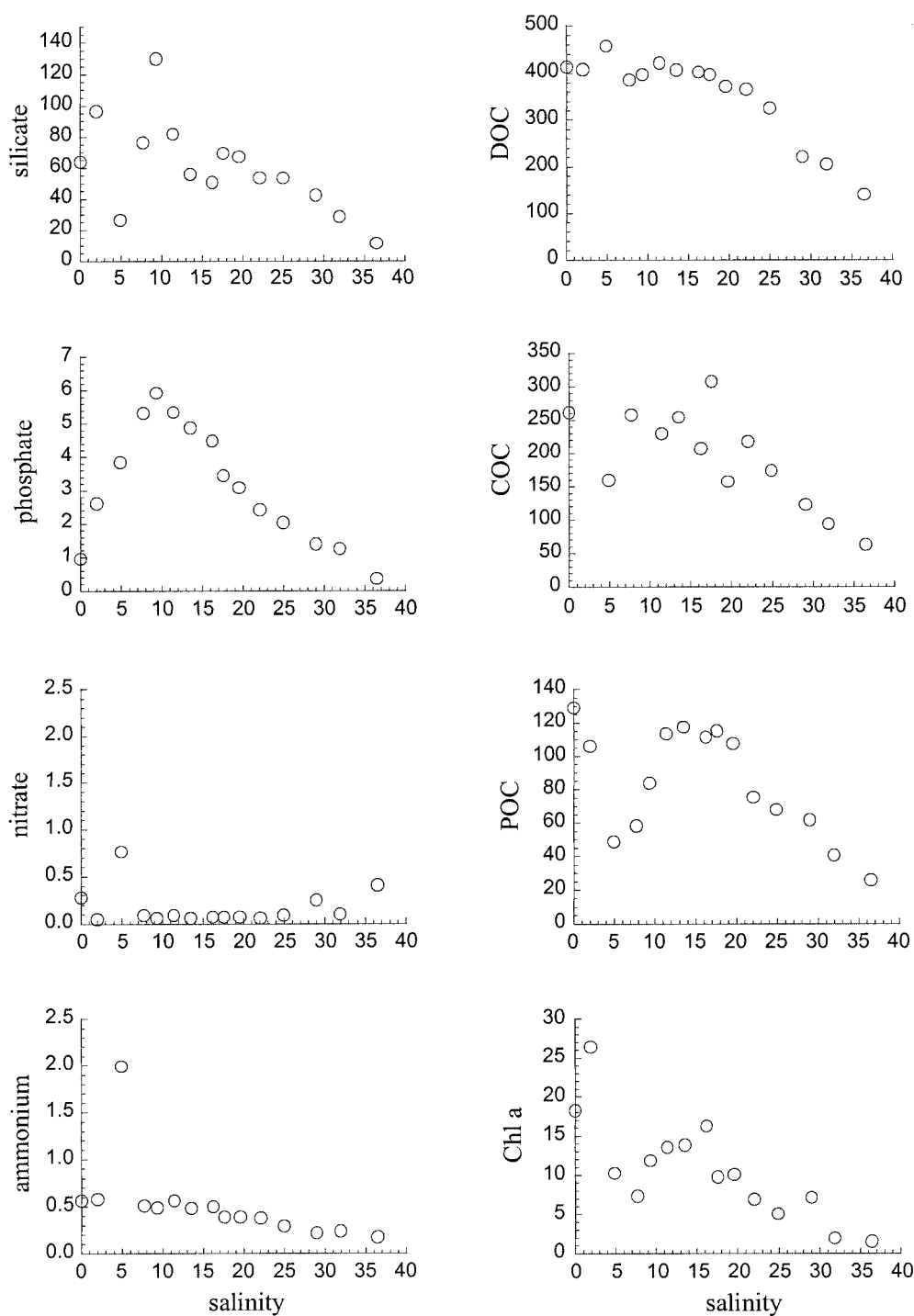


Fig. 3. The distribution of nutrients, organic carbon, and chlorophyll *a* (Chl *a*) in Galveston Bay surface waters. Units are μM for all but Chl *a*, which is $\mu\text{g L}^{-1}$.

tions are listed in Table 1. Since it is difficult to differentiate between colloiddally bound thiols and “artificially” retained LMW thiols, these operationally defined “colloidal” thiols should be interpreted with caution. The “colloidal” GSH amounted to only 10–20% of the dissolved GSH, indicating that LMW GSH could have been retained in the colloidal phase during the processing (Guo et al. 2000); alternatively,

this measure could be real and could be interpreted to indicate retention of combined LMW thiols. Particulate thiol concentrations (Table 2) are converted to volume-based concentrations in the filtered water so that direct comparison to the dissolved and colloidal thiols can be made.

