State of Hawaii Marine Mining Program

THE PREDICTED EFFECTS OF DISSOLVED MANGANESE IN THE PHOTIC ZONE

Department of Business and Economic Development

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A Baseline Study of Soil Geochemistry in Selected Areas on the Island of Hawaii

A Baseline Study of Ground Water Geochemistry in the Kawaihae and Hilo Areas on the Island of Hawaii

A Baseline Study of the Geochemistry and Sedimentology of Nearshore Marine Sediments in Selected Areas off the Island of Hawaii

Infrastructure Requirements for a Marine Minerals Processing Industry

The Predicted Effects of Dissolved Manganese in the Photic Zone

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FOREWORD

The State of Hawaii has long recognized the need for a more diversified economy. One area with excellent potential for new industry is the ocean. The ocean has great untapped food, energy, and mineral resources. The State of Hawaii Marine Mining Program was developed in an effort to examine fully the potential in the area of minerals. Other State programs are similarly examining ocean energy and mariculture possibilities.

Manganese nodules and crusts as sources of strategic minerals have generated interest on the part of government and industry for many years. Since 1972, Hawaii has been considering the advent of a marine mining industry in the Pacific early in the 21st century. Such an industry could use Hawaii as a minerals processing site, a supply base, a research center, a transshipment point, or any combination of these activities.

In 1975, the State Legislature provided funding to begin a manganese nodule program to assess the potential of a nodule processing industry in Hawaii. The goal of this program was to attract to Hawaii a manganese nodule industry which was environmentally sound, socially acceptable, and economically beneficial to the people of Hawaii. The program objectives included industry interaction to identify Hawaii's resources and the requirements of a new industry, assessment of environmental, social and economic impacts which the industry could have, and the dissemination of information about this potential new industry to government agencies, private businesses, and the general public.

In 1982, The Feasibility and Potential Impact of Manganese Nodule Processing in the Puna and Kohala Districts of Hawaii was released by the State of Hawaii and the National Oceanic and Atmospheric Administration. That report recounted the development of the industry and described several possible scenarios for a processing industry on the island of Hawaii. To provide a more detailed assessment of the potential impacts of a marine minerals processing industry, a team of researchers was assembled to conduct specific investigations. These studies were supported by the State of Hawaii, the County of Hawaii, the University of Hawaii, the University of Hawaii Sea Grant College Program, and the Ocean Minerals Company.

This report is the fifth in a series of environmental monographs sponsored under the State of Hawaii Marine Mining Program. It is based on work by Craig Rice completed for the degree of Master of Science from the

Department of Oceanography at the University of Hawaii. Rice's work, as well as being of scientific interest in its investigation of photochemical effects on manganese in seawater, is of major significance in its findings dealing with potential negative effects of dissolved manganese on microplankton. A potential problem with a surface discharge plume from the mining of manganese nodules or crusts, and especially the ocean disposal of tailings from marine minerals processing, is the dissolution of contained metals in the seawater and their effect on microplankton.

Manganese, known to be readily adsorbed onto particles in seawater and in high concentrations in both the minerals and tailings, is a prime target for such a study. Rice found that only very high manganese concentrations, above 5 ppm for several hours, have an inhibiting effect on plankton. Lower concentrations appear to stimulate growth. The concentrations which might be present due to mining or ocean disposal are likely to be in this lower range. Rice's work suggests that uptake of dissolved manganese may not be as significant an environmental problem as has been postulated in the past. The work involved in modelling and computer simulating the discharge plume for this study was completed by Dr. Charles L. Morgan of the Department of Business and Economic Development.

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During the course of this research, several individuals contributed their efforts without remunerative expectations. Their valuable help deserves recognition at this time. The thesis committee supervising this research was chaired by Dr. Keith Chave who provided guidance and was instrumental in helping to obtain the necessary financial support. Dr. David Karl, also a member of the committee, generously opened his laboratory for the preparation of biological samples, providing the necessary equipment and supplies. The third committee member was Dr. Fred Mackenzie. Dr. Jed Hirota allowed use of his laboratories at the Hawaii Institute of Marine Biology whose then acting director, Dr. George Losey, also generously provided a radiochemical processing laboratory at the incubation site. Bob Young, Jim Finn, and Anita Taff assisted in collection of the offshore water samples. A special thanks is also given to Virginia Greenberg who provided thorough instruction and timely advice on numerous chemical preparations, atomic absorption spectrophotometry and X-ray diffractometry. Updating of the original master's thesis manuscript was undertaken by Drs. John Wiltshire and Charles Morgan of DBED's Ocean Resources Branch.

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SUMMARY

Two aspects of the role of manganese in oceanic surface waters are investigated. To date, literature on the subject fails to explain ubiquitous maxima of dissolved Mn(II) concentrations measured in the photic zone. In addition, although Mn is known to be an essential micro-nutrient for aquatic organisms, minimum levels toxic to marine microbial populations have not been determined. Results of radiotracer experiments using ^{54}Mn clearly show that light and natural marine dissolved organic carbon (DOC) can interact to inhibit adsorption of Mn(II) onto pelagic carbonate sediment suspensions. Affinity of dissolved Mn for such particles remains high in the dark, but much slower adsorption occurs with exposure to sunlight in the presence of DOC. It is proposed that sunlight and DOC shift the partitioning of Mn within the photic zone toward the divalent species and thereby allow relatively high Mn(II) concentrations to persist in the surface ocean.

Natural microplankton assemblages were incubated using clean techniques in 4-liter polycarbonate bottles with added Mn(II). Over eight hours, RNA and DNA synthesis, adenosine 5'-triphosphate (ATP) concentrations and ^{14}C-uptake were monitored. The data do not delineate a discrete level at which Mn(II) limits growth. Additions less than 500 parts per billion (ppb) had no effect. Additions between 500 and 2000 ppb gave erratic results, but above 5000 ppb, Mn(II) became inhibitory. A computer-generated model of a discharge plume from a manganese processing industry suggests that levels of 5000 ppb would exist in the water column only within less than 30 meters from the outfall.

INTRODUCTION

Previous scientific research has revealed interesting properties of manganese in the marine environment. These observations include a concentration of manganese in pelagic sediments significantly in excess of that found in crustal rocks, a hydrothermal supply associated with active spreading centers, a potential for efficient scavenging of other metals because of large surface areas of manganese hydroxides, and susceptibility to reduction and mobilization from the solid phase under only slightly suboxic conditions. Water column profiles of dissolved Mn in the ocean generally show surface enrichment and low values at depth in contrast to other transition metals (such as nickel, copper, cadmium and zinc) that generally exhibit surface depletion and enrichment at depth. In addition, manganese oxides are the principal constituents of ferromanganese nodules and crusts, a valuable potential mineral resource. Future metallurgical processing of nodules or crusts, particularly if done on or near the ocean, could produce significant amounts of divalent, water-soluble manganese in waste streams. The fates and effects of this species in the marine environment are therefore potentially of great practical as well as scientific interest. Despite a plethora of literature on the subject, the unique geochemistry of manganese remains for the most part unexplained.

Most research to date has focused on manganese in connection with sediments, hydrothermal chemistry, or formation of ferromanganese deposits. This research focuses on the photic zone where divalent Mn predominates (Klinkhammer and Bender, 1980) and is exchanged rapidly with solid oxide phases (Sunda and Huntsman, 1985). A review of the geochemical literature indicates two major unresolved problems concerning manganese in the photic zone. The first concerns the oxidation state and speciation of manganese and its association with dissolved particulate organic matter and inorganic particles. The second directly involves marine biota and the role of divalent manganese as a nutrient or as a toxic substance.

Sampling improvements (Knauer and Martin, 1973; Fitzwater *et al.*, 1982) combined with flameless atomic absorption spectrophotometric techniques (Kingston *et al.*, 1978), provide improved accuracy and detail to our knowledge of the spatial distributions of dissolved manganese concentrations in seawater. The resultant data base has enabled investigators (Klinkhammer and Bender, 1980; Pedersen and Price, 1982; Grill, 1982) to propose kinetic and thermodynamic models of manganese solubility. The hexaquo manganous ion, $Mn(H_2O)_6^{++}$ has been suggested as the most likely form of Mn(II) in seawater. Other aqueous species might include: $[Mn(H_2O)_5OH]^+$, $[Mn(H_2O)_3(OH)_3]^-$, $[Mn(H_2O)_5HCO_3]^+$, $[Mn(H_2O)_5SO_4]^\circ$, etc. (Morgan, 1967).

Turner et al. (1981) proposed a significant (37%) association between manganous and chloride ions in model seawater. Furthermore, organic ligands may complex with Mn(II) and effectively keep it in solution. Thermodynamic considerations, however, favor the tetravalent Mn(IV)- particulate form assuming equilibrium at the pH and Po_2 of oxygenated seawater (Ahrland, 1975). Thus, one would expect precipitation, adsorption, active uptake and bioaccumulation processes within the photic zone to enrich the particulate fraction in Mn(IV) at the expense of the dissolved Mn(II)-pool. Considering all of these potential removal mechanisms, what kinetic barriers allow Mn(II) to predominate? Except in limited cases where the oxic-anoxic interface occurs within the water column, particulate manganese makes up less than 10% of the total Mn in seawater (Bischoff and Piper, 1979; Brewer et al., 1974).

$$Mn(II) = x/2\ O_2 \longrightarrow MnOx \qquad (1)$$

$$Mn^{++} + 2H_2O \longrightarrow MnOOH + 3H^+ + e^- \qquad (1a)$$

$$3Mn^{++} + 4H_2O \longrightarrow Mn_3O_4 + 8H^+ + 2e^- \qquad (1b)$$

$$MnOx + Mn(II) \longrightarrow Mn \cdot MnOx \qquad (2)$$

$$Mn \cdot MnOx + 4-x/2\ O_2 \longrightarrow 2MnO_2 \qquad (3)$$

Oxidation of divalent, aqueous manganese is known to involve complex reaction mechanisms (see Stumm and Morgan, 1981, for a review). Adsorption (equation 2) and oxidation (equations 1 and 3) take place simultaneously at the interfaces of the solid oxides. The value of x above varies between 1.3 and 1.9 in seawater. Thus, products of manganese oxidation are nonstoichiometric, yielding a mixture of ionic species at various degrees of oxidation. Specifically, equations 1a and 1b are thought to control manganese solubility in the oceans (Klinkhammer and Bender, 1980; Grill, 1982). The reaction is first order with respect to Po_2 and shows a strong dependence on pH. As oxidation proceeds, protons are liberated which lower pH and reduce the reaction rate. Overall, the reaction is autocatalytic. The various solid oxides that form as products, such as manganite (MnOOH) or hausmanite (Mn_3O_4), catalyze further oxidation (equation 3). In natural waters, these reactions may be catalyzed by manganese-oxidizing microorganisms (Emerson et al., 1982). Also, the presence of detrital organic matter (Martin and Knauer, 1980; Hunt, 1983) and certain inorganic surfaces, especially γ-FeOOH (lepidocrocite), can significantly increase the rate of scavenging of divalent manganese by surface catalysis (Sung and Morgan, 1981).

