RELATIONSHIP OF CERTAIN MACROSCOPIC MARINE ALGAE TO ZN^{65}

By

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	vi
CHAPTER	
I. INTRODUCTION	1
II. MARINE ALGAE -- THE SEAWEEDS	4
III. SCOPE OF PROBLEM	12
IV. LITERATURE REVIEW	21
V. EXPERIMENTAL PROCEDURE	40
VI. RESULTS AND DISCUSSION	54
VII. SUMMARY AND CONCLUSIONS	102
APPENDICES	110
BIBLIOGRAPHY	158
BIOGRAPHICAL SKETCH	165

LIST OF TABLES

Table		Page
1.	SPECIFIC ACTIVITY OF ZN^{65} CONCENTRATED BY SPHACELARIA . . .	55
2.	SELF-ABSORPTION CORRECTION FACTORS FOR ZN^{65} IN SEA WATER .	56
3.	THE APPROXIMATE MAXIMUM CONCENTRATION FACTORS FOR ZN^{65} TAKEN UP BY THREE MARINE ALGAE UNDER VARIOUS CONDITIONS OF LIGHT AND TEMPERATURE	68
4.	COMPARISONS OF THE APPROXIMATE MAXIMUM CONCENTRATION FACTORS OF THE EQUIVALENT ALGA TO THOSE OF THE ACTUAL ALGA	86
5.	COMPARISONS OF THE APPROXIMATE MAXIMUM CONCENTRATION FACTORS AS DERIVED FROM ORIGINAL AND REPLICATED UPTAKE EXPERIMENTS	87
6.	COMPARISONS OF THE APPROXIMATE DILUTION FACTORS OF THE MARINE ALGAE AS DETERMINED BY THE CONCENTRATION FACTOR EQUIVALENT VOLUME AND BY THE RATIO OF INITIAL AND FINAL WATER ACTIVITY	91
7.	COMPARISONS OF ASSAYED ALGAL CONCENTRATION FACTORS IN MEDIUM OF HIGHER ACTIVITY AS COMPARED TO REFINED CONCENTRATION FACTORS IN MEDIUM OF ABOUT 25 COUNTS PER MINUTE PER MILLILITER	96
8.	ACTIVITY OF SPECIFIC PORTIONS OF GRACILARIA FOLIIFERA USED IN THE PREPARATION OF THE AUTORADIOGRAPHS	101
9.	GRACILARIA UPTAKE DATA AT 25°C	117
10.	GRACILARIA UPTAKE DATA AT 18°C	120
11.	GRACILARIA UPTAKE DATA AT 5°C	124
12.	ENTEROMORPHA UPTAKE DATA AT 25°C	128
13.	ENTEROMORPHA UPTAKE DATA AT 18°C	131
14.	ENTEROMORPHA UPTAKE DATA AT 5°C	134
15.	SPHACELARIA UPTAKE DATA AT 25°C	138

Table Page

16. SPHACELARIA UPTAKE DATA AT 18°C 142

17. SPHACELARIA UPTAKE DATA AT 5°C 146

18. GROWTH RATE DATA FOR ENTEROMORPHA 151

19. GROWTH RATE DATA FOR SPHACELARIA 152

20. RETENTION DATA FOR SPHACELARIA AT 18°C IN PLAIN SEA WATER . 156

21. RETENTION DATA FOR SPHACELARIA AT 18°C IN ZINC ENRICHED SEA WATER 157

LIST OF FIGURES

Figure		Page
1.	SCHEMATIC FOR THE ARRANGEMENT OF AQUARIA, LIGHT SOURCE, AND ALGAE FOR A GIVEN UPTAKE EXPERIMENT	43
2.	ARRANGEMENT OF GLASS AQUARIA AND APPURTENANCES IN THE WATER RECIRCULATION SYSTEM FOR THE UPTAKE EXPERIMENTS	44
3.	ARRANGEMENT OF BEAKERS AND ALGA IN TRANSLOCATION EXPERIMENT	51
4.	THE SELF-ABSORPTION EFFECT AND CORRECTION CURVES FOR SEA WATER OF APPROXIMATELY 35 $^{o}/_{oo}$ SALINITY FOR THREE CONCENTRATIONS OF ZN^{65}	57
5.	CONCENTRATION FACTORS FOR GRACILARIA FOLIIFERA IN ZN^{65}-DOSED SEA WATER UNDER VARIOUS LIGHTING CONDITIONS AT 25°C	64
6.	CONCENTRATION FACTORS FOR GRACILARIA FOLIIFERA IN ZN^{65}-DOSED SEA WATER UNDER VARIOUS LIGHTING CONDITIONS AT 18°C	65
7.	CONCENTRATION FACTORS FOR GRACILARIA FOLIIFERA IN ZN^{65}-DOSED SEA WATER UNDER VARIOUS LIGHTING CONDITIONS AT 5°C	66
8.	THE APPROXIMATE MAXIMUM CONCENTRATION FACTORS OF GRACILARIA FOLIIFERA FOR ZN^{65} IN DOSED SEA WATER UNDER DIFFERENT LIGHTING AND TEMPERATURE CONDITIONS	67
9.	CONCENTRATION FACTORS FOR ENTEROMORPHA PROLIFERA IN ZN^{65}-DOSED SEA WATER UNDER VARIOUS LIGHTING CONDITIONS AT 25°C	70
10.	CONCENTRATION FACTORS FOR ENTEROMORPHA PROLIFERA IN ZN^{65}-DOSED SEA WATER UNDER VARIOUS LIGHTING CONDITIONS AT 18°C	71
11.	CONCENTRATION FACTORS FOR ENTEROMORPHA PROLIFERA IN ZN^{65}-DOSED SEA WATER UNDER VARIOUS LIGHTING CONDITIONS AT 5°C	72
12.	THE APPROXIMATE MAXIMUM CONCENTRATION FACTORS OF ENTEROMORPHA PROLIFERA FOR ZN^{65} IN DOSED SEA WATER UNDER DIFFERENT LIGHTING AND TEMPERATURE CONDITIONS	73
13.	CONCENTRATION FACTORS FOR SPHACELARIA SP. IN ZN^{65}-DOSED SEA WATER UNDER VARIOUS LIGHTING CONDITIONS AT 25°C	75
14.	CONCENTRATION FACTORS FOR SPHACELARIA SP. IN ZN^{65}-DOSED SEA WATER UNDER VARIOUS LIGHTING CONDITIONS AT 18°C	76

Figure Page

15. CONCENTRATION FACTORS FOR SPHACELARIA SP. IN ZN^{65}-DOSED SEA WATER UNDER VARIOUS LIGHTING CONDITIONS AT 5°C 77

16. THE APPROXIMATE MAXIMUM CONCENTRATION FACTORS OF SPHACELARIA SP. FOR ZN^{65} IN DOSED SEA WATER UNDER DIFFERENT LIGHTING AND TEMPERATURE CONDITIONS 78