A greater number of individual thiols were detected in the particulate phase (Fig. 2d); however, the total concentrations

Table 1. Thiol concentrations in dissolved (<0.45 μm) and colloidal (1 nm–0.45 μm) fractions. The u1 value is an unknown thiol, quantified as GSH.

Sta.	Salinity	Dissolved thiols			Colloidal thiols			
		GSH	u1	PC ₂	GSH	γEC	u1	PC ₂
		(nM)			(nM)			
1	36.4	0.23	n.d.	n.d.	0.21	n.d.	n.d.	n.d.
2	31.9	2.01	0.58	n.d.	0.27	n.d.	0.09	0.2
3	29.0	2.36	0.39	1.2	0.43	0.2	0.14	0.2
5	—	—	—	—	0.53	0.2	0.05	0.2
6	22.0	5.15	0.76	2.0	0.44	0.2	0.04	0.3
7	—	—	—	—	0.85	0.2	0.08	0.2
8	17.6	6.18	n.d.	2.7	0.30	n.d.	0.15	0.2
9	16.2	4.52	0.74	3.0	0.32	0.2	0.12	0.2
10	13.5	3.19	0.74	2.4	0.42	n.d.	0.13	0.4
11	11.4	4.81	0.70	3.0	0.98	0.2	0.15	0.5
12	9.3	2.64	0.71	2.9	—	—	—	—
13	7.7	2.01	0.57	3.2	0.45	0.2	0.09	0.6
14	4.9	1.02	0.34	2.8	0.25	0.3	0.12	0.4
15	1.9	2.56	n.d.	3.3	—	—	—	—
16	0.0	2.27	n.d.	1.4	0.21	0.3	0.17	0.3

n.d. = not detected; — = missing samples; GSH = glutathione; PC = phytochelatin; γEC = γ -glutamylcysteine.

of dissolved thiols were about 3–12 times higher than the total particulate thiol concentrations, except in the case of the seawater end member, in which the dissolved thiol concentrations were less than the particulate thiols (Tables 1, 2). Individually, most of the GSH and PC₂ were in the dissolved phase, and most γEC was in the particulate phase. GSH was present in all three fractions, with a major peak at a salinity of around 20 (Sta. 7–8) in LGB and a minor peak at a salinity of 10 (Sta. 10–11) in UGB (Fig. 4). Dissolved GSH concentrations ranged from 0.23 to 6.23 nM in surface water, whereas the particulate GSH concentrations ranged from 0.065 to 0.72 nM. Most γEC was present in the particulate

phase, with concentrations as high as 2.24 nM in the middle of the estuary. The particulate GSH, γEC , and PC₂ concentrations are shown in Fig. 5. As with GSH, a bimodal distribution was observed for γEC , with a concentration in LGB that was five times higher than that of GSH. The concentration of PC₂, however, only showed one peak at a salinity of approximately 16.

Discussion

GSH is the major LMW thiol in planktonic algae (Rijstbil and Wijnholds 1996). The production of GSH could

Table 2. Particulate thiols ($\geq 0.45 \mu\text{m}$) in Galveston Bay waters. The u1 and u2 values represent unknown thiol species, quantified as GSH.

Sta.	Salinity	Thiols					
		u2	GSH	γEC	u1	NAC	PC ₂
		(nM)					
1	36.4	0.052	0.33	0.094	n.d.	n.d.	0.006
2	31.9	0.037	0.32	0.69	0.049	n.d.	0.012
3	29.0	0.058	0.27	0.65	0.049	0.013	0.007
5	24.9	0.034	0.19	0.83	0.072	n.d.	0.010
6	22.0	0.045	0.18	0.90	0.072	0.025	0.015
7	19.5	0.14	0.47	2.24	0.18	0.073	0.022
8	17.6	0.33	0.48	2.17	0.15	0.072	0.016
9	16.2	0.023	0.14	1.46	0.12	0.060	0.073
10	13.5	0.018	0.14	0.86	0.062	0.053	0.052
11	11.4	0.030	0.15	0.77	0.053	0.033	0.019
12	9.3	0.016	0.095	1.29	0.10	0.035	0.013
13	7.7	0.015	0.065	0.59	0.045	0.033	0.017
14	4.9	0.030	0.094	0.19	0.011	0.031	n.d.
15	1.9	0.12	0.47	0.19	0.013	0.042	n.d.
16	0.0	0.22	0.72	0.054	n.d.	0.075	n.d.

n.d. = not detected; GSH = glutathione; γEC = γ -glutamylcysteine; NAC = *n*-acetyl-L-cysteine; PC = phytochelatin.