Pacific open-ocean surface waters (<20 meters (m) water depth) contain between 0.031 and 0.1 parts per billion (ppb) (0.57-1.8 nano-

mole per kilogram (nmol/kg)) dissolved (particle size less than 0.4 micrometers (μm)) manganese (Jones and Murray, 1985; Bender et al., 1977; Landing and Bruland, 1980). There follows a gradual decrease in dissolved Mn with depth to abyssal values of about 0.02 ppb. Higher values often occur close to the bottom from resuspension of fine-grained sediment-rich adsorbed manganese. Some investigators find mid-water Mn(II) maxima associated with zones of low dissolved oxygen (Klinkhammer and Bender, 1980), which might be expected because manganese is susceptible to reduction in suboxic conditions. This pattern, however, is not consistently observed. Jones and Murray (1985) detected other subsurface maxima which may be associated with hydrothermal emanations from the Juan de Fuca seafloor spreading center. Martin and Knauer (1982), investigating Mn fluxes in the Pacific Ocean, found maxima in suspended particulate Mn just above oxygen minima with no correlation between dissolved oxygen and Mn(II) concentrations. They suggest that manganese dissolves in nearshore sediments where the oxygen minimum intersects the continental slope. Divalent manganese is released and advected laterally along isopycnals out into the deep ocean where it is rapidly scavenged by sinking particles and subsequently accumulates in pelagic sediments. As sinking particles approach the top of this zone of low oxygen, the proportion of labile Mn associated with them increases sharply. Microbial activity may control this process because low O_2 concentrations favor oxidation of manganese by marine bacteria (Nealson, 1977). Thus, if particle flux is high where oxygen is low, the mid-water source of Mn(II) within the oxygen minimum zone may become depleted. In any case, the net flux is downward and cannot explain the observed ubiquitous surface maxima of dissolved manganese in the open ocean.

Both light and dissolved organic molecules are found in relative abundance in the photic zone. Many organic molecules such as phenolic compounds, carbonyl groups and conjugated systems containing aromatic rings can absorb light energy in aqueous solutions. The energy is transformed into excited electron states. Homolytic bond cleavage may result if sufficiently high energy quanta are absorbed by the molecule. Free radicals are formed by such molecular fragmentation. A transition metal with multivalent bonding characteristics would tend to collect such highly reactive species as free radicals and become reduced. Sunda et al. (1983) and Sunda and Huntsman (1985) have shown conclusive evidence for photoreduction of manganese oxides in seawater. Photoreduction would shift the thermodynamics of the system as previously described in favor of increased activity of the reduced species at redox equilibrium. This process would also delay the oxidation of Mn(II) species dissolving from aerosols deposited at the sea

surface. The net effect could explain the observed Mn(II) enrichment at the surface of the ocean.

The activity of divalent manganese that is toxic to natural populations of marine phytoplankton and bacteria has never been determined. The biochemical role of Mn as an enzyme co-factor and for electron transfer during photosynthesis is well-known. Manganese is an essential micronutrient for marine organisms (Vinogradov, 1953), but can also be toxic at high levels (O'Kelley, 1974). Measurements of total manganese in the organic fraction of phytoplankton (Martin and Knauer, 1973) show enrichment by as much as 10^6 over open-ocean surface concentrations. Nutrient-limited growth of the giant kelp, Macrocystis pyrifera, occurs in seawater media containing very low ambient Mn(II) concentrations typical of the deep sea (Kuwabara, 1982). Other researchers (Harvey, 1947; Sunda et al., 1981) have stimulated phytoplankton growth by adding small amounts of Mn(II) to algal cultures grown in deep, nutrient-rich seawater collected and brought to the surface. It has been hypothesized that, under certain upwelling conditions, patterns of production in the ocean may be governed by the availability of Mn(II).

This present study has been designed to provide data on two aspects of the biogeochemistry of manganese. The first is to find the level of divalent manganese at which phytoplankton and bacterial growth is inhibited, and to characterize the toxic response. From the general literature, my hypothesis is that this level will be high. The second is to determine whether light and natural levels of dissolved organics affect the partitioning of manganese between a solid phase, specifically pelagic calcite sediment, and seawater.

MATERIALS AND METHODS

General

Equipment and labware used in productivity experiments were of non-metallic, in most cases plastic, construction. Pyrex® vessels were substituted when plastic was not suitable (or not available) and because glass could be cleaned of all organic contaminants for experiments with ^{54}Mn. All labware was cleaned as follows: washed thoroughly in Liquinox®; soaked for at least one week in separate 10% hydrochloric and 10% nitric acid baths; and thoroughly rinsed with glass-distilled, deionized (Barnstead Ultrapure®) water (DDW) before and after each soaking period. When organic-free water was desired, DDW was redistilled under a filtered laminar flow hood at sub-boiling temperature in a still made entirely of quartz (QDW). Productivity bottles were given a final cleaning with 0.25 equivalents per liter (N) sub-boiling distilled HCl, then rinsed with 3.5 milli-mole per liter (mM) Na_2CO_3 in QDW to ensure that no residual nitrate or acid remained. All chemicals were Baker Analyzed® reagents.

Sample Collection and Manipulation

Samples of seawater were collected in November, February and May, 1982-83, from stations 8-14 kilometers (km) N.E. of Kaneohe Bay, Oahu. On the first two dates (Experiments I and II), southerly "Kona" wind conditions had prevailed for 48 hours (hr) prior to sampling and the sea state was calm. In May, very light trade winds preceded Experiment III, resulting in light chop. A standard Go Flo bottle (Ocean Dynamics) was lowered on nylon parachute chord or polypropylene line, weighted with 20 pounds of plastic-coated dive weights, to a depth of approximately 60 meters. The unmodified bottle was scrubbed out inside and filled with 10% HCl overnight, then rinsed and filled with DDW until just before use. Multiple casts were combined in a cleaned polyethylene tank and immediately transported back to the Hawaii Institute of Marine Biology on Moku o Lo'e Island (Figure 1). Collection of seawater took 20-30 minutes and the return trip to HIMB required 30-45 minutes (min).

Once in the lab, the sample seawater was screened through Nitex® mesh directly into clean four-liter polycarbonate bottles (Nalgene 2200). For the first two experiments, 35 μm mesh was used. The mesh size was increased to 102 μm for Experiment III, and only Experiment III (which was designed to measure ^{14}C-productivity and ^{3}H nucleotide synthesis simultaneously) included a dark bottle. The volume of each productivity bottle

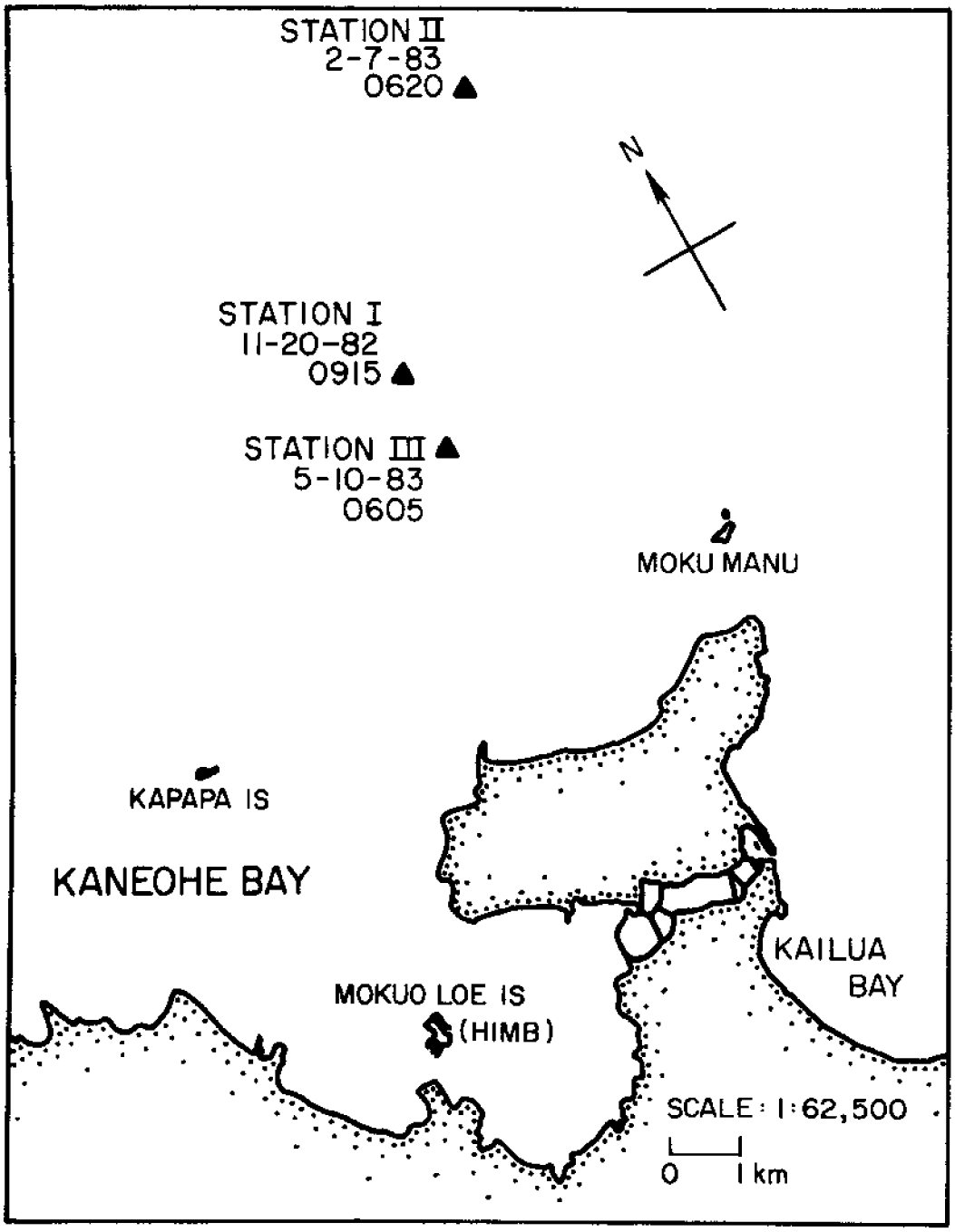


Figure 1. Locations of Collection Stations for Biological Experiments. Windward Oahu. Station Times are PST.

was determined by weight. Control samples received no added manganese while divalent manganese was added to a duplicate series of 3-4 bottles, from a concentrated stock solution of non-radioactive $MnCl_2$ in DDW. The volume of this first addition varied between 10 microliters ($\mu\ell$) and 5 milliliters (mℓ) to provide a wide range of ambient Mn(II) concentrations (1-5212 ppb) for the series of time-course measurements. Between samplings, the bottles were incubated in the small lagoon outside the HIMB facility. They were kept under a floating dock in the shade and suspended about one-half meter below the surface.

Addition of Radionuclides

Immediately after filling and adding Mn(II), all productivity bottles were spiked with 2.0 mℓ of a [2-^{3}H] adenine stock solution. The radiotracer stock was prepared by diluting one part [2-^{3}H] adenine in sterile water (1 millicurie per milliliter (mCi/mℓ), 15 curies per millimole (Ci/mmol), New England Nuclear) with nine parts filtered (1.22 μm pore size) autoclaved seawater. This stock was stored frozen until use (not longer than one month). The adenine addition was approximately 3.3 nanomoles/liter (nM).

During Experiment III, ^{14}C was added as sodium bicarbonate. Dual radiotracers were used only during Experiment III. Fitzwater et al. (1982) give conclusive evidence of adverse effects from trace metal contaminants inadvertently added during execution of the procedures of Strickland and Parsons (1972) for measuring ^{14}C primary production. Specifically, they warn against the use of soft glass storage ampoules and the diluted ^{14}C solutions that contain variable and unknown quantities of heavy metals. Therefore, no stock ^{14}C dilutions were prepared. Instead, 2 x 100 $\mu\ell$ aliquots from an ampoule containing 1 mCi/mℓ (52.4 mCi/mmol $NaHCO_3$) were injected just below the surface into each full bottle. The final procedure was to cap and invert each bottle several times and withdraw 2 mℓ to determine the total activity initially added. The bottles were then sealed and incubated as described previously.

Adsorption experiments were spiked in the following manner: To 200 mℓ of seawater, 50 $\mu\ell$ of a $^{54}MnCl_2$ stock solution were added. This stock was prepared by diluting 100 $\mu\ell$ of $^{54}MnCl_2$ (1 mCi/mℓ, 10 microcuries per micromole (μCi/μmol), New England Nuclear) to exactly 25 mℓ with filtered (0.22 μm pore size) artificial seawater (Sverdrup et al., 1946).