17. GROWTH RATE CURVES FOR SPHACELARIA SP. AND ENTEROMORPHA PROLIFERA UNDER VARIOUS CONDITIONS OF LIGHTING, TEMPERATURE, AND MEDIUM ENRICHMENT 79

18. THE CONCENTRATION FACTORS FOR SPHACELARIA SP. AT 25°C IN ZN^{65}-DOSED SEA WATER ENRICHED WITH KCL 83

19. TYPICAL WORKING GRAPHS DEPICTING THE DETERMINATION OF THE CORRECTED-MEDIUM CURVE 89

20. RETENTION OF ZN^{65} BY SPHACELARIA SP. AT 18°C, UNDER 500 FOOT-CANDLES OF LIGHT, IN PLAIN SEA WATER AND IN SEA WATER ENRICHED WITH STABLE ZINC 93

21. RESOLUTION OF THE RETENTION CURVE FOR SPHACELARIA SP. AT 18°C, UNDER 500 FOOT-CANDLES OF LIGHT, AND IN PLAIN SEA WATER 94

22. RESOLUTION OF THE RETENTION CURVE FOR SPHACELARIA SP. AT 18°C, UNDER 500 FOOT-CANDLES OF LIGHT, AND IN SEA WATER ENRICHED WITH STABLE ZINC 97

23. AUTORADIOGRAPHS OF CONCENTRATION SITES FOR ZN^{65} TAKEN UP BY GRACILARIA FOLIIFERA UNDER 500 FOOT-CANDLES AT 18°C. LEFT ORGANISM WITH FRUITING BODIES, RIGHT WITHOUT FRUITING BODIES 99

CHAPTER I

INTRODUCTION

The discharge of industrial and domestic waste to a receiving body of water has long been the keen interest of sanitary engineers throughout the world. Deep concern over proper waste discharge practices is evidenced because water is of vital importance to the well being and comfort of man and, therefore, its conservation and its protection against waste contamination are highly desirable.

In some areas the desire for maintenance of clean water arises principally out of the need for the conservation of this invaluable resource, in other areas the aesthetic appearance and recreational uses of the receiving water are the motivating forces which protect it against man's pollution. It is only in a few instances, such as the discharge of highly poisonous cyanide wastes, where these receiving waters are protected for the sake of man's health.

Most streams and other bodies of water can adequately handle reasonable amounts of untreated wastes with no detrimental effect to the water. The process of self-purification in a stream, for example, is sufficient to prevent the occurrence of a polluted situation. This process is essentially one of a biological nature operating under optimum conditions for its successful completion. In self-purification, potential organic pollutants are reduced by the biologic activity to harmless, non-pollutant mineral matter. There are, however, wastes

such as radioactive ones which can be reduced to simple mineral matter by this process and yet be considered as highly undesirable pollutants. Man's abhorrence of these radioactive pollutants stems not from the usual reasons associated with the dislike of pollution in general, but primarily from the high potential health hazard offered by these wastes. This is the main reason for man's great concern in the treatment of radioactive wastes and in their proper discharge to the environment.

Radioactivity is a complex phenomenon which is generally harmful to the well being of most living entities. Its ionizing radiations react with matter and alter the natural life functions of living creatures thereby causing radioactive wastes to be undesired in the environment. Biologic activity, which has proved to be successful in reducing most other wastes to innocuous mineral matter, cannot alter the nature of radioactivity, nor has any other method been found to be effective in this regard. Basically, the only remedy to protect man from the deleterious effects of radioactivity is to remove the radioactive substances from his environment and to make certain that they will not return.

Increasing usage of radioactive isotopes in industry, research and hospitals, coupled with production of power by atomic energy, has augmented the quantity of radioactive wastes in the environment. Atomic power at present is not confined just to land installations, but is now operative on a few ocean-going ships and submarines. It will only be a question of time before both land and marine atomic installations will grow in number. The reality is present that waste discharges from atomic plants located on land near the ocean, together with the waste

discharges from marine power plants, can create in the coastal waters a potential health hazard to man (34, 37, 60).

It would not be wholly justified for man to set aside atomic energy and sacrifice all the potential benefits that are within his reach just because radioactive wastes might constitute a menace to his well being. This, of course, would be the most positive method in protecting the environment from radioactive sources, but it is considered that adequate protection can be achieved by careful, deliberate control in the discharge of radioactive wastes. Accordingly, it is the purpose of this study to ascertain the merits of one small aspect of this control as it pertains to man's closest marine environment -- the coastal waters (52).

The fact has long been known that plants, algae, and many microorganisms can take up and concentrate both radioactive and non-radioactive elements from their immediate aquatic environment thereby reducing the concentration of such elements in their surrounding water (72). Is it not possible then to apply the same principle to the ocean? Why cannot some organisms, for example macroscopic marine algae, take up radionuclides from the ambient water and thereby reduce the aquatic concentration of radioactivity to allowable limits? Heretofore, very few investigations of this potential control mechanism have ever been made. It is felt that there is some merit in the decontaminating potentialities of these macroscopic marine alage which could possibly not only afford protection to the coastal environment, but also increase the efficiency of the ocean as a radioactive waste disposal medium.

CHAPTER II

MARINE ALGAE -- THE SEAWEEDS

Marine algae are photosynthetic plants, some of which grow completely submersed in water, while others thrive with only partial submersion. The size of these plants varies widely from one species to another ranging from one-celled microscopic flagellates to giant multicellular kelp. Like terrestrial vegetation, the algae possess chlorophyll by which they are able to utilize the sun's energy to synthesize their food. The myriad chemical compounds ever-present in the oceans, are used by the algae in this process of photosynthesis. However, unlike spermatophytes the algae have no flowers, leaves, seeds, or roots (80, 36).

The plant kingdom, although highly ramified, consists of four primary divisions: Thallophyta, Bryophyta, Pteridophyta, and Spermatophyta. Only the Thallophyta and Spermatophyta divisions are represented in the sea with the algae belonging to the former (75). Although most algae usually require in their environment similar physical factors such as air, light, and moisture for existence, they differ in cell structure, reproductive processes, size, shape, color and habitat. Therefore, it is possible for many algae to look alike and yet belong to different genera, families, and even orders (28).

Marine algae have been generally classified, according to the color manifestations of their chromataphores, into four principal groups which are Chlorophyceae (green), Myxophyceae (blue-green), Rhodophyceae

(red), and Phaeophyceae (brown). There is also a nebulous fifth classification comprising the yellow-green algae which are a heterogeneous group. The yellow-green algae for the most part are floating or planktonic forms whereas the others, except for some of the blue-green algae, are primarily attached forms. The macroscopic forms of these attached algae, which bear a close physical resemblance to terrestrial plants, are collectively known as seaweeds and species of these were used in this study.