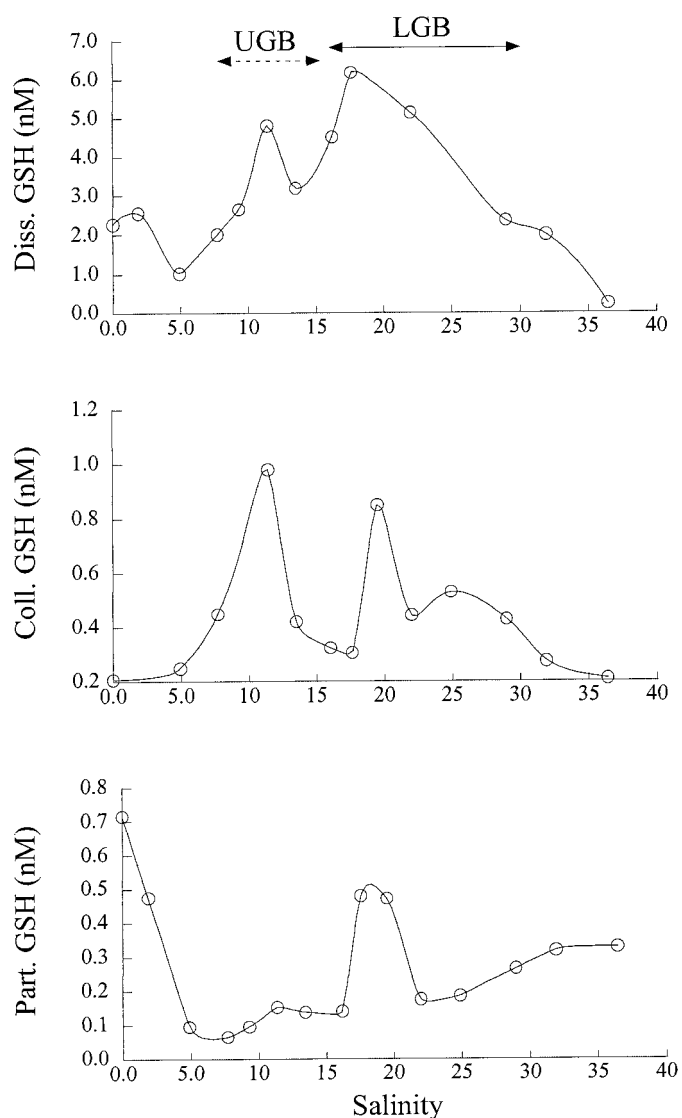


Fig. 4. Distribution of GSH in dissolved ($<0.45 \mu\text{m}$), colloidal ($1 \text{ nm} - 0.45 \mu\text{m}$), and particulate ($\geq 0.45 \mu\text{m}$) fractions in Galveston Bay. The Upper Galveston Bay (UGB) region was dominated by a cyanobacteria bloom and the Lower Galveston Bay (LGB) region by the presence of other phytoplankton species.

be influenced by light availability, nutrient levels, and toxicant concentrations (trace metals and organic contaminants). In other estuarine/coastal environments, reported particulate GSH concentrations ranged from 20 to 100 pM in Saanich Inlet, British Columbia, and from 100 to 600 pM in Southern California Bight (Matrai and Vetter 1988). In Saanich Inlet, Matrai and Vetter (1988) showed that the vertical distribution of GSH corresponded to both POC and Chl *a*. In their laboratory incubation experiments, particulate GSH increased or remained constant under elevated light levels and decreased at lower light conditions, except when nitrate concentrations were elevated. Particulate GSH occurred at similar concentrations (65–720 pM) in Galveston Bay, despite differences in location and sampling time.