Sample Preparation: Toxicological Investigations

Experiments I-III were designed to determine the level of divalent (aqueous) manganese at which the growth of microbial populations endemic to Hawaiian waters was affected, and to characterize the response. During time-course incubations over eight hours, the response of the natural community to added Mn(II) was monitored in terms of changes in concentrations of adenosine 5'-triphosphate (ATP) associated with the particulate fraction, rates of total microbial RNA and DNA synthesis and, in Experiment III, ^{14}C-bicarbonate uptake into organic matter.

Samples for ATP analysis (500 mℓ) were filtered by low vacuum (<15 pounds per square inch) through glass fiber filters (Whatman® GF/F). As the top of the meniscus reached the filter, it was immediately removed from the filtration manifold and extracted by plunging into a test tube containing 5.0 mℓ of boiling phosphate buffer. The method follows that of Karl and Craven (1980). A stock buffer solution was prepared by combining equal volumes of 0.6 molar KH_2PO_4 and 0.6 molar K_2HPO_4. The stock was diluted by a factor of 5 with DDW just prior to heating (pH 6.8). Tubes were kept stoppered in order to minimize evaporative loss. Hypodermic needles were used to vent pressure. No subsequent volumetric corrections were made. After extracting for 5 minutes, needles were removed and the tubes frozen until assayed.

Samples for ^{14}C analysis were prepared in accordance with the procedures for measurement of primary productivity outlined by Strickland and Parsons (1972) with previously discussed modifications suggested by Fitzwater et al. (1982). After at least four hours of incubation, 250 mℓ aliquots were vacuum filtered as in the ATP procedure. To remove excess inorganic ^{14}C, unfiltered seawater left over in the polyethylene tank which held the entire water cast was used to rinse each cylinder and filter approximately 10 mℓ of the samples. Instead of acid fuming, filters were placed inside glass scintillation counting vials to which 1 mℓ of 10% (volume per volume) hydrochloric acid was added. The vials were left open and placed under a fume hood at room temperature for 30 minutes, then sealed and kept at 4°C.

At least three times during each incubation period duplicate 250 mℓ aliquots were poured out into separate volumetric flasks and filtered in the same manner as for ATP and ^{14}C sampling. The unrinsed filters were frozen in test tubes and later processed for the isolation of ^{3}H-labeled RNA and DNA macromolecules, following the procedure developed by Karl (1979 and 1982). To ensure efficient recovery of the acid insoluble materials, 200 μℓ

of stock solutions containing 5 milligrams per milliliter (mg/mℓ) sterile water of yeast RNA and calf thymus DNA (Sigma Chemical Company) water were added to each sample tube immediately before extracting the filters overnight in 5% (weight per volume) trichloroacetic acid (TCA) at 4°C. All trichloroacetic acid solutions were mixed from crystalline stock immediately before use and precautions were taken to ensure that wash solutions remained near 4°C. These precautions included use of ice baths during homogenation, pre-chilling all solutions, and refrigerated centrifugation (IEC Centra 7-R). Residual ethanol washes were evaporated in a water bath (95°C). Nucleic acids were first hydrolyzed in warm (37°C) 1 N sodium hydroxide for one hour, then acidified with excess HCl in 5% TCA and chilled (4°C) to separate RNA from DNA (Munro and Fleck, 1966; Karl et al., 1981). Subsequent hydrolysis of DNA was done in boiling 5% TCA for 30 minutes (Karl, 1982).

In order to obtain a meaningful estimate of the rate of nucleic acid synthesis, it is necessary to measure the specific activity of the immediate precursor: intracellular and extracellular pools of structurally related compounds which serve to dilute the radioactivity of the labeled molecules incorporated into chemically stable nucleotides (Karl, 1982). In this case, RNA and DNA are labeled via ATP and deuterated ATP (dATP), respectively. Activity of [^{3}H]-ATP (nanocuries per milliliter (nCi/mℓ)) was determined by thin layer chromatographic separation of a 10:1 concentrate (40°C, in vacuo) of the 60 mM phosphate buffered ATP extract described previously. The methodology follows that of Karl et al. (1981), Karl (1979), and Cashel et al. (1969). A total of 20-50 μℓ of the concentrate, along with a 20 μℓ portion of non-radioactive ATP stock (3.3 mg ATP/mℓ solution), was added to a 5 mℓ teflon beaker. After mixing, two 10 μℓ portions were carefully spotted onto PEI plates (Brinkman Instrument Company). The plates were rinsed twice with DDW, air-dried, developed in 0.85 M PO_4 buffer (pH 3.4), visualized under UV and subsequently eluted with a magnesium chloride solution in a glass scintillation vial. Intracellular specific activity (nanocuries per picomole (nCi/pmol)) of the ATP pools was then calculated by comparison of these data with corresponding total ATP concentrations as determined by the method of Karl and Holm-Hansen (1978).

Sample Preparation: ^{54}Mn Sorption Studies

The partitioning of manganese between a solid phase and the solvated divalent state was investigated by methods similar to those of Duursma and Bosch (1970). Artificial seawater prepared as described previously and natural surface seawater from Kaneohe Bay served as the experimental test media. Both solutions were equilibrated with peroxide-cleaned reagent

calcium carbonate (10 grams per liter (g/ℓ)) to a final pH of 8.2-8.3, then filtered (Millipore® HAWP, 0.22 μm pore size) directly into autoclaved Pyrex® flasks fitted with ground-glass stoppers. A suspension technique was employed using pelagic carbonate ooze (HIG 79-2, PC 09, 580-600 centimeters (cm) as the solid phase. The sediment was first cleaned of organic matter by heating to 550°C for several hours, rinsed with QDW, and then constantly stirred in 30% hydrogen peroxide for two hours in direct natural sunlight. Analysis by X-ray diffraction following this treatment showed the sediment to be essentially pure calcite with trace amounts of quartz. After another QDW rinse, filtering (Millipore® HAWP, 0.45 μm pore size) and oven drying at 105°C; the cleaned sediment was dry-sieved through Spex® #400 mesh and suspended in artificial seawater to a concentration of 30 mg/ℓ in a 25 mℓ graduated cylinder. This stock calcium carbonate suspension was kept sterile by lowering the cylinder inside a tubular low pressure mercury vapor ultra-violet lamp. A 200:1 dilution (150 mg sediment/kg seawater) was normally used in the reaction flasks.

One set of flasks was kept in a darkened hood. Red light was used for illumination during sampling. Another set was exposed to constant illumination from a sunlamp (Sylvania® RSM, 275 watts). The radiation spectrum of this source closely resembles that of natural sunlight (D. Crosby, personal communication). A constant-flow water bath fashioned out of a Pyrex® tray was imposed between the light source and the reaction flasks to absorb infrared radiation and prevent warming of the flasks above room temperature. A block of insulation 1.5 cm thick separated the flasks from a four-place multi-magnetic stirrer (Lab-Line Instruments, model 1262). Duplicate samples were taken immediately before addition of sediment for measurement of total ^{54}Mn activity. Additional aliquots were withdrawn one hour after adding sediment and at various times during a ten day period. Solid and aqueous phases were separated by filtration. Several brands and types of filters were tried including Millipore® HAWP and HAVP, and Gelman® metrical. All gave erratic and relatively large blank readings because of adsorption and retention of variable and excessive amounts of the spiked seawater. This problem was completely eliminated by the use of Nucleopore® polycarbonate membrane filters (0.4 μm pore size). After three minutes of suction, these filter discs (25 millimeter (mm) diameter) retained little of the liquid phase and were easily blotted dry on the non-filtering side. Each filter was placed inside a glass scintillation vial along with 0.5 mℓ of 10% HCl, capped and allowed to stand for 30 minutes before adding the liquid scintillation cocktail. Blank values using this procedure did not exceed twice the background level of 10-20 counts per minute. In order to calculate the "radiotracer distribution coefficient" (Kd), and so that a complete radiochemical inventory could be kept, samples

were also prepared from 1 mℓ each of the filtrates (activity in solution). Radiochemical budgets agreed to within ±5%.

Sample Analysis

Concentrations of total ATP in the phosphate-buffered cell extracts were determined by the firefly bioluminescence assay (Holm-Hansen and Booth, 1966; McElroy, 1947). Refinements in the preparation of crude luciferin-luciferase reagent (FLE-50) developed by researchers Karl and Holm-Hansen (1978) were adopted. For all samples, a 200 μℓ portion of extract was injected into 1 mℓ of enzyme mixture. Light emission was quantified using an ATP photometer (SAI Technology, model 2000) by measuring maximum peak heights on a strip chart recorder (Hewlet-Packard, model 7155B) during initial (0-3 seconds (sec)) mixing of reactants. The average values obtained from three such measurements were compared to mean peak height values obtained from standard solutions of adenosine 5'-triphosphate (di-sodium salt, Sigma Chemical Company). These standards were prepared by quantitative serial dilutions of a stock solution containing 1 μg ATP/mℓ. All dilutions were made up in phosphate buffer identical to that used in the initial ATP extraction. Concentrations of standards ranged from 1 to 10 nanogram per milliliter (ng/mℓ), and they were assayed before and after each set of seawater extracts.

All radiochemical samples (those prepared for measurement of [^{3}H]-ATP, [^{3}H]-RNA, [^{3}H]-DNA, ^{14}C-organics and ^{54}Mn) were analyzed with a scintillation counting system (Packard TriCarb®, model 4640). Channel settings pre-programmed by the factory were used for single and dual label experiments with ^{3}H and ^{14}C. Samples containing ^{54}Mn were counted for ten minutes over a 2-625 KeV energy range. To correct for variations in quench among samples, a series of 7-10 vials was spiked with equal activity and a variety of quenching agents added including filters, seawater, 10% HCl, TCA, etc. An unquenched sample was prepared from high activity stock and used to calculate maximum machine efficiency compared to standards of known activity. Efficiency data were correlated, electronically stored, and used to correct counts per minute (cpm) to disintegrations per minute (dpm). A standard for ^{54}Mn was not available and not considered necessary because the value of Kd depends on the relative proportion of activity in the solid phase to the activity in solution. Thus, in the case of ^{54}Mn, the data were corrected for quench with the assumption that counting efficiency remained constant. In all cases, the scintillator used was Aquasol II® (New England Nuclear): 10 mℓ into each glass vial.

Dispersion Modeling

One of the motivating factors for this investigation, as outlined above, is the potential for future man-made sources of divalent manganese to be introduced into the marine environment in the form of wastes from marine ore processing plants. To derive a first-order estimate of the primary fates and effects of this manganese, a two dimensional finite element computer dispersion model was constructed using the algorithm for passive dispersion developed by Brandsma (Brandsma et al., 1973; Brandsma and Divoky, 1976). Advection with surface currents, turbulent dispersion, and removal by zero order processes are included in the model and described below.

Primary Algorithm: A 130 x 130 matrix of elements is defined with each element representing a cube of surface water 15 m on a side. A constant amount of Mn(II) is introduced into the center of the upper left quandrant of the matrix at the beginning of each time step (90 sec). During the time step the appropriate amounts of Mn(II) are transferred from each matrix element to each adjacent element to represent advection by currents and turbulent dispersion. A flux of 266 grams per second (g/sec) of Mn(II) is used in this simulation. This represents the estimated untreated output from a full-scale (1 million metric tons per year throughput of ore) processing plant for ferromanganese crust deposits (see Harvey and Ammann, 1987, p.181). After these transfers, a constant amount Mn(II) is removed from each element which contains sufficient Mn(II). This simulates zero-order oxidation and adsorption removal processes. To simplify the book-keeping, two matrices are used in these calculations, representing the previous and present data configurations. The constants selected to control these transfers and removal are as follows:

Turbulent dispersion: As discussed in Nihoul (1975), natural dispersion coefficients are highly variable, but appear in some studies to be roughly proportional to some power of the length scale under consideration. In particular, Okubo (1968) notes that $A = k\ell^{1.15}$, where A is the dispersion coefficient (units of length squared per unit time), k is a proportionality constant, and ℓ is the length scale. Lavelle and Ozturgut (1981) and Lavelle et al. (1980), in a study of dispersion of particles from an experimental deep-sea mining reject discharge, found that a value of 10 meters squared per second (m^2/sec) for A was consistent with observations made of mining system tests with a length scale of about one km. This value, scaled using Okubo's relationship to a 15 m length, is used in this simulation (0.08 m^2/sec).