The growth of algae occurs chiefly in the littoral region of the ocean. The boundaries of this region extend continuously from the high tide level onshore to approximately the 200 meter depth offshore. This particular depth is generally accepted by scientists to approximate the position of the outer edge of the continental shelf. They also consider 200 meters to be the depth which separates the lighted portion of the sea from the dark regions. It therefore follows that this depth could conceivably represent the limiting extent to which photosynthetic organisms can survive. However, the depth through which sufficient light can penetrate in order to sustain the growth of attached plants (seaweeds) is approximately the 50 meter depth offshore. The stretch of ocean between this depth and the high tide level is known as the eulittoral or upper littoral zone (75). Since seaweeds often are both sessile and photosynthetic, they can only grow in a region which will afford both light and attachment. Such conditions are provided in the eulittoral regions and, only under special circumstances, on the high sea (49, 62).

As algae are photosynthetic organisms, the complete deletion of light at great ocean depths restricts the growth of algae to the shallow waters of the littoral regions which comprise about 2 per cent of the ocean floor (75). The depth to which sunlight can penetrate so as to be used effectively in plant photosynthesis usually varies from one oceanic area to another and also varies with the latitude. The lighted depth is greater in the low latitudes and less in the higher latitudes. In the northern seas the lower limit of the lighted depth is approximately 40 to 50 meters (28). In the warm seas, where there is usually less sediment in suspension, this depth ranges between 100 to 130 meters. In any event, the maximum depth at which algae seem able to live has been arbitrarily set at 200 meters (67).

The growth of seaweed in the littoral region exhibits a very general tendency towards a zonal distribution along the ocean floor. The sun-forms (primarily Chlorophyceae), grow in the upper portions of the eulittoral region, commonly known as the intertidal belt, and utilize strong light for their photosynthesis, while much of the shade-forms of seaweeds (mainly Rhodophyceae and Phaeophyceae), grow in the lower reaches of the littoral section where they utilize the weaker light in their photosynthesis.

High up on the intertidal belt, seaweeds grow in very shallow water or in no water at all except for water sprays they might receive by the action of waves or wind. Here, the green and blue-green algae thrive abundantly as all spectral light is available to them. As the sun's light penetrates into the deeper water, portions of its spectrum, the red, orange and yellow components, are filtered out by the water.

At this depth, the resultant light is efficiently utilized in photosynthesis by the brown algae and it is in this zone where the browns grow luxuriously. When after penetrating into still deeper water where all that is left of the sun's light is the blue component, the red algae appear in greater abundance with the obvious reduction of other forms. The filtering of the sunlight is a function of the turbulence of the surface water and also of the amount of materials in suspension. It is not wholly a function of depth. In other words, it is possible for red algae to thrive in turbid water only a few meters deep as well as in clear water many meters deep. In both situations, the resultant light reaching the red algae would be about the same.

The zonation of seaweeds is not clean cut but exists with much overlapping. This unique type of distribution is generally attributed to the principal accessory pigment contained in the algal chromataphores. There is, however, no absolute correlation between the color of the algae and the depths at which they live or of the intensity of the illumination they receive (75).

Members of the Chlorophyceae are found mainly in the warmer waters of the upper littoral zone, the depth of which extends from high tide level to approximately the 10 meter depth offshore. These algae thrive best in well lighted habitats and of all the marine algae, they are most closely related to the fresh water algae. The green algae vary in size from microscopic to large broad fronded (comparable to terrestrial leaves) specimens. The blue-green algae consist solely of small, poorly organized plants of both single and multicellular variety. The blue-greens are found in both fresh and brackish warmer waters and are the organisms which usually cause sliming of these waters.

Red algae vary in size from single-celled microscopic plants to filamentous, branching forms of broad fronds one or two meters long. The red seaweeds are widely distributed but are most abundant in temperate climates. Generally they occur in water ranging in depth from the intertidal zone to depths of 130 meters or more. The brown seaweeds are structurally more complex than the red and grow at shallower depths than the red. The browns are also widely distributed, being more abundant in the temperate and even cooler waters and vary in size from small filamentous forms to giant kelps. Both the browns and reds are found predominantly in the marine environment with only a few occurring in fresh water (75, 26).

In appearance, large marine algae grow very much like flowering plants. The marine organisms seem to grow out of the ground or out of whatever substratum to which they are attached. This illusion is created by the algae's root-like appendage -- the holdfast. It is by means of the holdfast that seaweeds attach themselves to rocks, sand, shells, and other substrata. The substratum is not exclusively inorganic since some algae grow while attached to animals, wood, and even to other algae. Different species of algae are found on different substrata but this is, for the most part, due to the variations in roughness, hardness, and other physical differences of the substrata and not to their chemical characteristics. The holdfast merely holds the seaweed to the substratum and is not an organ used to take in nourishment for the plant (67, 48). Nourishment is taken in by the seaweeds directly through their cell walls from the ambient waters. Algae attached to substrata do not move except to sway with currents, but a constant change in water provides

nutrients. Floating algal forms may not receive as much food because they move along with their food and do not have too much opportunity to make contact with other potential food as do the attached algae. It is for this reason that large size is attained only by the attached forms. The ocean currents also tend to stabilize the temperature, salinity, and pH of the ambient water and thereby tend to create a more favorable growth environment for the seaweed. As seaweeds can withstand large changes in these physical factors, it is not acutely essential that they be controlled within narrow limits (49).

The physical factors affecting sea water exhibit but small variations out in the open sea. Organisms which inhabit the open sea are so very much adapted to their surroundings, which for all practical purposes can be considered fairly constant, that they cannot adapt themselves to an ever changing environment. These organisms are, therefore, very nonresistant to their environment and readily succumb to significant changes. There is, however, a zone of the sea which experiences very large variations in its physical state and thereby gives rise to the propagation of the more highly resistant marine forms. This zone, previously mentioned, is the intertidal belt and is bounded by the extreme high and low tide levels.

The shore in the intertidal belt is alternately covered and uncovered by the ocean tides. Not only are the seaweeds which grow on this shore subjected to the tidal effects, but also to pooling effects. Along the shore and inlets algae may become isolated in pools of water which eventually could become highly saline by the evaporation of the water or become very dilute by rain or extremely warm by the sun and

perhaps even become very basic or acidic by the photosynthetic action of the algae. The effects of the tide upon the seaweeds is, therefore, to expose the plants to desiccation, to severe temperature change, and to wide variation in both salinity and pH. The seaweeds that thrive on this shore, therefore, must be very resistant to withstand desiccation, and extreme sudden changes of temperature, salinity, and hydrogen-ion concentration (28, 63).

