14. A mascon is a positive gravity anomaly located in a topographic depression. They were first discovered on the moon and are associated with circular basins > 200 km in diameter.
15. There is a possibility that one exists under the polar cap but it would be difficult to prove even with low-altitude data.
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19. Disks were infinitely thin ellipsoids with no surface curvature. Their centers were placed at a 3393-km radius from the center of gravity. Only ten disks near the orbit were used, for disks farther away would be secondary and highly correlated with the primary ones. The mass solution for a particular feature was derived from the one orbit that passed nearest the feature. Simultaneous solutions based on the use of all orbits with hundreds of surface masses have not been carried out as of this date.
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22. The pressure $p = Mg/A$, where the mass $M = 8.7 \times 10^{21}$ g, the gravitational acceleration $g = 372$ cm/sec^2, 10^6 dyne/cm^2 = 1 bar, and $A = 2/3\ \pi r^2$ (where the radius $r = 6 \times 10^7$ cm and the 2/3 factor is used to account for the disk being an ellipsoid and not a uniform plate and the actual feature is conical). The mass value of 8.7×10^{21} g is considerably larger than what one would obtain by directly using the results in Fig. 3. For example, a point mass estimate would be 4.0×10^{21} g, based on the simple expression $\Delta M = \Delta g r^2/G = (0.3)\ (3 \times 10^7)^2/6.67 \times 10^{-8}$. For a disk the expression is somewhat more complicated but the result, for the same 300-mgal Δg and 300-km altitude, produces 5.9×10^{21} g. This estimate is 32 percent lower than the direct fit result and is consistent with the earlier statement that the least-squares filtering effect reduces the true acceleration amplitude by 30 percent.
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25. I thank J. Brenkle of the radio science team for his relentless efforts to acquire data. He even obtained Spaceflight Tracking and Data Network (STDN) tracking when the Deep Space Network station was shut down for modification. I thank G. Cowdrey for compiling the data tapes, E. Klumpe for plotting the data coverage, Z. Shippony for determining the initial conditions of orbit, and B. Wood for his efforts with the STDN tracking network. I thank the host of Deep Space Network operations personnel who made special efforts to ensure the success of this experiment. I especially thank R. Wimberly for his gigantic efforts in the data reduction. I am indebted to A. Ferrari, R. Phillips, M. Ananda, and B. Bills for their comments and review of this report. The research described in this paper was carried out by the Jet Propulsion Laboratory, California Institute of Technology, under NASA contract NAS7-100. This is Jet Propulsion Laboratory planetology publication No. 315-78-05 (JPL internal document).

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The Estuarine Surface Microlayer and Trace Metal Cycling in a Salt Marsh

Abstract. *The aqueous surface microlayer in a Delaware salt marsh carries an average of 10 percent of the copper, 19 percent of the zinc, and 23 percent of the iron relative to the total metal flux including the dissolved and seston components. Such trace metals cycle in the salt marsh by net import on the surface microlayer and net export in the dissolved and seston components during maximum monthly tides.*

Salt marshes represent an important interface between the land and the sea (*1*), and those dominated by the macrophyte *Spartina alterniflora* are foci for intense biological activity (*2*). Although nutrients and dissolved organic carbon are important exported elements (*3, 4*), the salt marsh also seems to be a source for dissolved and particulate organic materials such as those that collect at the surface microlayer (*5*). A surface microlayer resides on top of most natural bodies of water, and normally it is enriched in organic and metal-bearing materials (*6, 7*). Moreover, the surface microlayer appears to be a source of atmospherically mobilized aerosols through turbulent bubble fractionation (*8*). However, the surface microlayer is a complex region whose thickness (typically less than 100 μm) changes in response to environmental conditions and whose composition comprises a collection of hydrophobic or surface-active materials, particulates, and even biological organisms (*9*). Often the surface microlayer is operationally defined depending on the mode of collection with devices such as meshes, activated drums, or booms.

We examine here the tidal budgets of the trace metals copper, zinc, and iron, carried as (i) dissolved, (ii) particulate, and (iii) microlayer components fluxing in and out of a salt marsh with the tides. One can evaluate the capacity of the surface microlayer to concentrate and transport trace metals in a salt marsh by collecting and analyzing dissolved and seston materials separated by filtration, together with the total surface microlayer. These three sample types (water, seston, and surface microlayer) were gathered periodically over both ebbing and flooding tides in Canary Creek Marsh, Lewes, Delaware (Fig. 1) in the spring, summer, and fall of 1975 at the terminus of the marsh (Pilot Town Road). Canary Creek salt marsh is relatively undisturbed and quite typical of those salt marshes bordering many estuaries and bays of the U.S. East Coast.