Current speed: Current speeds in surface waters are also highly variable, and would have to be measured extensively at any specific site for adequate prediction of discharge plume dispersal. A speed of 0.1 m/sec is typical in many open ocean sites and selected for use here.

Removal of Mn(II): As discussed above, the detailed fate and effects of Mn(II) in marine surface waters are not fully understood. It is clear that large inputs from terrestrial run-off (e.g. Riley and Chester, 1971 Ch.4, Martin and Knauer, 1982) and hydrothermal sources (Jones and Murray, 1985) are rapidly removed in some manner to result in the low ambient levels observed in marine waters. In an investigation of a fjord pycnocline, Emerson et al. (1982) found that the Mn(II) mixed upwards from below the pycnocline was removed from the oxygenated surface waters at a rate of 60 x 10^{-3} mol/m^2-day (3.82 x 10^{-6} g/m^2-sec). Though this rate is likely to be much lower than that which would occur in relatively well-mixed and oxygen-supersaturated open ocean surface waters, it is used in this simulation to provide a conservative first estimate.

In taking conservative values for current speed and Mn(II) removal coupled with high values for Mn(II) influx, the resulting model will tend to err on the side of overestimating the amount of Mn(II) which would be introduced into marine waters.

RESULTS

Field Incubations with Added Manganese

The time-course measurements of RNA, DNA, and ATP are summarized individually by experiment in Tables 1-3. Synthesis rates are extrapolated from incorporation of ^{3}H-label and specific activity data, and represent mean production of RNA and DNA by the microbial community over the time indicated. In general, the data show that high concentrations of Mn(II) can be tolerated by oceanic microbial populations without significantly affecting either total or biomass-specific (not shown) rates of polynucleotide synthesis during exposure periods as long as eight hours. Only the highest ambient Mn concentration (5212 ppb, 9.5×10^{-5} M, Table 2, Bottle IV) shows a marked decrease in growth by this method. The lower Mn(II) additions from 1 to 485 ppb (1.8×10^{-8} to 8.8×10^{-6} M) either have no effect or slightly stimulate the rates of RNA synthesis. Syntheses of DNA seem to proceed at a uniform rate despite the presence of manganese or the absence of light (Table 3, Bottle V). Intermediate values of added Mn(II) result either in a synthesis rate reduction at latter time points (1042 ppb, Table 2, Bottle III) or show virtually no difference from the control (Table 3, compare Bottles I and IV). The general trend of the DNA/RNA rate ratios (expressed as percent) is increasing throughout the course of each experiment. Ratios that range from 0.1-0.2, increase by 30-50% after 5-8 hours. The "dark bottle" in Experiment III exhibits a marked increase in the rate ratio values, the result of a reduction in the RNA rates while rates of DNA synthesis are not affected. Manganese additions have little effect on these ratios relative to control samples.

Despite an order of magnitude variation in the range of total label incorporated between experiments, individual experiments show a remarkably constant uptake rate. All but the most Mn-enriched sample take up ^{3}H-adenine into RNA and DNA pools in a linear manner. Figures 2 and 3 are shown as examples. Production of ^3H_2O (Figure 4), a metabolic by-product, is also linear. This pattern is observed during all ^{3}H-labeling experiments and indicates that turnover rates of adenine are much greater than the time of the incubations.

Enough ^{3}H-radiotracer stock was added initially to spike the working solutions in all experiments to a calculated activity of approximately 50 $\mu Ci/\ell$. If this initial spike becomes exhausted, a drop in specific activity would occur; however, no such phenomenon was observed. Actual measurements of total activity were lowest during Experiment II (25-31)

Table 1

Synthesis of RNA, DNA, and Concentrations of ATP
The Data are from Experiment 1

Bottle Number	Time (hrs.)	^{3}H-RNA Produced (nCi/l)	^{3}H-DNA Produced (nCi/l)	DNA/RNA (%)	ATP Pool Specific Activity (nCi/pmol)	ATP Concentration (ngram/l)	Rate of RNA Synthesis (pmol/l/hr)	Rate of DNA Synthesis (pmol/l/hr)	Mn(II) Added (ppb)
I	1	130.9	8.7	6.6	0.57	26	230	15.3	0
II	1	141.2	9.6	6.8	0.24	12	588	40.0	1
III	1	150.5	11.5	7.6	0.30	25	502	38.3	10
IV	1	135.9	11.2	8.2	0.25	19	544	44.8	100
I	3	335.0	30.5	9.1	0.56	18	199	18.2	
II	3	305.1	31.3	10.2	0.50	15	203	20.9	
III	3	342.2	36.9	10.8	0.47	18	243	26.2	
IV	3	361.0	31.8	8.8	0.44	18	273	24.1	
I	5	524.6	56.3	10.7	0.53	18	198	21.2	
II	5	585.0	64.1	11.0	0.78	24	150	16.4	
III	5	597.2	58.3	9.8	0.74	28	161	15.8	
IV	5	598.3	58.7	9.8	0.76	20	157	15.4	

Table 2

Synthesis of RNA, DNA, and Concentrations of ATP
The Data are from Experiment II

Bottle Number	Time (hrs.)	^{3}H-RNA Produced (nCi/l)	^{3}H-DNA Produced (nCi/l)	DNA/RNA (%)	ATP Pool Specific Activity (nCi/pmol)	ATP Concentration (ngram/l)	Rate of RNA Synthesis (pmol/l/hr)	Rate of DNA Synthesis (pmol/l/hr)	Mn(II) Added (ppb)
I	1	17.3	1.9	11	0.15	42	115	12.9	0
II	1	22.6	2.7	12	0.17	29	133	16.0	104
III	1	16.6	1.4	8	0.19	37	87	7.4	1042
IV	1	14.6	1.7	11	0.21	32	69	7.9	5212
I	3	23.1	2.6	11	0.23	18	33	11.3	
II	3	34.3	3.7	11	0.25	21	46	14.8	
III	3	24.8	3.0	12	0.24	17	34	12.5	
IV	3	19.9	1.7	8	0.26	28	25	2.1	
I	5	33.5	4.5	14	0.10	21	67	9.1	
II	5	61.0	9.6	16	0.17	29	72	11.3	
III	5	32.2	3.8	13	0.18	14	36	4.2	
IV	5	25.3	3.3	13	0.26	15	19	2.5	
I	8	55.6	9.3	17	0.12	27	58	9.7	
II	8	78.0	13.6	17	0.20	29	49	8.5	
III	8	48.1	6.6	14	0.26	12	23	3.2	
IV	8	5.4	1.3	24	0.47	11	1	0.3	

Table 3

Synthesis of RNA, DNA, and Concentrations of ATP
The Data are from Experiment III

Bottle Number	Time (hrs.)	^{3}H-RNA Produced (nCi/l)	^{3}H-DNA Produced (nCi/l)	DNA/RNA (%)	ATP Pool Specific Activity (nCi/pmol)	ATP Concentration (ngram/l)	Rate of RNA Synthesis (pmol/l/hr)	Rate of DNA Synthesis (pmol/l/hr)	Mn(II) Added (ppb)
I	4	139.8	27.8	20	0.59	32	59	11.8	0
II	4	135.9	30.0	22	0.68	36	50	11.0	97
III	4	125.5	25.8	20	0.72	41	44	9.0	485
IV	4	137.0	25.0	18	0.87	23	39	7.2	1942
V	4	98.0	26.2	27	0.79	27	31	8.3	dark (0)
I	6	170.7	38.5	22	0.69	49	41	9.3	
II	6	182.2	43.9	24	0.72	51	42	10.2	
III	6	174.7	37.0	21	0.69	46	42	8.9	
IV	6	188.5	42.1	22	0.72	46	44	9.7	
V	6	128.0	42.2	33	0.67	41	32	10.5	
I	8	217.0	65.3	30	0.70	44	39	11.7	
II	8	220.8	65.0	29	0.77	36	36	10.6	
III	8	240.7	63.1	26	0.71	61	42	11.1	
IV	8	223.1	66.6	30	0.67	43	42	12.4	
V	8	163.3	60.4	37	0.74	39	28	10.2	