Some species of Fucus, for example, can survive out of water for periods of 24-48 hours (49). Other seaweeds are known to live out of water for more than 15 consecutive days (38) and still others have been discovered which were able to withstand desiccation for such a long time that they were still alive even after becoming brittle and easily pulverizable. Such desiccation is tolerated only by the resistant littoral forms. As a matter of fact, some of these forms require desiccation in order to live, otherwise they will die (67, 2).

In conjunction with the temperature of the ocean water, it is fairly well established that the thermal variations are much greater for shallow water, i.e. the intertidal zone, than for the deeper water of the open sea. Temperature variation is, therefore, a function of depth. Not only does the temperature vary with depth, but it also varies from one geographical location to another. These temperature variations govern the distribution of algae both to species located in a certain geographical site and to vertical location in the ocean depths. Some algae are known to inhabit the higher levels of the ocean in the winter and migrate to lower depths in the summer when the surface and near surface water becomes warmer (67). The Fucales as well as many

other seaweeds common to the intertidal region have been known to withstand temperatures as low as -20°C and as high as 34°C (25).

Species of seaweeds thriving in the high tide level and which become exposed when the water is at low tide can tolerate salinity concentrations of 20 to 300 per cent of normal sea water for 24 hours. Seaweeds of the mid tide and lower levels can tolerate a salinity range of 20 to 200 per cent of normal sea water and 40 to 150 per cent respectively. These figures indicate that seaweeds living in the intertidal zone are able to withstand a wider range of salt concentration than those growing in deeper water (27).

With regard to the hydrogen-ion concentration, present data indicate that again the algae growing closest to the shore can tolerate the wider pH range. Some seaweeds can withstand pH as high as 10 while others thrive in pH as low as 5. During low tide, photosynthesis of algae may change the pH of the water to a value as high as 9.9. Generally, many algae can exist in pH ranging from 6.8 to 9.6. In contrast to this range, the pH in the open sea ranges from 8.1 to 8.3 and is strongly buffered (49).

CHAPTER III

SCOPE OF PROBLEM

Ever since the introduction of the atomic bomb approximately sixteen years ago, the peaceful uses of atomic energy have been slowly but surely growing. From its original use as a means to wreak destruction, atomic energy has steadfastly grown into a boon to man and is now not only operable to supply electrical power for domestic use but is also employed as the source of propelling power for both merchant and naval vessels (37, 60). As present applications of atomic power prove to be just as effective, if not more effective than conventional power based on fossil fuels, and as the economical cost of atomic energy production becomes comparable to that of conventional power production, then its use as the chief fuel in power plants will increase. As of this writing, no less than one hundred land-based power reactors have been built and are in operation or are being planned for construction (59). Within the next ten years an estimated three hundred nuclear marine vessels will be in operation (60).

In addition to the use of atomic energy for power, other uses are being made of it in such places as industry, research institutions, hospitals, and agriculture. These are only a few of the broad categories in which the usefulness of radioactivity is applied in one way or another. The uses are greatly varied and multiplied in the many-fold subclassifications contained within these broad categories. From all the

application of atomic energy as outlined above, there result many varied waste products which must be cared for properly. The amount of waste produced in the environment is staggering and extreme care and caution must be exercised in the disposal of these radioactive products because of their potential health hazard to both man and his environment.

It has been estimated (15) that by the year 2000, if 700,000 megawatts of installed heat capacity are in operation, there will result about 280 metric tons of fission products waste alone! This waste will be largely contained in some 50,000,000 gallons of high-level wastes per year. The volume of low level waste will no doubt be somewhat larger. The question arises then, can the environment safely consume this amount of waste or should the production of such wastes be stopped and hence sacrifice the untold potential benefits of atomic energy? Man has chosen to continue his peaceful uses of atomic energy and has planned to reduce the hazards afforded by the wastes with adequate treatment and disposal.

The methods of waste treatment and disposal are not many and are generally similar to the methods used in the treatment of ordinary chemical wastes (74). Of course, the most ideal form of waste treatment here would be to actually and positively stop the radioactivity. To date no such treatment is possible nor does it seem probable in the near future. Present treatments consist generally of putting the radioactive waste in a suitable form for either dilution in water or air, or long time storage whereby the natural decay factor of the waste will eventually bring the radioactivity to a harmless level. The available treatment methods now employed are:

1. Disposal by dilution including both liquid and chemical means are frequently used. Liquid dilution is merely the dilution of the radioactive waste by its mixing with a large volume of water (i.e. the ocean) or some other suitable liquid. Chemical dilution, however, involves the mixture of the radioactive isotope, whether it be a compound or element, with the stable isotope.
2. Some wastes lend themselves quite well to the process of evaporation. Here a large volume of waste can be concentrated into a small volume of slurry or sludge which can be stored, or buried either on land or in the sea.
3. Coagulation such as used in water treatment can be used to remove certain radioactive isotopes from the water. Here also, the resulting precipitate can be stored or buried.
4. Ion exchange resins are used in column type units through which the radioactive wastes are passed. The resins have the capacity to exchange a non-radioactive cation or anion for the corresponding radioactive ion in the waste. Sometimes the wastes are discharged to underground cribs from which they leach out gradually into the soils. Where the soil conditions are favorable, the radioactive contaminants will attach themselves to the soil and thereby become fixed.
5. Biological processes have been found to be quite effective in the removal of certain radioactive isotopes from a given waste. It has been ascertained that microscopic organisms have a very high capacity to concentrate certain isotopes whether radioactive or not. After concentration has occurred, the organisms can be collected and disposed.

6. Sand filtration has been used for some radioactive wastes. In most cases only limited success has resulted from this method. The chief mechanism of this process is the adsorption of the radioactive isotope on the sand grains. By appropriate chemical washes the adsorbed waste is then easily eluted from the sand grains. This small volume of wash solution is then further treated for ultimate disposal.

It can be seen from the above that treatment is essentially containment coupled with isolation from man's environment or dispersion so that the probability of return to man is extremely small.

Of the above treatments, ocean disposal of low level radioactive wastes is the major concern in this study. It has been reported that ocean disposal is not employed to any great extent, but there is no indication that it will not be. Wolman and Gorman (84) have stated that low level wastes can be rendered safe by the high dilution of the ocean. However, they further indicate that ocean disposal of high level wastes is not recommended because of the uncertainty of the effect in the ocean and its further use to man. Land disposal of radioactive waste is practiced to a greater extent than ocean disposal. Land disposal at the present time is adequate, but as atomic wastes increase, other disposal areas must be used. When that time comes, there is no doubt but that the oceans will be used for such disposal purposes. The ocean affords advantages uncommon to land disposal. For example, generally any land area used for such purposes cannot be used for anything else by man until the radioactive waste is rendered harmless. As a result, further use of such areas is no longer available to man.