Water samples were collected with an uncontaminating plastic pole with a bottle opening at depth and filtered through 0.3-μm, acid-washed glass fiber filters. We analyzed the samples for dissolved trace metals, using ammonium pyrrolidine dithiocarbamate as a chelating agent and methyl isobutyl ketone for solvent extraction (*10*). The water extracts were dried and then digested with hot nitric acid. The surface microlayer was collected during tidal flows with an acid-cleaned, V-shaped polyvinyl chloride boom (*7*), which acts to rapidly collapse hydrophobic and particulate material associated with the surface microlayer at its apex in convenient, water-free gram-size quantities. Solid samples of freeze-dried microlayer material and filtered seston were dried at 75°C and leached with hot nitric acid. All nitric acid digests were made to a constant volume and analyzed by atomic absorption spectrophotometry.

We calculated the trace metal budgets for the marsh (*11*) from the integrated mass of the water multiplied by the trace metal concentrations in the water, seston, and surface microlayer materials collected periodically over the tidal cycle. We monitored the salt marsh only during maximum monthly tides so as to consistently sample during periods of complete inundation and flushing of the marsh. The amount of freshwater drainage in Canary Creek during the sampling periods was negligible, with total salinity excursions of less than 3 per mil. During sampling times, the marsh is assumed to be flooded with the measured quantity of water and seston to greater than 98 percent of its area, which is covered with a uniform surface microlayer to an average thickness of 1.5 μm. The thickness of the surface microlayer is extremely difficult to quantify in situ under all sampling conditions; we judged it on several occa-

Table 1. Trace metal concentrations at the mouth of the Canary Creek salt marsh, averaged over the spring, summer, and fall of 1975; ppb, parts per billion.

Averages for the tidal cycle	Copper (ppb)	Zinc (ppb)	Iron (ppb)
Water, flood	1.2 ± 0.07	1.3 ± 0.07	14.3 ± 0.8
Water, ebb	1.3 ± 0.08	1.6 ± 0.08	39.0 ± 2.0
Microlayer, flood	14,800	103,400	13.9×10^6
Microlayer, ebb	13,800	90,400	15.4×10^6
Particulate, flood (39.4 mg/liter)	37,200	169,000	20.1×10^6
Particulate, ebb (65.4 mg/liter)	26,000	139,000	18.4×10^6

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sions, using polarized light color scales, to well exceed the assumed value of 1.5 μm on a water-free basis.

Tables 1 and 2 present the average trace metal budget over three seasons for the sampling station at the terminus of the marsh. The average percentage of trace metals distributed between microlayer, water, and seston for the three seasons are summarized in Fig. 2. On the average, a microlayer 1.5 μm thick carries 10 percent of the copper, 19 percent of the zinc, and 23 percent of the iron associated with the waters of Canary Creek Marsh.

Table 2 also includes calculations of the net import and export residuals for the three trace metal tidal components in the salt marsh (*12*). On an average net basis for three seasons in 1975, trace metals appear to be cycled in this salt marsh by being imported on the surface microlayer and exported by dissolved and seston phases. The net export of trace metals as seston in this study may be due in part to our sampling only on maximum monthly tides that could resuspend particulates, during complete flushing of the salt marsh, that are normally entrained in the floodplain.

The surface microlayer must be acknowledged as an important vehicle for the transport and cycling of trace metals in the salt marsh. Certainly the particulates entrained within the microlayer may be as significant as the dissolved constituents in concentrating and transporting trace metals in the estuarine surface microlayer. The relative enrichments of trace metals in the estuarine surface microlayer of this salt marsh relative to the dissolved components are greater than those observed in other studies in more open marine waters (*6*, *8*). Such enrichments may be due either to the greater organic contents of salt marsh waters and perhaps their microlayers or to the inclusion of particulates entrained in the microlayer. In any case, a significant proportion of the trace metal burden for the salt marsh resides in the surface microlayer at any one time and suggests that the surface microlayer acts as an important agent for the concentration, cycling, and transport of trace metals in estuarine waters.

The surface microlayer shows increased affinity for organic or biological components of the marsh ecosystem, causing it to become a region of increased algal productivity and metabolism (*13*). In fact, the surface microlayer interacts with *Spartina alterniflora* in the marsh in a direct way such that dead *Spartina* litter is capable of sorbing the microlayer and thus becoming enriched

Table 2. Trace metal partitioning during flood and ebb tide at the mouth of the Canary Creek salt marsh, averaged over the spring, summer, and fall of 1975.