PCs, the metal-detoxifying peptides of plants, fungi, and

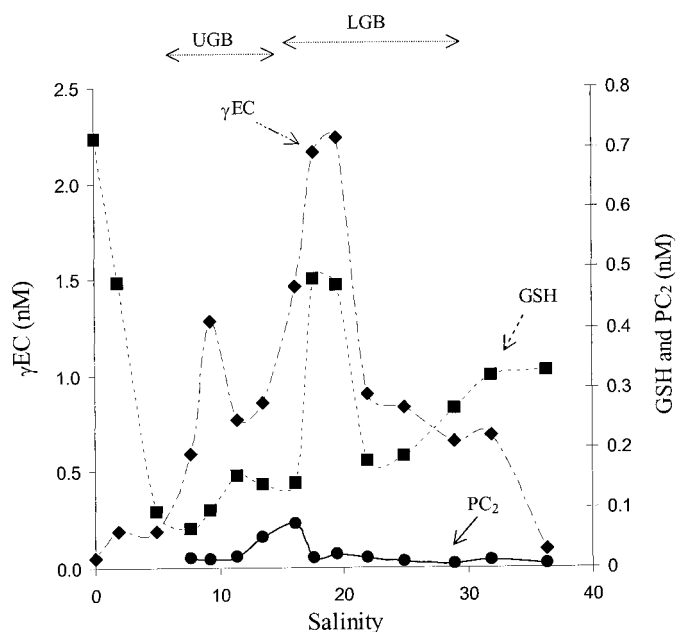


Fig. 5. Particulate ($\geq 0.45 \mu\text{m}$) thiol distribution in Galveston Bay.

algae (Zenk 1996), are ubiquitous in surface waters. Culture studies of the diatom *Thalassiosira pseudonana* have shown that PCs can be produced even when trace metals are at ambient concentrations, as long as nutrient concentrations are high (Rijstenbil et al. 1998). The Chl *a*-normalized PC₂ concentration (up to $6.3 \mu\text{mol g Chl } a^{-1}$) determined here is comparable to previous data collected from uncontaminated coastal and estuarine regions (e.g., average of $7.9 \mu\text{mol g Chl } a^{-1}$ in Galveston Bay [Tang et al. 2000] and of 2–4 $\mu\text{mol g Chl } a^{-1}$ in the New England coast [Ahner et al. 1997]). Higher concentrations (up to $50 \mu\text{mol g Chl } a^{-1}$) of PC₂ in particulate samples collected from the equatorial Pacific have recently been reported (Ahner et al. 1998). Since the ambient concentration of trace metals in the Pacific is extremely low, the unusually high levels of PC₂ may function to maintain intracellular metal homeostasis, thereby ensuring that essential trace metals, such as Cu and Zn, are present in sufficient quantities to supply the catalytic and structural proteins necessary for algal metabolism (Grill et al. 1988; Ahner et al. 1998).

In the particulate fraction, γEC is present at much higher concentrations than GSH (Fig. 5), whereas available data from estuarine diatom culture experiments in the exponential phase indicate that GSH should be the major thiol pool (Rijstenbil and Wijnholds 1996). GSH is synthesized from Cys via γEC by two enzymes: γ -glutamylcysteinyl synthetase and GSH synthetase. Since the former is the rate-limiting enzyme, cells could regulate the GSH concentration by controlling the activity of γ -glutamylcysteinyl synthetase (Meister and Anderson 1983). Evidence shows that the γ -glutamylcysteinyl moiety in PCs are synthesized directly from GSH, not from γEC (Grill et al. 1989). The higher γEC concentrations reported here suggest that synthesis of GSH was impeded. This could be a phytoplankton species-related phenomenon, or it could have resulted because γEC

functions as other than as a substrate in the synthesis of GSH, or it might be the result of some environmental parameters that are suppressing the GSH synthetase activity.

Since GSH is involved in more metabolic processes than are PCs, PC:GSH ratios should be maintained at a certain level in eukaryotic phytoplankton. Previous studies show that the intracellular PC:GSH ratio can reach as high as 0.26 in phytoplankton cultures under strong PC production, e.g., in *Phaeodactylum tricoratum* (Rijstenbil and Wijnholds 1996). The PC₂:GSH ratios measured in Galveston Bay were 0.018–0.52, which is reasonable, because the production of PCs in a healthy cell cannot decrease GSH below a level that is essential for cell metabolism. Ahner et al. (1997) suggested that PC synthesis via GSH consumption in New England coastal waters could represent a significant biochemical cost to phytoplankton, but the GSH concentrations used for the comparison were taken from Matrai and Vetter (1988). Therefore, it is important to simultaneously measure both GSH and PCs to address this issue.