On the other hand, the oceans are not used as much by man. Therefore, should portions of the ocean become contaminated by radioactive wastes, the inability to use these areas is of no great inconvenience to man. Since it seems problematical that the ocean will come into greater use for the disposal of radioactive wastes, it behooves man to find out the limitations of this vast water area. One small aspect pertinent to this sort of investigation is the relationship of macroscopic marine algae to radionuclides.

The ocean is a natural place for disposal of most any kind of waste. It has been frequently considered that the ocean affords limitless opportunities for waste disposal. The sea is one of the most attractive areas available to man for use as a receiving medium for his wastes. Its vast volume affords a very large dilution factor for most liquid wastes. Saddington and Templeton (64) have indicated that the oceanic dilution factor will range from 10,000:1 to 1,000,000:1 after 2 and 9-12 hours respectively. However, the high dilution was effected only when there existed the mixing action of the sea water as caused by wind movement. Usually, most liquid wastes will mix rapidly along horizontal planes of comparable viscosity and density and mix rather slowly in the vertical direction because of the stratifying variations of both viscosity and density (57).

Portions of the ocean seem aptly suited for waste disposal, especially in the deep oceanic trenches where the currents and the water turbulence might be negligible. Here then would be the most likely location for radioactive wastes to be stored and allowed to decay with no adverse affect upon man's environment.

In contrast to this optimistic view, oceanographers have discovered that all the ocean depths are not quiescent but are in some places alive with motion (76). In deep canyons, for example, mud flows occur which move at velocities from 15 to 50 miles per hour. The fate of wastes stored in such canyons cannot be too easily determined nor can the desired containment of the waste be assured (56).

Currently some low level wastes are fixed in dense concrete cylinders and blocks which are dropped into the ocean. It was commonly believed that these concrete containers penetrated the soft ocean floor and sunk deeply into the bottom ooze where the radioactive materials became isolated forever from man's environment. This conception for the most part is in error for the ocean floor is generally hard and covered with only a thin layer of ooze. Areas of thick ooze do exist but exist on the continental shelf near land and hence much too close to man's environment to be used as burial sites (57).

Along with the theory of quiescent trenches and thick bottom ooze, it has been suggested that the mixing of the colder waters at the ocean floor with the warmer waters near the surface would take about 1,500 years. Present evidence in some ocean areas indicate that a much shorter time, approximately 100 years, is required (56). Accordingly, waste placed on the ocean floor may rise to the surface and hence return to man long before it has decayed to a safe value. Right now this problem is small and of not too much concern. However, with the reliable knowledge that radioactive wastes to the marine environment will sharply increase, waste disposal to the sea will become a real problem. It becomes necessary then in ocean disposal of

radioactive wastes that the fate of these radioactive materials be known so that man can determine more accurately whether the wastes will be returned to his immediate environment by ocean currents and wave action washing ashore, whether the waste material will be carried to fishing banks and cause contamination among edible fish, or whether the waste will be sequestered on the ocean floor harmlessly out of contact with man (65). Therefore, precaution and controls must be employed in dispersing wastes even to the vast volumes of the ocean.

In addition to the ocean being purposely and specifically used as a waste disposal area for radioactive materials from land operations, it is also the area which receives the radioactive wastes directly from nuclear powered ocean vessels. Further, some rivers entering the ocean probably carry the discharged wastes from land based radioactive sources. Unlike the radioactive waste purposely buried at sea, these two sources of waste pose a greater threat to man because they are closer to his environment. Rivers entering the ocean will deposit most of their wastes in and around the estuarial area as well as in the areas along the shores and beaches. Nuclear powered vessels while on the high seas are of no immediate concern; however, when they are inshore and docked in harbors, the possibility of contaminating coastal waters becomes very high (58).

Due to the uncertainties of various dynamic physical factors of the ocean depths and to the increased production of radioactive wastes from nuclear powered vessels and land based installations, man's coastal environment in certain locations will be subject to the hazards of radioactivity.

It is entirely feasible that any dangerously high levels of radioactivity in these coastal areas could be detected and decontaminated by certain marine organisms which inhabit the coastal waters. In the past few years, some studies have been conducted using seaweeds and other algal forms in radioactive uptake experiments from which it was learned that algae are capable of concentrating fairly high quantities of both radioactive and non-radioactive elements (4).

The only apparent disadvantage to the use of seaweeds is that they are living organisms and subject to whatever toxic constituents might exist in the waste. Conceivably, under such toxic conditions, seaweeds would die, thereby releasing whatever activity they had concentrated back to the water.

If further studies prove seaweeds to be highly effective concentrators of non-toxic radioactive nuclides, then there is every possibility that macroscopic marine algae will not only effectively protect the coastal environment, but will also render the ocean a more attractive area for waste disposal. Currently seaweeds are used to advantage in this respect in the Irish Sea. It is known that most fission products discharged to the sea usually suffer an isotopic dilution by the non-radioactive species present, thus reducing somewhat the hazardous nature of the radioactive species. However, some fission products such as plutonium do not have a stable isotope in the sea and, therefore, do not undergo an isotopic dilution (14). The discharge of active plutonium must, therefore, be made with extreme care. The seaweed Porphyra umbilicalis is known to concentrate plutonium by a factor of

several hundred and thereby aids in reducing the potential danger afforded by this long-lived nuclide (20).

Theoretically, the ocean can accept safely large quantities of radioactive wastes provided they are discharged in accordance with tested procedures (57, 52). However, some radioactive waste disposal to the ocean is not performed in accordance with the rules and regulations commonly accepted. An example of this is the burial of wastes off the New England Coast in only fifty feet of water when the accepted code calls for burial in no less than six thousand feet (6, 7). Wastes from the Windscale plant in England are discharged, at the estimated daily rate of 100 curies of mixed fission products, directly into the Irish Sea from which the Welsh were accustomed to gathering edible seaweed (20). Man cannot be so sure that the sea is an absolutely reliable and safe disposal area without exercising judicious care in the discharge of these wastes. But with proper planning now, and with the ascertainment of the limitations of the ocean as a disposal area, man can effectively and safely employ this tremendous area and volume of water for his radioactive wastes (78).

As a probable means of increasing the potential of the sea as a disposal area for low-level radioactive wastes, seaweeds no doubt can be used advantageously for not only removing the radioactive nuclides from the water but also as a safety factor against careless or accidental discharge of these hazardous wastes. Since little has been done along these lines with reference to marine algae, it is thought that this present study will be both worthwhile and justifiable.