Element	Total (g)	Water (g)	Microlayer (g)	Particulate (g)
		Flood		
Copper	6.41×10^2	2.91×10^2 (45%)	6.86×10^1 (11%)	2.81×10^2 (44%)
Zinc	2.47×10^3	2.99×10^2 (12%)	4.78×10^2 (19%)	1.69×10^3 (68%)
Iron	2.41×10^5	3.31×10^3 (1%)	6.46×10^4 (27%)	1.73×10^5 (72%)
		Ebb		
Copper	7.55×10^2	3.04×10^2 (40%)	6.42×10^1 (9%)	3.87×10^2 (51%)
Zinc	3.15×10^3	3.72×10^2 (12%)	4.18×10^2 (13%)	2.36×10^3 (75%)
Iron	3.51×10^5	8.90×10^3 (3%)	5.60×10^4 (16%)	2.86×10^5 (81%)
		*Differences**		
Copper	-1.15×10^2	-0.13×10^2	$+0.44 \times 10^1$	-1.06×10^2
Zinc	-6.83×10^2	-0.73×10^2	$+0.60 \times 10^2$	-0.67×10^3
Iron	-1.10×10^5	-5.59×10^3	$+0.86 \times 10^4$	-1.13×10^5

*Positive numbers indicate import, and negative numbers indicate export.

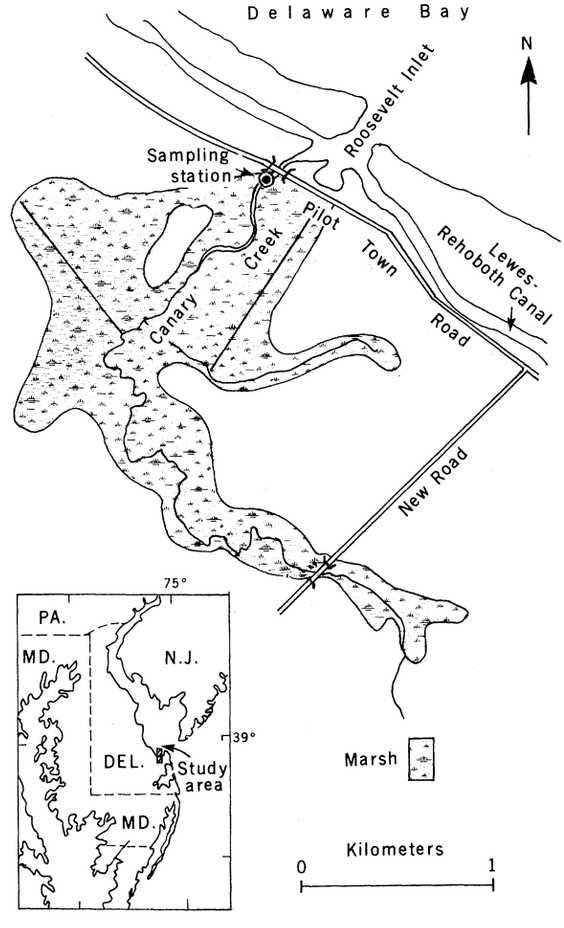

Fig. 1. Site of the trace metal study in the Canary Creek salt marsh, Lewes, Delaware. The salt march drains 2 km^2 of a small and rather pristine wetland at the southern terminus of Delaware Bay. This marsh is typical of many in the area and has been the site of numerous studies over the past three decades.

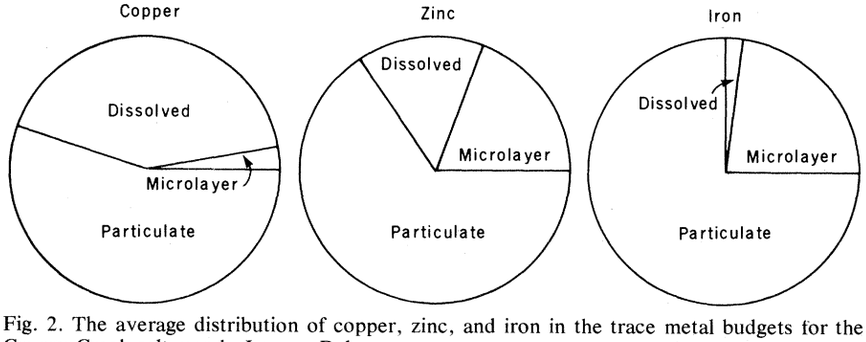

Fig. 2. The average distribution of copper, zinc, and iron in the trace metal budgets for the Canary Creek salt marsh, Lewes, Delaware.

in trace metals (*14*). *Spartina* litter also releases fulvic acids, or precursors, during decomposition, and such organic decomposition products could chelate dissolved trace metals which collect at the surface microlayer and become available for scavenging by the litter of the marsh (*14*). Thus, *Spartina* litter appears to both sorb surface microlayer components and release organic materials which comprise an integral part of the microlayer. Such organic materials could provide natural soluble chelators for trace metals and account in part for net export as dissolved components.