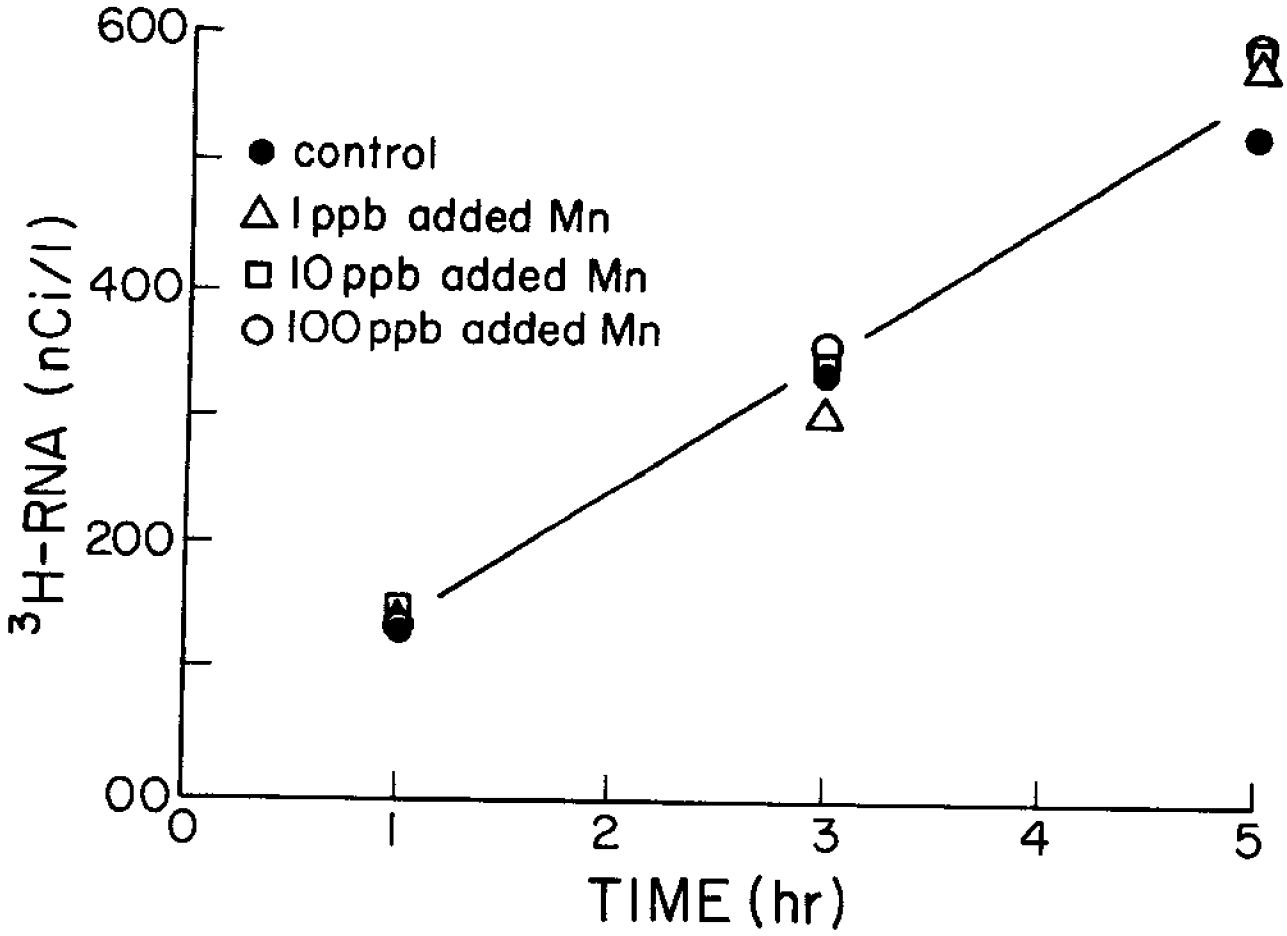


Figure 2. Total Microbial ^{3}H-RNA Production
Data from Experiment I

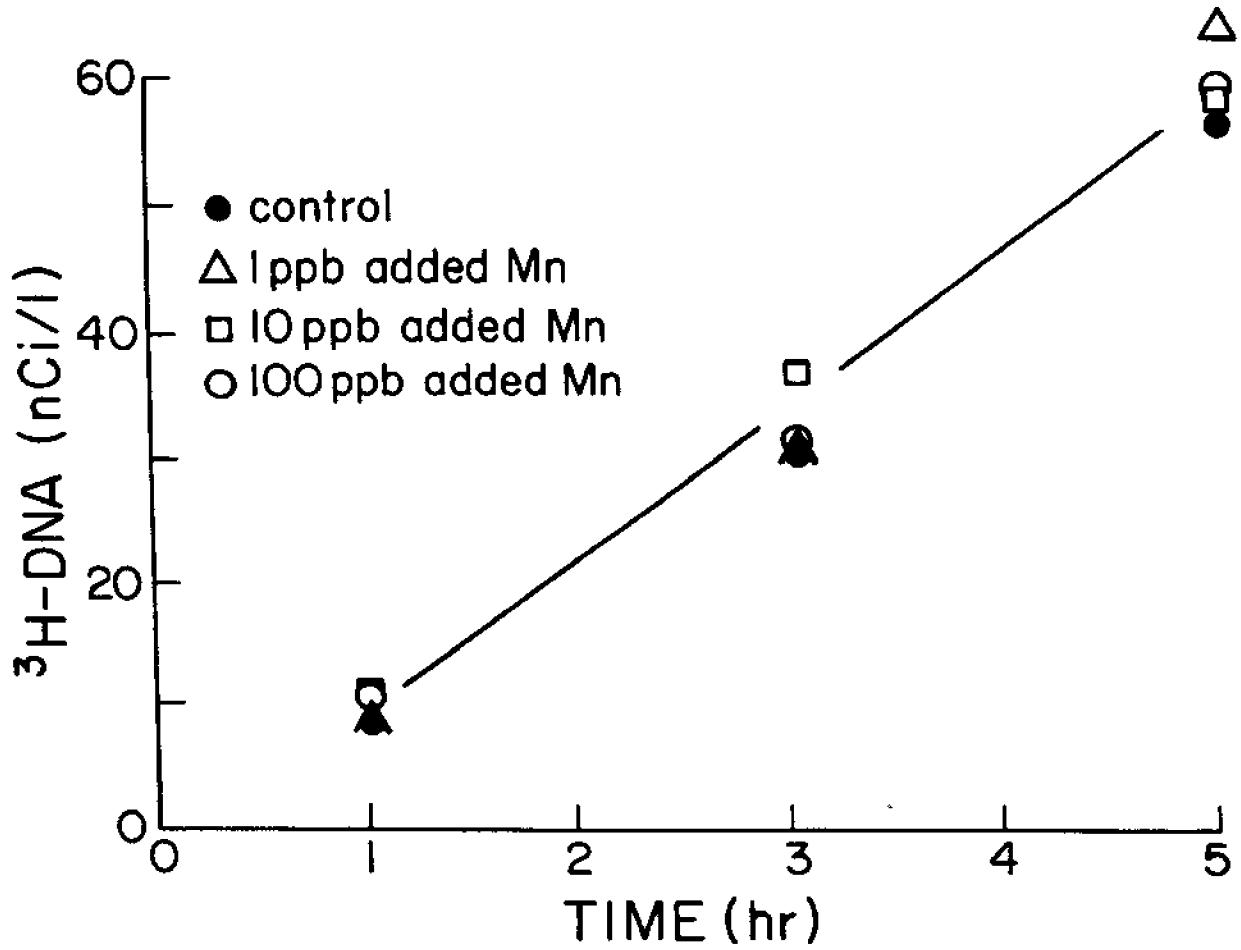


Figure 3. Total Microbial ^{3}H-DNA Production Data from Experiment II

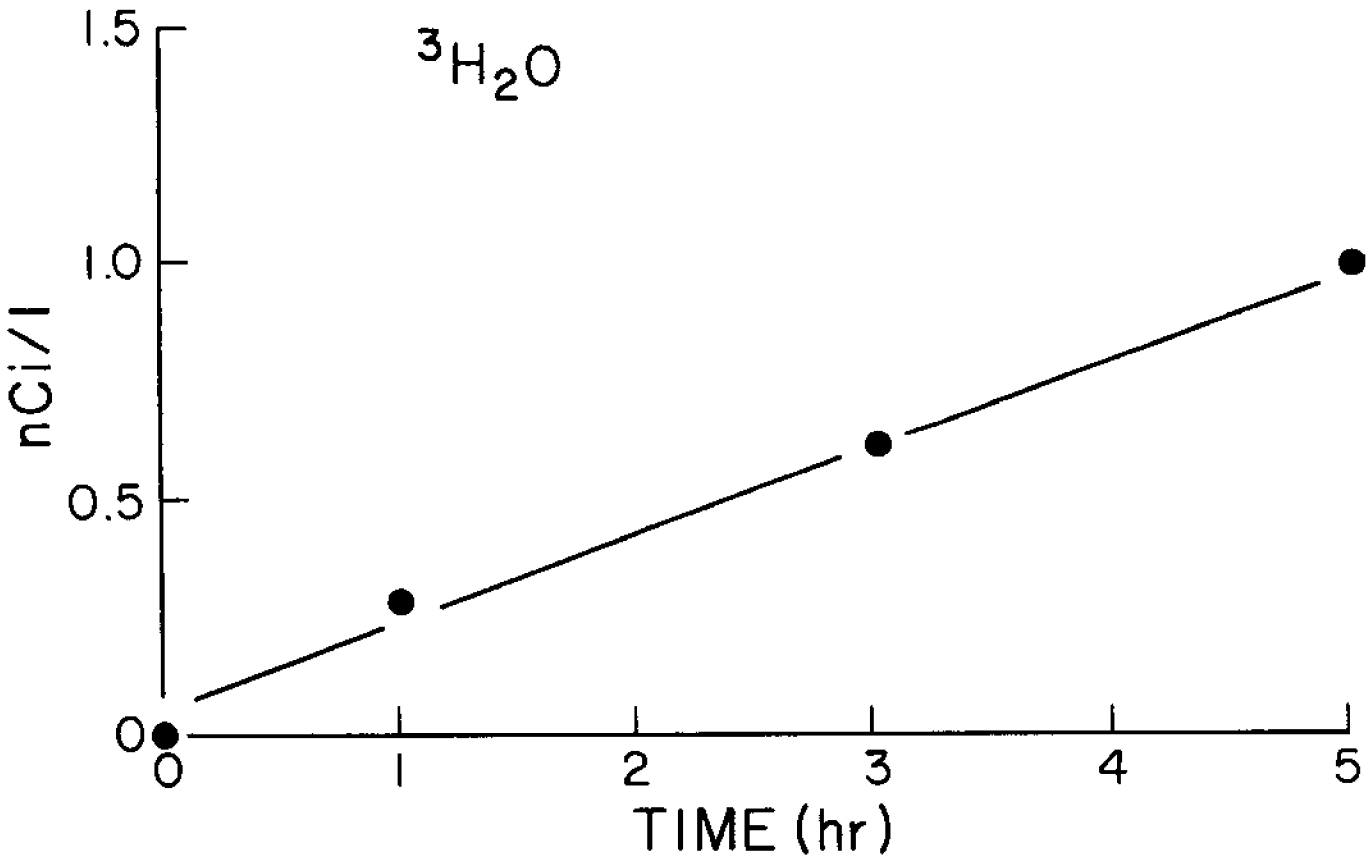


Figure 4. Mean Activity of All Bottles
Data from Experiment I

mCi/ℓ), whereas bottles in Experiments I and III contained 60-70 mCi/ℓ. This low level of activity during Experiment II caused problems with measurements of ^{3}H-ATP and ^{3}H-DNA. Measured counts from the purified ATP extracts and hydrolyzed DNA solutions were often so low (less than 100 and less than 1000 cpm respectively) as not to be considered reliable. Unfortunately, the duplicate RNA and DNA sample set was lost during sample processing. The specific activity and DNA data from Experiment II should therefore be interpreted with caution. Labeling of all samples proceeded rapidly during the period between adding radiotracer stock and the first time-course sampling. For the control samples (Tables 1-3, Bottle I), subsequent specific activities remained essentially constant. The specific activities of bottles with added manganese were more variable and tended to increase during the incubation such that by the last sampling period, values for the controls were generally lower than the Mn-treated replicates. The bottle with the highest Mn content increased dramatically in specific activity between five and eight hours after adding label.

There appears to be no significant pattern to the ATP values other than that they are low and typical of previous data from oceanic environs (Karl and Winn, 1984). Concentrations of ATP remain relatively constant during the first experiment, show a steady decrease at the higher Mn additions made during Experiment II, and in most cases, increase very slightly during the final experiment. No influence is observed on ATP until more than 1000 ppb Mn(II) is added.

Dual radiolabels were added during Experiment III to compare production based on autotrophic carbon fixation to total microbial growth estimates extrapolated from rates of DNA synthesis (Table 4). A linear regression of the three time-course DNA measurements (Table 3) yields an hourly rate of adenine incorporation into DNA per liter. This value is then multiplied by a factor of 6.62×10^{-2} to change the units to micrograms of carbon per liter per hour (μgC/ℓ/hr), and again by 24 to obtain a daily rate of community carbon production (ΔC). The ^{14}C-uptake data (in nCi/ℓ) are converted to carbon production by linear regression as above, divided by 4.4×10^{4} nCi/ℓ (the amount of ^{14}C label initially added) and then multiplied by 2.0×10^{-3} M, the natural concentration of inorganic carbon assumed to be present. Inorganic carbon addition from the label (8.4×10^{-7} M) is negligible in comparison to natural levels. The product is then multiplied by an isotopic discrimination factor of 1.05 (Strickland and Parsons, 1972) and units converted to μgC/ℓ/hr. No correction for respiratory loss was made. A factor of twelve was used to obtain daily ^{14}C-production (ΔC). For biomass estimates (C_0), the three corresponding ATP concentration measurements from Table 3 are averaged and

Table 4

Carbon Production and Community Specific Growth Rates
Based on ^{14}C-productivity and Rates of DNA Synthesis

Bottle Number	Mn^{+2} Added (ppb)	Total Primary+ Production (mgC/l/hr)	Specific+ Growth Rate (day^{-1})	Total Microbial* Production (mgC/l/hr)	Specific* Growth Rate (day^{-1})
I	0	0.57	0.50	0.76	1.01
II	97	0.53	0.48	0.67	0.94
III	485	0.98	0.67	0.88	1.00
IV	1942	0.36	0.38	1.17	1.39
V	dark (0)	----	----	0.80	1.15

+Extrapolations based on ^{14}C-uptake rates and ATP biomass data (see text)
*Extrapolations based on DNA synthesis rates and ATP biomass data (see text)

converted to carbon based on a C/ATP ratio of 250. Community specific specific growth rates (day^{-1}) are then calculated according to the formula:

$$\mu = \ln [(C_o + \Delta C)/C_o]$$

To obtain "doublings per day" (not listed), two is used as the logarithmic base.