CHAPTER IV

LITERATURE REVIEW

The need for more basic information concerning marine seaweeds as well as other marine biologic entities has been urgently increased since the introduction of atomic energy into man's environment. This desire for more information was brought about through necessity as aptly stated by Roger Revelle (61):

> "Our knowledge of just what share of these fission products can be safely introduced into the oceans is woefully incomplete because we simply do not know enough about the physical, chemical, and biological processes. If the sea is to be seriously considered as a dumping ground for any large fraction of the fission products that will be produced even within the next ten years, it is urgently necessary to learn enough about these processes to provide a basis for engineering estimates. . . .
>
> "In the next decade we should attempt to learn far more about the ocean and its contents than has been learned since modern oceanography began 80 years ago."

Prior to the atomic era, fundamental information such as chemical composition, the biology and ecology, the rates of growth and the rates of harvest, the means of reproduction, whether vegetative or sporogenous, the seasonal development of the algal species, and whether they were annual or perennial, and the oceanic distribution of seaweed was greatly lacking (63, 80). The situation at present, however, is not much improved although it is now receiving greater recognition than ever before. There were several possible reasons for this apathetic attitude towards these macroscopic algae. One of the main reasons undoubtedly

was that man has had very little use for the seaweed and another likely reason was that man's methods of analysis were crude and not as well instrumented as they are today. Due to the advances in the field of electronics, instrumentations are possible which are able to solve many of the complex biological problems considered hitherto unsolvable.

At the present time, seaweeds have found extensive application in such diversified fields as the production of agar, cosmetics, drugs, prepared foods, cattle feed, fertilizer, and paper manufacturing. The great potential of ocean farming regarding seaweeds has only been partially realized. It is not inconceivable that as the world's population increases more of man's food supply will come from these marine forms (1, 11, 77). Destroying the usefulness of these fertile productive areas by the improper discharge of radioactive materials to the ocean is undesirable and of paramount concern to man (12). It is for this reason that more studies of not only marine algae but other aspects of the aquatic environment have been and are being conducted and especially under radioactive experimental conditions. The following discussion will be concerned primarily with some of these investigative studies.

Laboratory Research

The uptake of certain elements by some algae is quite dependent upon the plant's respiration. Kelly (39), from her experiments with the brown alga, Ascophyllum nodosum, showed that iodine uptake by this organism was dependent upon its respiration. She placed several 10 millimeter (mm) segments of this alga in a solution of van't Hoff sea water

containing radioactive I^{131}. When the alga's respiration was stimulated by the addition of substances known to serve as respiratory substrates such as glucose, sucrose, malic and succinic acids, I^{131} uptake increased. When compared to the control solution, sugars at pH 6.8 and acids at pH about 4.5 increased the alga's oxygen uptake up to 20 and 25 per cent respectively. As caused by the sugar effect, the corresponding increases in I^{131} uptake ranged from about 180 to 400 per cent of control values. To lend more reliability to her findings, Kelly injected known oxidation inhibitors such as sodium azide and potassium cyanide and found that not only was oxygen uptake inhibited up to 87 per cent but that I^{131} uptake was correspondingly inhibited up to 96 per cent of the control value. When the algal segments were exposed to a non-oxygen atmosphere such as nitrogen, I^{131} uptake decrease ranged from 50 to 75 per cent of the control. These tests indicate quite well that iodine uptake is an aerobic process and that maximum I^{131} uptake is achieved in the pH range 4.5 to 5.5. This latter finding indicates that only in a limited area of the ocean will maximim I^{131} uptake by A. nodosum be achieved since the common pH range of the ocean is 6.5 to 10.

In all her samples, Kelly determined the algal uptake by immersing a number of alga segments for a period of about 20 minutes in a flask of the radioactive solution. The segments were then removed, washed with a similar solution but not radioactive, dried with paper towels and counted. This analytical approach was employed by other investigators as will be seen later in this discussion. The method although not precise was evidently accurate enough to produce qualitative data.

Shaw (66) also worked with I^{131} but used the brown alga Laminaria digitata. He employed sampling and experimental techniques similar to those of Kelly. In his studies, Shaw proposed to ascertain the mechanism whereby L. digitata was capable of concentrating iodine by a factor of about 30,000 (activity per gram of alga/activity per gram of water). From his studies he concluded that iodine was taken up by the alga as HIO. Examination of the plant tissues showed that the accumulation was iodide and not iodine. Shaw believed that the iodine compound HIO had been converted to iodide within the plant's cells. This mechanism was deemed valid because HIO, being an undissociated molecule and not in ionic form, could easily penetrate the alga's cells. The supporting evidence for this conclusion was found in Osterhout's work completed in 1925 (50).

The uptake of iodine by the alga was found to vary markedly with the concentration of I_2 present in the water. When Shaw exposed his experiment to an atmosphere of pure nitrogen, the formation of I_2 in the water was greatly reduced and the uptake of I^{131} was reduced. However, when I_2 was introduced into the experiment even under a nitrogen atmosphere, the uptake of I^{131} increased. It was evident then that I^{131} uptake was an aerobic process -- a conclusion reached by Kelly in her experiments.

It is known that marine algae discriminate strongly between the various elements of the alkali metals group. A good example of this is the larger concentration of potassium over sodium by the algae in spite of the fact that sodium exists in sea water to a greater extent than potassium. Since this discrimination exists, and since the alkali metal cesium is formed in appreciable quantities in nuclear fission products,

it becomes important to determine the amounts and mode of accumulation of this radioactive specimen by the marine algae. Scott (65) undertook such a study using various species of brown and red algae and conducted his experiments by immersing ten pieces of each algal species in separate glass aquaria containing 15 liters of filtered sea water enriched with Cs^{134}. Temperature of the water was maintained at $10^{o}C$ and illumination was provided for eight hours per day by two fluorescent tubes of the daylight type suspended directly above the aquaria. At intervals, pieces of the algae were removed from the aquarium, fresh-air dried, weighed and then wet-ashed with a mixture of nitric and sulphuric acids. The activity per gram of the residue was then compared to the activity per gram of the radioactive sea water sampled at the same time from the same aquarium. This ratio yielded the concentration factor for the various algae studied. The species studied were Ascophyllum nodosum, Laminaria digitata, Laminaria saccharina, Polysiphonia fastigiata, Rhodymenia palmata and Ulva lactuca.

Again, like both Kelly and Shaw, Scott noticed that the uptake of Cs^{134} was increased by the addition of certain chemicals. In this case, the addition of the potassium salts of phosphate and nitrate stimulated the algal uptake to a slight degree. It was noted, however, that a marked uptake of Cs^{134} was achieved when the potassium content of the sea water was decreased. In this situation, the alga in all probability absorbed more Cs^{134} to compensate for the lack of potassium. The concentration factors thus found ranged from 200 to 50,000 for the various species examined.