Earlier surveys on salt marshes to the south (*4*) suggested less of a range of trace metal tidal fluxes. At either locale, there is probably little long-term net export or import of most trace metals, except possibly iron. In the Delaware salt marsh, iron appears to undergo large (100×) dissolved export during periods of the summer; this export is due to acid-producing reactions of sulfide oxidation at the salt marsh sediment surface (*15*). Tidal heights in this salt marsh are moderate (1.5 m), and often inundations of large areas of the salt marsh surface are only monthly. Thus the export of trace metals entrained in the marsh by the action of the surface microlayer or arising from oxidation at the sediment surface may be more periodic. Where there is greater tidal height and thus complete inundation more often, exports of surface-entrained materials might be more regular. For any salt marsh, however, reactions at the tidally regulated salt marsh surface seem critical to the flux and cycling of trace metals and other elements.

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12. The differences between the fluxes of the three metal components (flood minus ebb) yield positive (import) or negative (export) residuals. However, these residuals are probably significant only in sign, relative to the components sampled (surface microlayer, dissolved components, and seston). These signs were consistent for all three seasonal tides sampled. Whether they are representative on a longer term basis or for salt marshes in general cannot be determined until more data have been acquired.
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β-Galactosidase and Selective Neutrality

Abstract. *Three hypotheses to explain the amino acid composition of proteins are inconsistent* ($P \simeq 10^{-9}$) *with the experimental data for β-galactosidase from* Escherichia coli. *The exceptional length of this protein, 1021 residues, permits rigorous tests of these hypotheses without complication from statistical artifacts. Either this protein is not at compositional equilibrium, which is unlikely from knowledge about other proteins, or the evolution of this protein and its coding gene have not been selectively neutral. However, the composition of approximately 60 percent of the molecule is consistent with either a selectively neutral or nonneutral evolutionary process.*

Although the amino acid composition is a readily measured characteristic of protein structure, the factors that contribute toward determining this composition are not well understood. Various hypotheses have been put forward to explain the experimental compositions. If the replacement of one amino acid by another is a neutral process with respect to Darwinian selection, then at compositional equilibrium the amino acids would be, within statistical scatter, equally abundant in proteins at a frequency of 1/20. In contrast, if selection occurs at the gene level and is a neutral process, the four nucleotides in the structural gene would be approximately equally abundant at a frequency of 1/4 each, that is, in a ratio of 1:1:1:1, at each of the three coding triplet positions. In this case, the expected amino acid compositions would be very close to that in the genetic code table, exclusion of the chain-terminating codons perturbing this result only slightly. Finally, independent of the extent to which evolution has been selectively neutral, the observed amino acid composition in individual proteins could be considered a sampling from the average natural abundances of amino acids in proteins. For brevity, these three explanations will be designated the amino acid–random, genetic code–random, and natural abundance–random hypotheses, respectively.

In the aggregate, the proteins that have been sequenced so far do not support any of these three hypotheses (*1–3*). A more sensitive examination of each would be possible if the amino acid composition of individual proteins could be tested against them. In some of the referenced studies such a sensitive test could not be made because the distribution of the test statistic was not known with certainty, or because the number of amino acid residues in particular proteins is so small as to reduce the force of the inferences drawn. The latter consideration is particularly relevant with respect to determining whether a particular amino acid type is or is not present in excess or deficit of theoretical expectation. The most satisfactory way to avoid these problems is to test each hypothesis against a protein of very long length so that (i) the effect of short finite length is statistically negligible; (ii) the assumptions under which the distribution of the χ^2 test statistic are valid, are satisfied; and (iii) in testing a particular amino acid against these hypotheses, the binomial distribution (see below) is well approximated by the normal distribution.

The amino acid sequence of β-galactosidase from *Escherichia coli* reported by Fowler and Zabin (*4*) provides an opportunity to examine the several hypotheses about the compositional structure of proteins. The unusual length of this protein, 1021 residues, permits the testing of each, statistically free of the sampling error introduced by small sample size. Also, as there are 20 amino acid types, each is represented in the sequence by the order of 50 residues, which is a sufficiently large number in itself to make meaningful statements about each amino

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