Carbon production values for the control and for the least Mn addition (Table 4, Bottles I and II) are essentially the same over the course of Experiment III. Estimates based on DNA synthesis are generally about 130% greater than estimates based on ^{14}C-uptake. The bottle containing 485 ppb Mn(II), however, shows a higher rate of production based on ^{14}C-uptake than the rate based on DNA synthesis. For this bottle, ^{14}C-counts are significantly lower than control values at the four-hour sampling period and significantly higher after eight hours. Unlike the DNA data, no duplicate ^{14}C-samples were taken and the higher rates of production in this case may be an experimental artifact. In general, ^{14}C-productivity data show more deviation from the linear pattern characteristic of ^{3}H-adenine uptake into DNA over time. The largest Mn addition shows a decrease in autotrophic growth while total microbial production increases. Of particular interest are the dark production values which indicate that microbial production of reduced carbon based on extrapolations from DNA synthesis is the same as in the light while, at the same time, no inorganic carbon fixation occurs.

Sorption of Mn onto Suspended Calcite Sediment in Seawater

Measurements of the partitioning of ^{54}Mn in a system of seawater and fine calcite sediment show that dissolved Mn(II) has a high affinity for these particles. Previous literature has documented the particle-reactive behavior of aqueous manganese on surfaces such as siliceous clays, metal oxides and hydroxides, especially those rich in iron (Wilson, 1980). The distribution coefficients (Kd) obtained for calcium carbonate also show a strong pattern of adsorption, where:

$$Kd = \frac{\text{activity in solid phase}}{\text{activity in solution}} \times F$$

F is a factor that normalizes the measured ^{54}Mn-activities per unit volume to mass so that the units of Kd are dimensionless. The measured values of Kd obtained in the dark are shown in Figure 5. In the natural seawater treatment, dark sorption occurs more rapidly over the first hours of the

experiment. A period characterized by a more gradual linear uptake follows for approximately 5 days. At this point, the value of Kd is greater than 4×10^3 without any evidence to indicate that equilibrium has occurred. The same general pattern was observed for artificial seawater treatments except for an apparent lag in uptake.

A marked shift in the distribution coefficient occurs when the system is exposed to continuous artificial sunlight. The effect is most pronounced on the natural seawater treatment (Figure 6). Under light conditions, the value of Kd rises to just over 1.3×10^3 and remains fairly constant during the remainder of the experiment while after ten days in the dark, the value of Kd for the natural seawater system is over 4.4×10^3. A less pronounced but significant effect is also observed on dark vs. light treatments in the artificial seawater-calcite system (Figure 7). In both cases, values of Kd in the dark significantly exceed those obtained in the light.

After ten days in the dark, artificial and natural seawater suspensions adsorbed 43(±1.9)% and 41(±2.1)% of the ^{54}Mn-spike, respectively. Thus, there was essentially no difference between adsorption of divalent manganese from artificial and natural seawater onto suspended calcite sediment in the dark. For samples exposed to continuous illumination, however, adsorption of Mn(II) both from the natural seawater-calcite system and, to a lesser extent in the artificial seawater treatment, was inhibited. At the conclusion of the light experiment, only 16(±1.4)% of the initial ^{54}Mn-spike in natural seawater had been removed by adsorption while counts removed from the artificial seawater suspensions averaged 30(±3.4)%.

The salinity of Kaneohe Bay seawater averages 3.5% (Smith et al., 1981), compared to 3.43%. for the artificial seawater. Measured pH values were all between 8.2 and 8.3 and temperatures in the dark averaged 3°C less than in the light treatments that were maintained at 25°C. Both seawater samples were analyzed for total dissolved organic carbon (DOC) after equilibration with peroxide-cleaned reagent calcite but before addition of the ^{54}Mn-spike. The seawater from Kaneohe Bay contained 0.64(±0.1)mgC/ℓ and the artificial seawater contained 0.23(±0.1) milligrams-carbon per liter (mgC/ℓ).

Dispersion Model

Figure 8 represents an essentially steady-state dispersion plume generated using the finite-element algorithm described above. In this run, a time increment of 1.5 minutes was used for 85 iterations (total duration 21.25 hr). Runs of 600 iterations produced virtually identical results.

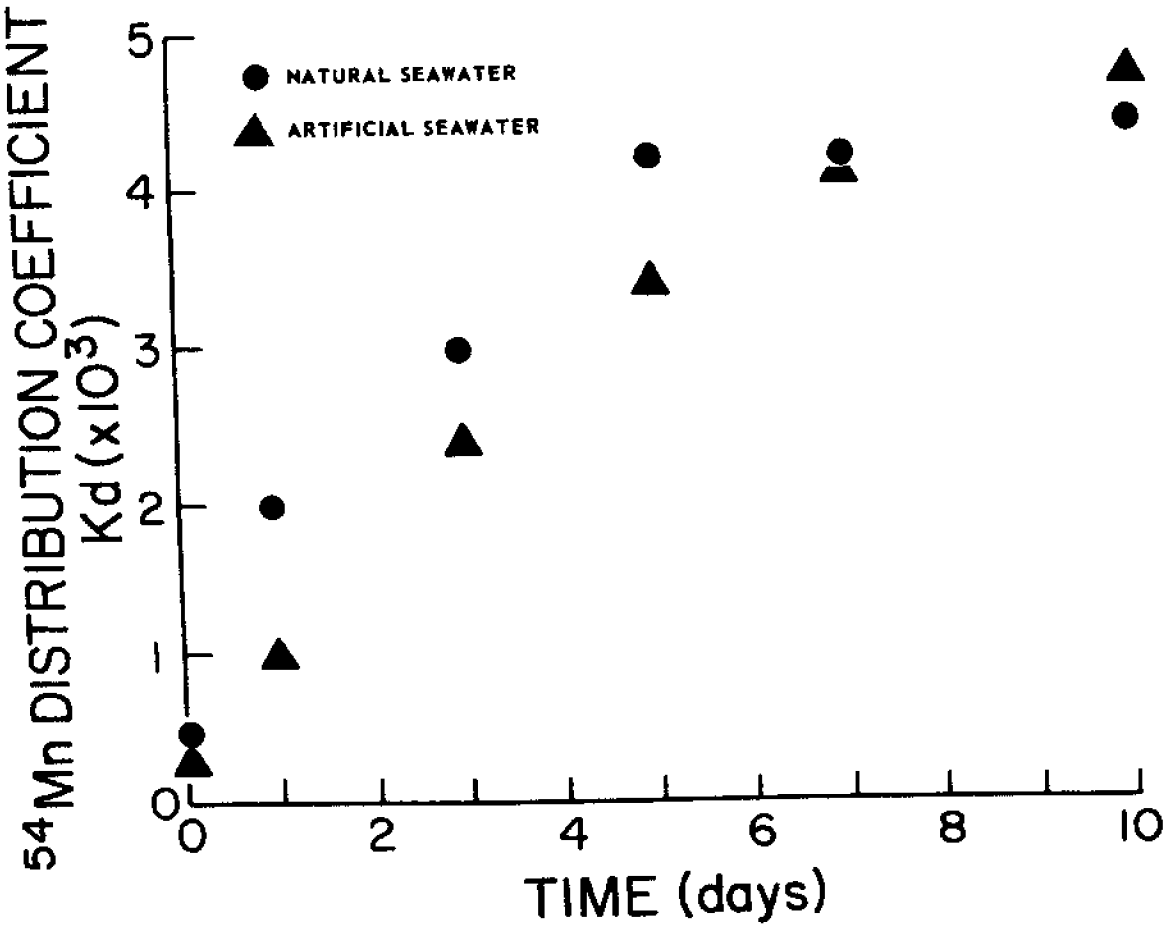


Figure 5. Partitioning of Manganese in Suspensions of Calcite Sediment with Natural Seawater and with Artificial Seawater Kept in the Dark

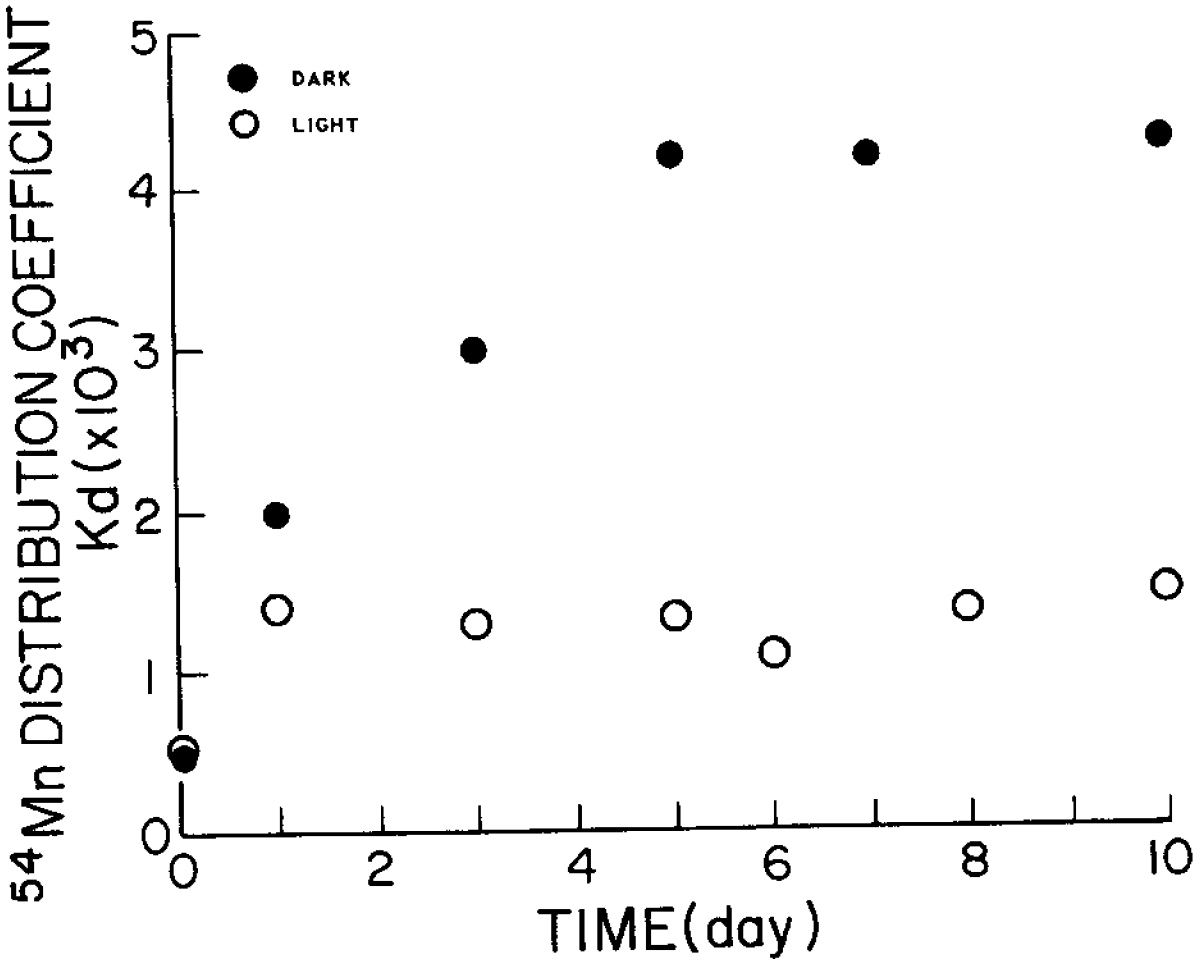


Figure 6. Partitioning of Manganese in Natural Seawater-Calcite Suspensions in the Dark and with Artificial Sunlight