Rhodymenia palmata, the alga with the high concentration factor of 50,000 was the subject of further tests. It was found that with no illumination R. palmata virtually excluded Cs^{134} but immediately commenced to concentrate it on being illuminated. The accumulation of Cs^{134} was, therefore, found to be dependent upon the illumination. Scott also noted that no region of the visible light spectrum was more effective in increasing uptake than any other portion of the total light spectrum. He further discovered that the concentration factors were independent of and did not vary with the concentration of the Cs^{134} initially present in the water.

R. palmata retained its radioactive cesium for weeks even while it was kept in natural sea water. The addition of stable cesium to the same sea water failed to release the radioactive species from the algae. Cs^{134} was retained by the plant in the presence of another plant which contained no detectable radioactivity. However, when the plant containing the Cs^{134} was placed in boiling water or was otherwise killed, it released its concentrated activity almost immediately.

The concentration factors have been found to vary from one algal species to another and also from one radioactive nuclide to another. As already mentioned, the red alga, R. palmata, has a concentration factor of 50,000 for Cs^{134}. Spooner (68), in his studies found that R. palmata concentrated strontium-90 and yttrium-90 by a factor of only 2. It is therefore evident that the results as found for one alga and a given nuclide cannot be faithfully applied to other algae and other nuclides. Each must be ascertained by direct experimentation. This is an awesome task when one considers that there are approximately 40,000 (79) algal

species to choose from and almost 2,000 radioactive nuclides with which to contend.

With R. palmata in a solution of sea water containing Sr^{90}, Spooner (68) found that as the Sr^{90} decayed to its daughter, Y^{90}, the alga concentrated the daughter nuclide until equilibrium with the Y^{90} in the sea water was attained and then the Y^{90} in the plant followed a decay scheme according to the natural aspects of that nuclide. The alga absorbed very little of the Sr^{90} and seemed almost to selectively exclude Sr^{90} at the expense of selectively concentrating its daughter. Approximately 99 per cent of the activity found in the alga was due to the Y^{90}.

Using other algae such as the brown Fucus serratus and Fucus vesiculosus, it was found that Sr^{90} was concentrated at the expense of Y^{90} and that the concentration factors for these algae were 40 and 30 respectively. Spooner's studies point out the fact that red and brown algae react quite differently in their uptake of both strontium and yttrium.

An interesting aspect of Spooner's method of analysis was that he placed only one piece of a given alga in his radioactive solution and instead of assaying the alga he assayed the water. As the alga concentrated the active nuclide from the water, what amounted to a dilution of the radioactive species was taking place. From his assay data, Spooner determined the equivalent weight of water which would cause the same dilution effect as the alga. Then the concentration factor was equivalent to the number of milliliters of water required to cause the same dilution as one gram of alga. This approach in determining the factor is correct, unique, and no doubt quite rapid as

compared to assaying the alga for radioisotopes. Any errors brought in by extraneous uptake caused by the aquarium walls or foreign organisms were canceled by a carefully monitored control.

In a non-radioactive study, Wassermann (83) has attempted to explain an aspect of the uptake mechanism of various cations by the brown algae Ascophyllum nodosum, Laminaria digitata, and Laminaria saccharina. From his experimental studies he contended that because alginic acid and other water-insoluble acids occurred in the cell tissue of brown algae in the form of various metal salts and not in a free state, then ion exchange between the algal acid salts and ions in solution was possible. To prove this, he treated the algae with 1 N HCl. The metals were dissolved, while the H^+ were taken up from the HCl solution. When this HCl extracted algae was brought into contact with a metal salt solution, the reverse happened; namely, the adsorption of the metallic cation from the salt solution and the release of the H^+ back to the salt solution. Wassermann conducted his studies under both continuous flow conditions where only the cations appeared to be absorbed by the algae and under static conditions where anion absorption also could be detected. So long as the alga was alive to produce alginic acid then the above chemical reactions occurred to effect the uptake.

Rediske and others (54, 55) have found that with terrestrial plants such as red kidney bean, tomato, wheat, and Russian thistle, radioactive uptake was proportional to the concentration of radioactive substances available to the plant and also to the pH of the radioactive solution. A similar situation has been noted with uptake by seaweeds, i.e., the uptake in general is proportional to the radioactive concentration.

Field Studies

Perhaps to date the greatest opportunity to reap experimental data concerning several different types of organisms and their relation to radioactivity has been in the Pacific area around the Marshall Islands. Ever since the first nuclear bomb was set off in March of 1954 in this area, many studies have been conducted concerning the uptake of radioisotopes by algae as well as by other marine organisms (17). Studies and surveys of this nature were generally under the auspices of the Atomic Energy Commission and were written up in several reports.

Of these reports, only a few dealt with the uptake of radionuclides by seaweeds. Two reports in particular indicated that some algae do concentrate radioisotopes (16, 53). In some of the island areas, it was also discovered that algae found in isolated pools onshore contained more radioactive isotopes than those algae growing on the ocean floor at depths of 22 to 50 feet. The exact reverse, however, was noted on other islands in the same atoll (53).

In all these reports concerned with monitoring the Marshall Islands, not too much work was done with respect to macroscopic seaweeds as compared to the other environmental entities. Most of the studies dealt with uptake by fish, invertebrates, birds, plankton, land plants, soil, and water.

A vast monitoring program for radioactivity in the Pacific Ocean began in February, 1955, and expired in May, 1955 (31). The area monitored extended from the west coast of the United States to the waters around Japan. Some of the significant conclusions reached from this operation were as follows:

1. Radioactive assay of sea water and plankton samples indicated widespread activity in the Pacific Ocean as a result of the atomic bomb tests conducted in the spring of 1954. Activity of sea water ranged from 0 to 570 disintergrations per minute per liter (d/min/liter) and of plankton from 3 to 140 d/min/gram wet weight.
2. Concentrations of radioactive elements were found in the main current streams which transported the radioactive contaminations over a wide area.
3. Uptake by plankton offered a sensitive indication of activity in the ocean. It was further noted that algae and other marine organisms concentrated more radioelements in the contaminated areas than in the other areas.

Algae sampled from the Eniwetok area, showed concentration values ranging from 260 to 223,000 d/min/gram wet weight. Elsewhere in the Pacific area, the algal uptake varied from 12 d/min/gram of wet weight to 1,760,000 d/min.

Approximately 2 1/2 years subsequent to the bomb tests of 1954, Lowman and others (46) performed monitoring surveys in the Marshall Islands. They examined plants and animals from both land and sea for radioactive contamination by Mn^{54}, $Fe^{59, 55}$, Zn^{65}, $Co^{60, 58, 57}$. It was found in general that these elements occurred in the sea and not on land, and in marine animals not in marine plants. Only in marine plankton, invertebrate filter feeders and fish were the radioisotopes found in measurable amounts. Of course, not all the algae were examined as only 6 species were investigated in this survey. Of these examined, some were found to have detectable amounts of Zn^{65} and $Co^{60, 58, 57}$;

none contained Mn^{54} or $Fe^{55, 59}$. Hiyama (35) in his investigation of radioactive fallout from the hydrogen bomb also found that the concentration of radioactivity was higher in the microplankton than macroplankton. This common finding of more activity in microorganisms than in macroorganisms is in part explained by the fact that since microorganisms have more surface area per unit body weight, more radioactive materials can be adsorbed on these forms than on the larger organisms.