In Galveston Bay, most of the GSH and PC₂ were in the dissolved (<0.45 μm) phase, whereas γEC was present mostly in the particulate phase (Tables 1, 2). As a substrate for GSH, γEC is likely a very reactive thiol in cells, possibly leading to a very low concentration in the dissolved phase. This could also indicate that sloppy feeding by zooplankton accounts for only a minor portion of the total thiols released from phytoplankton. The bimodal distribution of thiols along a salinity gradient in Galveston Bay (Figs. 4, 5) likely indicates that thiols were produced biogenically in situ and were not a result of inputs from riverine or seawater sources. An independent study of the spatiotemporal distribution of phytoplankton species in Galveston Bay during this sampling period showed that a cyanobacteria bloom occurred in UGB and TR/AC, whereas chrysophytes, prasinophytes, and cryptophytes were more abundant in the salty waters of LGB (Pinckney pers. comm.). This pattern indicates that the bimodal distribution of thiols observed here could have resulted from the presence of different phytoplankton species in these two regions of Galveston Bay. For instance, culture studies have shown that the production of thiolic compounds is dependent on phytoplankton species (Rijstenbil and Wijnholds 1996).

The concentrations of particulate thiols are closely related to Chl *a* within LGB and UGB (Fig. 6). The ratio of slopes from the linear regressions between GSH and Chl *a* concentrations for LGB and UGB is about 7.4 (Fig. 6a), which indicates that seven times more GSH was produced per unit of Chl *a* in LGB than in UGB. Similarly, γEC was produced five times more efficiently in LGB (Fig. 6b). However, using the same approach for PC₂, the ratio of the two slopes indicates that more PC₂ was produced in UGB (Fig. 6c). The ratio between GSH and γEC slopes is 4.6 in LGB and 6.5 in UGB, indicating that for each mole of GSH produced, 5–6 mol of γEC are produced.

The release of GSH from cells can also be enzymatically controlled (Meister and Anderson 1983). In natural waters, sloppy feeding by zooplankton can also release thiols. However, the fate of these thiols in the water column is still largely unknown. Thiols seem to be very reactive and could undergo further modifications, which would be partly me-

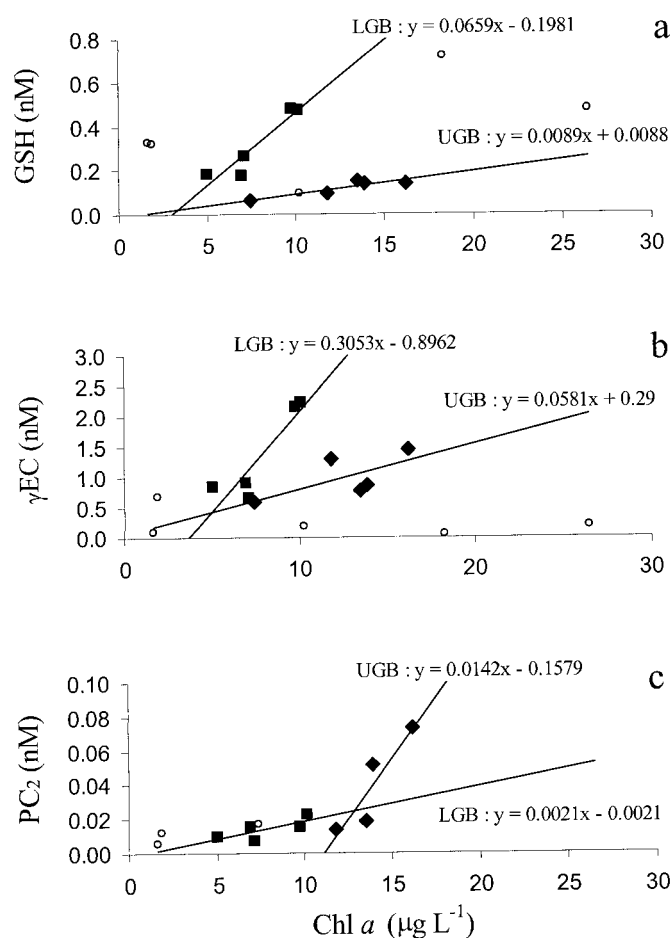


Fig. 6. Relationship of chlorophyll *a* (Chl *a*) and particulate thiol concentrations in the two regions (Lower Galveston Bay [LGB] and Upper Galveston Bay [UGB]), where concentration maximums were observed. The stations with open circles (river and seawater end members) were excluded in the regression.