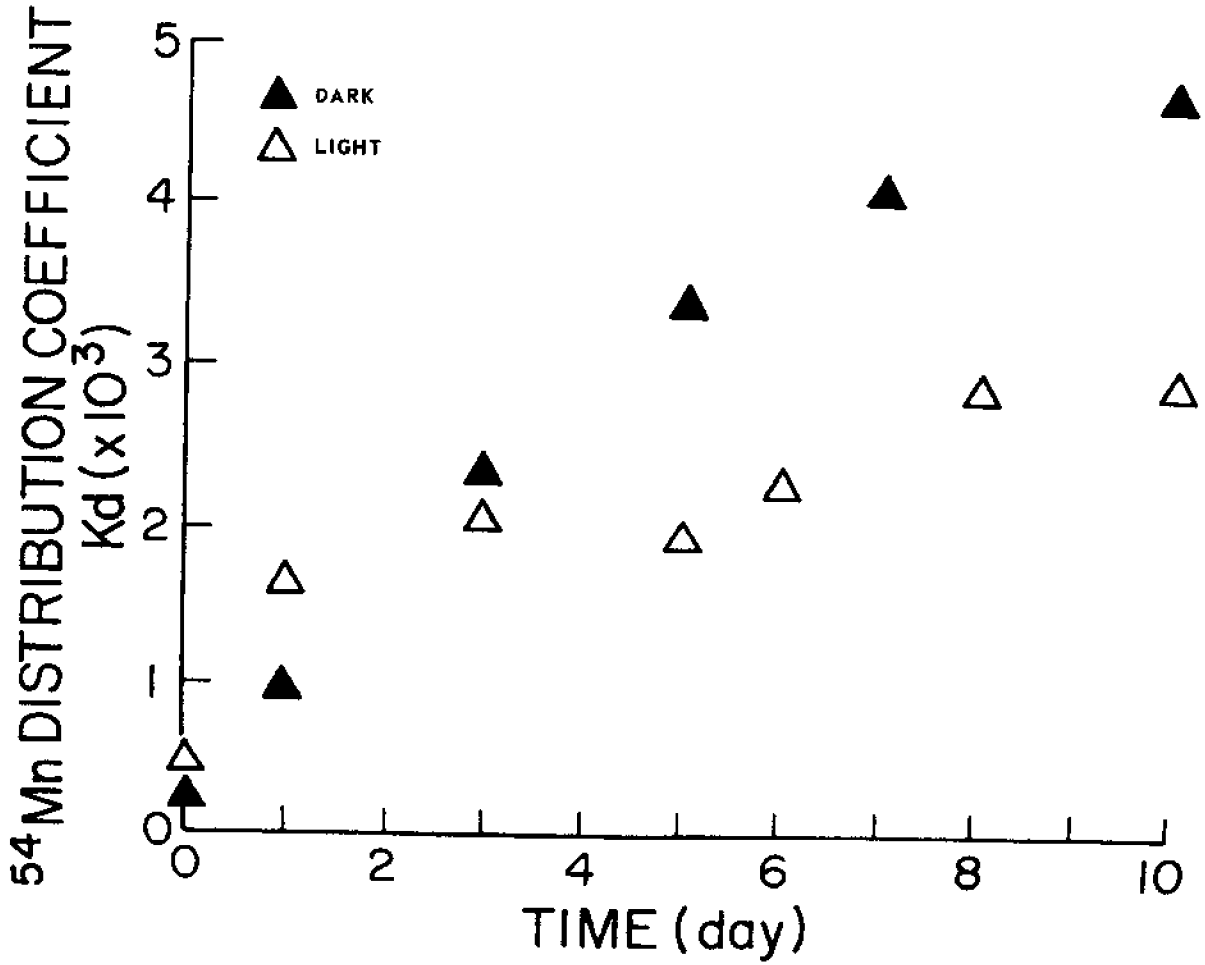
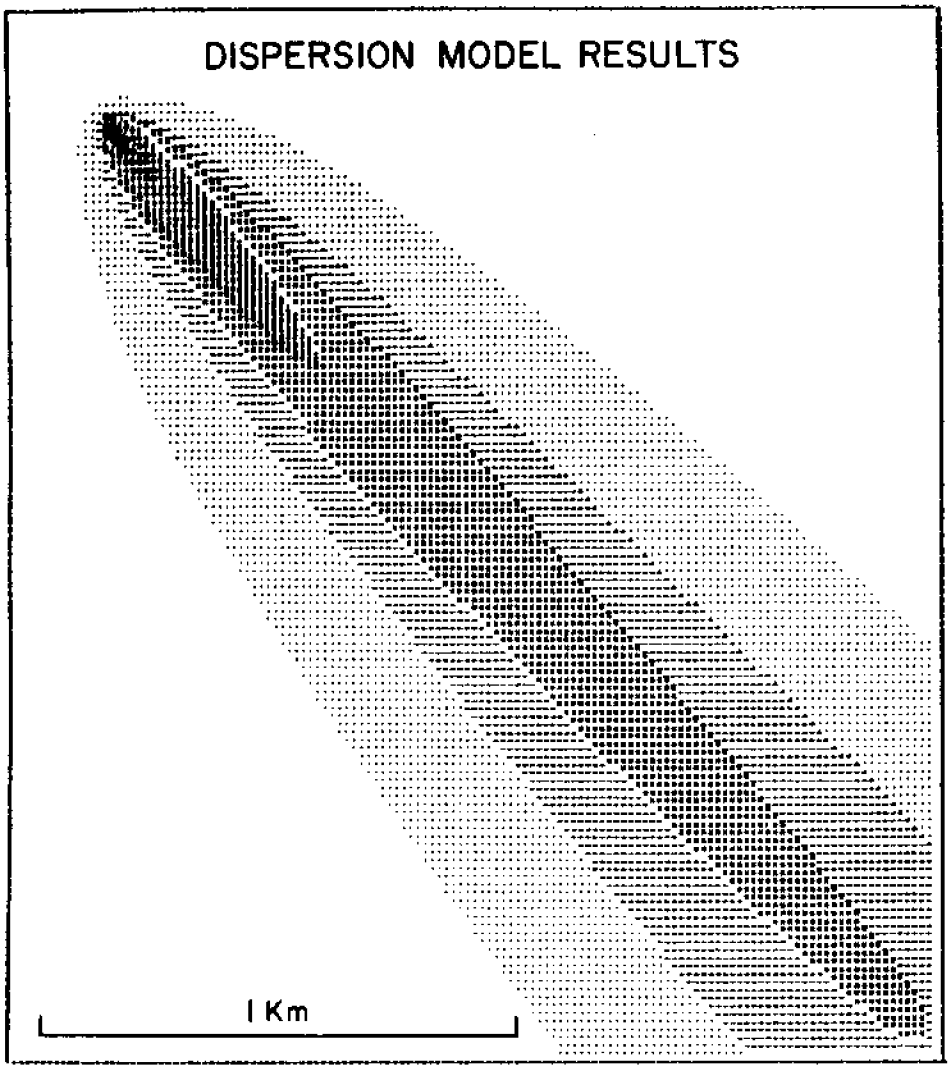


Figure 7. Partitioning of Manganese in Artificial Seawater-Calcite Suspensions in the Dark and with Artificial Sunlight



LEGEND

blank=0

· = < 100 ppb

- = < 396

" = < 792

: = < 1190

+ = < 1580

= = < 1980

/ = < 2380

o = < 2770

• = < 3170

= < 3560

■ = ≥ 3560

Figure 8. Dispersion Model Results

The discharge point in this figure is in the upper left-hand corner and the 0.1 m/sec current moves diagonally toward the lower right-hand corner. There is a slight elongation of the plot in the y-direction (up-down) due to a limitation on the lineprinter pitch and line-spacing controls. The ratio of the x to y increments in the plot is 0.898.

DISCUSSION AND CONCLUSIONS

Franklin and Morse (1983) measured the adsorptive uptake of manganous ions onto pure calcite surfaces at concentrations of Mn(II) from 10^{-5} to 10^{-9} M in distilled water and seawater at saturation with respect to $CaCO_3$. They determined that variations in initial Mn(II) concentrations did not affect the percent of Mn(II) adsorbed over time. Concentrations of adsorbate ranged from 2.5 to 10 grams of $CaCO_3$ per liter of suspension, much more than the 150 mg/ℓ calcite sediment used in this study. Nevertheless, the data are closely comparable. Such agreement supports the conclusion that the percentage of manganese removed from seawater is independent of the initial Mn(II) concentration (approximately 10^{-7} M in this study) and of the amount of solid calcite added over at least two orders of magnitude. Adsorption in seawater differs from Mn-uptake in distilled water in that the latter adsorption isotherm is affected by the ratio of adsorbate to solution volume (McBride, 1979). A close similarity in adsorption characteristics between reagent calcite and pelagic carbonate sediment is also recognized.

Adsorption of Mn(II) onto the mineral surface presumably occurs by coordination bonding between the metal ion and surface hydroxyl ligands. A study by Wilson (1980) determined that a variety of surfaces, including α-FeOOH, particulate organic matter and siliceous clays, all catalyzed the oxidation of manganese in natural waters to some extent. Surface complexation purportedly satisfies the electronic (valence) requirements of a metastable oxy-hydroxide intermediate species (perhaps MnOOH) that, under favorable conditions, may undergo further oxidation. Alternatively, Mn(II) may substitute for Ca(II) in the rhombohedral calcite lattice. Solid solutions of Mn-$CaCO_3$ are known to occur during co-precipitation of these species in seawater (Garrels and Christ, 1965; Pedersen and Price, 1982). These two mechanisms are not mutually exclusive, however, and probably occur simultaneously. In any case, competition with Mg(II) for available bonding sites slows the reaction in seawater by factor of 4×10^3 compared to adsorption rates measured in distilled water (Franklin and Morse, 1983).

An initial period of relatively rapid uptake was observed in the present study. It is assumed that this represents adsorption of Mn(II) onto the surface of the suspended sediment. In natural waters below pH 8.5, the kinetics of manganese oxidation (equation 1) are too slow to account for the observed uptake rate (Stumm and Morgan, 1981). In the dark, this adsorption phase lasted approximately five days and resulted in the removal of 40% of the ^{54}Mn-spike. Illuminated samples adsorbed only about 20%, although this varied between artificial and natural seawater treatments (see RESULTS) and the duration of the first phase was approximately one day in the light

treatments. Thereafter, values of Kd in all cases increased very much more slowly. A reduction in the reaction rate may be due to precipitation of manganese oxides (equations 1 and 3) after initial adsorption of Mn(II) onto the mineral surfaces. This second phase lasted throughout the remainder of the experiment and removed about 1% of the initial spike per day.

Similar work with dilute solutions of Mn(II) and calcite (McBride, 1979) suggested that the system may take on different solubility characteristics with respect to both manganese and calcium carbonate depending on the relative proportions of aqueous manganese and calcite surface area. If the latter greatly predominates, Mn(II) concentrations may fall below values calculated from pure mineral equilibria. When Mn(II) concentrations are high relative to calcite surface area, the surface bonding sites, once occupied, become coated with the forming oxide and are thus effectively isolated from the system. Once all the calcite crystals are coated in this manner, the system may become under- or over-saturated with respect to $CaCO_3$. Surface complexation with organic matter allows the latter condition to exist in most surface seawater (Chave, 1965; Chave and Suess, 1970). Ultimately, the amount of Mn(II) that remains in solution is controlled, in part, by the solution chemistry of the incipient mineral phase. Other physico-chemical variables affecting manganese solubility and oxidation rate are temperature, pressure, pH and Po_2. The data presented in this study indicate that light may also play a significant role in determining the partitioning of manganese in the photic zone.

An enormous amount of energy flux in the form of electromagnetic radiation enters the ocean through the air-sea interface and is more than 90% absorbed within the photic zone. This energy interacts with aqueous molecules and is converted to other forms of energy such as heat. Individual radiation quanta possess energies in excess of the activation energies required for most relevant chemical reactions (Zafiriou, 1977). For example, photosynthesis, the most extensively studied photochemical process in the sea, has had a profound effect on the biology and chemistry of the biosphere and yet photosynthesis involves only a miniscule fraction of organic matter that is living. The ocean contains vast quantities of dissolved organic and inorganic molecules capable of interacting with light. Despite the obvious potential significance of photochemistry in the marine environment, however, the current literature contains no examples (aside from photosynthesis) of well-understood organic photochemical systems. Even rudimentary knowledge of the spatial and temporal variability of the marine light field (especially in the ultraviolet) is lacking, although interest in remote sensing may soon change this situation. As a necessary prerequisite to the interpretation of satellite data, the optical properties of seawater

are becoming better understood with the aid of computer modeling. Algorithms thus derived are currently employed to relate multiband spectral data to important biological parameters such as surface chlorophyll concentration (Wilson et al., 1978; Wilson and Kiefer, 1979). Compounding the problem is the nature and structure of marine DOC which has yet to be resolved in any rigorous chemical way. These compounds are often termed "Gelbstoffe" or "humic substances", a term borrowed from soil science, and are known collectively by their presumed effects rather than by quantitative scientific investigation. Therefore, because of the inadequacies in our picture of the ocean as a photochemical system, and because the field of marine photochemistry is just beginning to emerge from infancy, much of this discussion will be of necessity, speculative.

The process of photochemistry begins when a quantum of sunlight radiation interacts with the vibrational electronic system of the receptor atom, ion or molecule (hereafter referred to as a chromophore). Chromophores are chemical entities whose quantized electronic energy levels are so configured that, under favorable conditions, adsorption of photon energy can occur. This process results in excitation of one of the bound electrons to a higher energy state. Although ionization can occur, the initial products of light absorption, in general, are electronically excited molecules. One characteristic of photo-excitation is that it is a selective process active only within a narrow range of wavelengths that depend on the nature and structure of the reactant. The electrochemical nature of aqueous chromophores, however, is poorly described. In seawater, H_2O, DOC and both living and detrital POC are believed to be the principal chromophores (Zafiriou, 1977). Most simple organic constituents of seawater that have been isolated (Williams, 1975) are not good absorbers of light but the high reactivity of efficient chromophores would tend to keep their concentrations low, perhaps below our present ability to detect them. Vitamin B_{12} is one example of a marine organometallic complex that is a good absorber and rapidly degrades in seawater exposed to sunlight (Carlucci et al., 1969).

A variety of primary photochemical reactions may dissipate or transfer the energy from the excited chromophore. For a net chemical change to occur in the system, excitation followed by primary and/or secondary photochemical reactions must be operative. The energy may be transferred to another chromophore, dissipated as heat (intra-chromophore degradation), released as luminescence (fluorescence or phosphorescence) or transferred by chemical reactions of the electronically excited states and their dissociation products such as free radicals. The latter mechanism is often termed a secondary or "dark" reaction.

In the context of the present study, it appears that natural levels of dissolved organic matter initially serve to increase the dark adsorption rate of Mn(II) onto calcite surfaces (Figure 5). If adsorption kinetics generally follow a first-order heterogeneous and autocatalytic rate equation for manganese oxidation in natural waters (at constant pH and Po_2) as derived by Morgan (1967) of the form:

$$-\frac{d[Mn^{+2}]}{dt} = k_o[Mn^{+2}] + k_1[Mn^{+2}][MnOx]$$

where k_o is the first-order rate constant and k_1 the heterogeneous rate constant, then complexation between DOC and aqueous manganese, partially removing some of the free metal ion from the system, would tend to reduce the overall reaction rate. An inverse dependence of the reaction rate on organic ligand concentration was observed by Wilson (1980) when uncharacterized "humic acid" extracted from soils was added to suspensions of clay minerals in borate buffered solutions (pH 9) of low ionic strength (0.01 M). These results may not be valid when applied to seawater systems. It has been shown that calcite suspensions in seawater selectively adsorb surface-active organic compounds such as amino acids, proteins, lipids, fatty alcohols, esters and polypeptides (Suess, 1970). The method of calcite equilibration employed in the present study may therefore have removed as much as 14% of the organics from the seawater prior to adding ^{54}Mn (Suess, 1970). Loss of DOC is suggested by the relatively low concentration (0.64 parts per million (ppm) measured in the surface seawater from Kaneohe Bay following equilibration with cleaned calcite compared to typical values of 1-1.5 ppm (T. Walsh, personal communication). In this regard, when changing the quality or quantity of natural DOC is undesirable, a better method of achieving $CaCO_3$ saturation might be by alkalinity adjustment with HCl and atmospheric CO_2 (Franklin and Morse, 1983). It should also be noted that the artificial "organic-free" seawater used in this study did contain measurable amounts of DOC, presumably as contaminants inadvertently added along with the various inorganic reagents. The quality of these organic contaminants was not determined.

The data presented here indicate a direct relationship between organic matter and rate of adsorption. The evidence suggests that higher rates of adsorption from natural seawater are due to a greater abundance of organometallic complexes with strong affinity for calcite surfaces. The measured Kd's in both artificial and natural seawater treatments kept in the dark ultimately attained approximately the same value in both cases, the incipient mineral phases appear to have equivalent solubilities. This is interpreted as evidence that neither the nature nor the abundance of surface bonding sites was significantly altered relative to the potential for Mn(II)

adsorption in the artificial treatments. It is also possible that complexation takes place while manganese is in the solution phase (the Mn-spike was added 15-30 minutes prior to adding sediment).

In the light (Figures 6 and 7), a significant shift is observed in the values of Kd. The magnitude of the light effect appears to be related to DOC concentrations although the data are not sufficient to resolve the nature of the relationship. Adsorptive uptake is significantly inhibited in both natural and artificial treatments relative to parallel samples maintained in the dark. Sunda et al. (1983) and Sunda and Huntsman (1985) clearly demonstrate that exposure to sunlight greatly enhances the dissolution of manganese oxides in laboratory experiments performed with offshore and coastal near-surface seawater. Dissolution rates were found to increase in proportion to light intensity and with additions of isolated marine "humic acid". The data presented in the 1983 study support the hypothesis that photo-activation of organic compounds found in seawater results in reduction of manganese to the divalent state. Photochemical mechanisms for reduction of other transition metals such as Cu(II) and Fe(III) have been proposed (Langford et al., 1973; Miles and Brezonik, 1981). Possible mechanisms of photo-activated reduction may include ligand-to-metal charge transfer within a photo-excited organometallic complex (Balzani and Carassiti, 1970) or perhaps indirectly by means of redox reactions between the transition metal and the dissociation products of the initial excitation such as free radicals (Zafiriou, 1977) or solvated electrons (Swallow, 1969). From the data presently available, it cannot be determined whether these reactions occur at the calcite-seawater interface or in solution.

Manganese enters the surface ocean from riverine influx (Turekian, 1971; Gibbs, 1977) by atmospheric fallout (Klinkhammer and Bender, 1980) and hydrothermal inputs (Jones and Murray, 1985). The relative contributions of these sources are still in debate (Landing and Bruland, 1980; Jones and Murray, 1985). The atmospheric input is significant, especially in locations remote from land (Klinkhammer and Bender, 1980). Hodge and others (1978) have shown that perhaps 50% of the manganese associated with aeolian particles is in a labile form that readily leaches into seawater as Mn(II). Divalent manganese is removed from the photic zone by relatively rapid adsorption onto particles of all kinds followed by much slower oxidation as they transit the water column. Where a well-developed oxygen minimum is encountered, remobilization may occur. The relatively high particulate flux characteristic of surface waters would be expected to reduce Mn(II) concentrations were it not for the presence of light and DOC that appear to interact in such a way that adsorption is inhibited while dissolution is

enhanced (Sunda et al., 1983). A balance among these principal sources and sinks serves to maintain the relatively high concentrations of dissolved manganese ubiquitously measured within the photic zone.

Future actions by man may perturb this dynamic system. If seafloor mineral deposits are mined and processed for their economically and strategically important metals, wastes high in dissolved and particulate manganese could be created (Haynes and Law, 1982). Disposal scenarios for these wastes could include ocean dumping (Keith, 1979). Substantial deposits are known to exist near the Hawaiian Archipelago (Craig et al., 1982; Wenzel, 1987; U.S. Dept of the Interior, 1987). If waste slurries of processing plant tailings are simply pumped into oceanic surface waters, Mn(II) concentrations might become significantly enriched, perhaps long enough to inhibit the growth and production of natural phytoplankton and bacterial populations. It was therefore considered worthwhile to investigate the toxic effects of elevated concentrations of divalent manganese on aquatic microbial communities endemic to Hawaiian waters.

In critically evaluating these results, it should be noted that the amount of Mn(II) added to each productivity bottle was determined by standard methods of flame-atomic absorption spectrophotometry on the concentrated $MnCl_2$ stock solution and then corrected by a volumetric dilution factor (see MATERIALS AND METHODS) to yield the concentrations shown in Tables 1-4. The effective activity of divalent manganese actually present in the bottle solutions was not directly measured but was undoubtedly less than the concentrations listed. Turner et al. (1981) calculated that 58% of the total aqueous manganese in model seawater exists as the uncomplexed manganous ion. Measured total activity coefficients for Mn(II) in seawater average approximately 0.25 (Klinkhammer, 1980; Morgan, 1967).

The stock was also analyzed for possible Cu, Ni, and Fe impurities. Nickel and iron were not detected by graphite furnace AAS (<1 ppb). A very small amount of copper (≈ 5 ppb) may have been present in the stock but the effects would be negligible after diluting to the concentrations actually present during the incubation. In addition, Ortner et al. (1983) and Stauber and Florence (1985) observed that elevated concentrations of Mn(II) ameliorate copper toxicity at low copper concentrations.

The productivity data (Tables 1-4) do not clearly delineate a discrete level at which Mn(II) becomes inhibitory. The data appear to indicate that concentrations of Mn(II) less than 500 ppb (9.1×10^{-6} M) do not adversely affect total production of the microbial communities sampled in Hawaiian waters, however, exposure periods of several hours to Mn(II) concentrations above 2000 ppb (3.6×10^{-5} M) may inhibit community growth

as measured in this study.

Because of the low ^3H-activity in the only experiment (II) that clearly indicates Mn-toxicity and the lack of duplicate samples in this case, these data provide only an approximation of manganese toxicity in the sea. Future investigations should be undertaken in an effort to determine *in situ* levels of divalent manganese that significantly inhibit marine microbial growth.

The dispersion model used to produce Figure 8 has an initial concentration of 7090 ppb Mn(II), determined by the 15 m matrix element size, the fixed input flux (266 g/sec) and the 1.5 minute time increment. In a real discharge situation, this initial concentration would be carefully controlled by pre-dilution and proper discharge configuration. If the waste stream consists only of process water with no pre-discharge dilution (5.4×10^6 metric tons/year; Harvey and Ammann, 1987, p. 205), the entire outfall flux would be 0.17 m^3/sec, and the initial dilution assumed with this particular set of model assumptions would be about 1:220. This is a conservative estimate for initial dilution, given the vigorous mixing processes found in most marine surface waters. More sophisticated means of estimating initial dilution (cf. Brandsma and Divoky, 1976) in similar situations characteristically produce similar or higher initial dilutions.

If the above model is a reasonable estimate of a real discharge, it implies the following:

1. Surface biota drifting with the current along the axis of the discharge plume will be exposed to Mn(II) levels above the inhibitory level of 5000 ppb for less than 5 minutes.
2. Surface biota drifting with the current along the axis of the discharge plume will be exposed to Mn(II) concentrations above the 2000 ppb level for about 30 minutes.
3. These drifting organisms will be in the intermediate zone between 2000 and 500 ppb for about 380 minutes (6.4 hours).
4. The total surface area in the ocean subject to concentrations of Mn(II) in the intermediate zone or greater would be 0.4 km^2.

Thus, based upon these first-order estimates, it appears that the near-field, short-term effects of Mn(II) from a ferromanganese crust metallurgical processing plant would be virtually insignificant. Much work remains to be completed, however, before long-term chronic effects can be evaluated confidently and before an adequate biogeochemical explanation of Mn(II) behavior in surface waters is achieved.

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