diated by microbial activities (Le Gall and van den Berg 1998) or by photochemical reactions. Previous studies have shown that PCs were released as cadmium complexes from a marine diatom, *Thalassiosira weissflogii*; however, these complexes rapidly dissociated in water (Lee et al. 1996). Thiols could also be stabilized by complexing with other soft metals, forming metal-thiolate polymers (Bell and Kramer 1999), which in turn can include sulfide-producing nanoparticles (Steffens 1990). This provides a plausible explanation for the presence of trace levels of sulfides in oxic waters. The free thiols could also be reversibly oxidized to disulfides, which are more resistant to further oxidation. Laboratory studies showed that OCS was produced from free thiols (e.g., GSH, Cys, and others), whereas disulfides, sulfones, thioethers, and tertiary sulfonium contributed negligibly to OCS production in natural aquatic environments (Flock et al. 1997). The results from this study provide field evidence for the presence of thiols as OCS precursors in the dissolved phase of natural water.

The dissolved GSH concentrations positively correlated with DOC concentrations in LGB (Fig. 7), indicating that

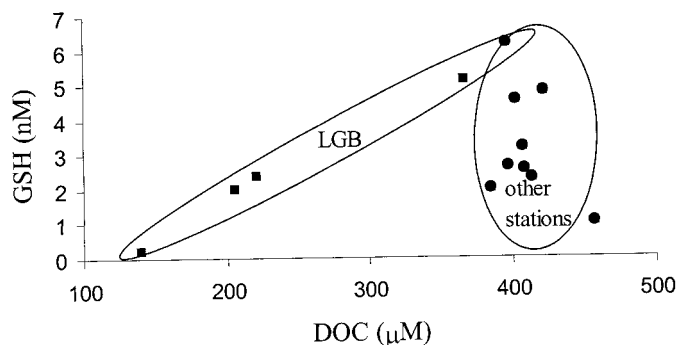


Fig. 7. Relationship between dissolved (<math><0.45\ \mu\text{m}</math>) GSH and dissolved organic carbon (DOC) concentrations.

DOC and dissolved GSH from the same sources were diluted through mixing within LGB. However, when DOC was equal to or greater than $400\ \mu\text{M}$, which was the case in UGB, GSH concentrations were unrelated to DOC. This likely indicates that there are different sources of GSH and DOC and different reactivities and residence times in these waters, because a large fraction of DOC in this region is likely composed of more refractory compounds. A linear decrease of GSH-equivalent thiols with increasing salinity was also observed in the Mersey Estuary in the United Kingdom (10–300 nM; Le Gall and van den Berg 1993) and in Galveston Bay (10–100 nM; Tang 2000) when voltammetry was used as the detection method. The higher values determined by voltammetry can be attributed to the procedure's lower specificity, which can include other thiols and dissolved sulfides in the same peak. However, dissolved sulfides are a minor portion of the total reduced sulfur (TRS) species present in Galveston Bay, because their concentrations are two orders of magnitude lower than that of TRS (Tang and Santschi 2000). The difference between TRS and the total identifiable thiols measured here could also be related to the fact that the HPLC method was optimized for the LMW thiolic compounds of interest, which left other thiols undetected.

To date, most of what we know about thiols comes from culture studies of phytoplankton; these studies usually emphasize intracellular thiol production as a detoxification mechanism. In a recent voltammetric study, Leal et al. (1999) showed that the dissolved thiols (GSH and other unidentified thiols) were released from marine microalgae (*Emiliania huxleyi*) growing in nutrient-enriched seawater in response to Cu and that thiols can account for most of the released Cu-complexing ligands. Lack of information about the dissolved concentrations of thiols in oxic waters limits our understanding of the role that thiolic compounds play in controlling trace-metal speciation at ambient levels. The fate of dissolved thiolic compounds as well as the extent of OCS production from these compounds in natural waters are still poorly understood. This study provides a solid basis for further exploring reduced sulfur speciation, cycling, and transformation questions.

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Received: 14 January 2000

Accepted: 23 May 2000

Amended: 1 June 2000