The Marshall Islands area serves as an excellent large scale radioactive experiment from which most useful data can be collected (19). However, there are other places, although not as large in extent, which serve the same purpose. One such area is the Irish Sea which receives the raw atomic wastes from the atomic reactors of the Windscale Power Plant in Cumberland, England.

In most coastal areas of the United Kingdom, the problem of radioactive uptake by seaweeds would ordinarily be of very little significance. The problem, however, is significant in the Irish Sea off the Cumberland coast because a seaweed growing there is harvested for human consumption. The seaweed in question and upon which radioactive assays were made was Porphyra umbilicalis. Seligman's examination (20) showed that the concentration factors for this alga based upon wet weight for various isotopes to be as follows:

Isotope	Concentration Factor
Strontium	0.5
Yttrium	30.0
Cesium	5.0
Cerium	25.0
Ruthenium	10.0 (assumed)
Plutonium	500.0 (assumed)

The concentration factors of yttrium, cerium, and ruthenium from Seligman's data were lower by a factor of 10 than the findings made by Spooner (68). Seligman believed that the factor should have been closer to 50 but because of a paucity of data, the factor of 10 was assumed to be correct.

To afford protection to those who ate this and other edible seaweeds, maximum permissible concentrations were established and taken to be 1/10 the allowable concentration for any radioisotope in drinking water (22, 47). The allowable limits in units of curies and microcuries (μc) were as follows:

Nuclide	Mean Activity Discharge (curies)	Sample	Mean Activity of Sample (μc/gm)	Assumed Criterion of Safety (μc/gm)
Pu^{239}	0.05	Fish	4×10^{-7}	3×10^{-5}
		Seaweed	3×10^{-8}	3×10^{-5}
Sr^{90}	7.0	Fish	2×10^{-7}	8×10^{-6}
		Seaweed	2×10^{-7}	8×10^{-6}
Ru^{106}	16.0	Fish	1×10^{-5}	1×10^{-3}
		Seaweed	3×10^{-5}	1×10^{-3}

The mean activity was determined from many months of sampling from the Irish Sea. The criterion set forth in the table was established by considering that both fish and seaweed were edible and therefore more controllable than the external hazard. If it were not for this risk of ingestion, the amount of radioactive wastes released to the Irish Sea could be greatly increased to thousands of curies. Uptake of radioisotopes

by seaweeds was tantamount to a radioactive dilution of the contaminated water. Dunster has attributed a dilution factor of about 2 for this uptake (21). This would not only indicate the potentiality of macroscopic algae as decontaminating agents but would also emphasize the precaution to be exercised over the harvesting of edible seaweeds. It is unlikely that the discharge of small amounts of radioactivity could cause any significant disturbance of the marine environment. However, since the effects of internal and external irradiation as would develop in man by exposure to contaminated fish and seaweeds are not too well understood, elaborate precautions must be exercised to protect the environment.

Fresh Water Studies

It has been stated that in the ocean the phytoplankton concentrated more radioelements than most other organisms. Krumholz (43, 44), in his survey of White Oak Creek, Roane County, Tennessee, found that filamentous algae concentrated radioactive isotopes by a greater factor than phytoplankton. For example, the phytoplankton Euglena and Pandorina showed concentration factors of 100,000 and 285,000 respectively. The filamentous alga Spirogyra, however, was found to have a concentration factor of 850,000. Krumholz estimated that a mat of Spirogyra weighing approximately one ton contained well over 100 millicuries (mc) of radioactive material. This study further indicated that in most of the algae, planktons, and other organisms, radiophosphorous was selectively concentrated in greater amounts than any other radionuclide. Other radioelements selectively concentrated were strontium, some rare earths and cesium.

In another study, Coffin, et al. (13), dosed a fresh-water lake with 100 mc of P^{32} and noted the uptake by various aquatic organisms. It was found that zooplankton, fish, algae and water lilies concentrated P^{32} by factors of 40,000, 13,000, 1,000, and 100 respectively. It is seen by these data that concentration was greater for the animals than the plants, but more important is that the concentration was greater for the lower plants than the higher plants.

Foster and Davis (24), during a period when the aquatic animals of the Columbia River were most abundant, found that the P^{32} content of some small fish was about 150,000 times that of the water in which they were thriving; in caddis fly larve the concentration factor was about 350,000. They attributed these different concentrations in part to the dissimilarities in chemical composition and to the physiological demands for specific elements. The isotopes were concentrated by particular tissues according to need. Bones and scales, for example, were found to contain more P^{32} than muscle and fat. Krumholz, as was earlier stated, also discovered such selective concentration in filamentous algae.

Adsorption to biological surfaces plays some part in this concentration ability of organisms. Some radioactive materials readily diffuse through living membranes and are thereby readily taken in by the organisms. Plants, for instance, build up protoplasm and thereby can concentrate many inorganic ions and some organic compounds. Since animal membranes are more selective, only a few radioisotopes are absorbed directly in significant amounts.

Lackey (45), in his examinations of the microscopic algal population of the Clinch River in Roane County, Tennessee, found that many algal species concentrate radionuclides. He too, like others already mentioned, found that filamentous algae have high concentration factors. He cites, for example, the concentration factor of the filamentous alga Oedogonium as ranging from 10,000 to 21,000.

Steel and Gloyna (72) examined the possibility of employing microscopic algae in oxidation ponds to effect decontamination of sewage containing radioisotopes. Several different species of green and blue-green algae ranging from unicellular to filamentous were used. In these studies, no practical effects were noticed by using the many different organisms. It was observed, however, that the uptake varied greatly with the particular nuclide in question. It was found, for example, that about 23 per cent of the original radioactive content could be removed by the metabolic action of the algae upon wastes containing mixed fission products only. With Sr^{89}, a 67 per cent removal of the original radioactive material could be realized in a twenty day period whereas with Ce^{141}, 45 per cent removal was noted in the same time period.

Physical Characteristics

Ketchum and Bowen (40, 41) have found that the circulation of isotopes in the sea is different from the natural circulation of the ocean waters. When marine organisms take up isotopes, the isotopic circulation is altered not only by the migrational habits of these living organisms but also by the definitly established patterns of movements for dead organisms. After the death of organisms, elements will be

released from their bodies and the elements which will most readily become well distributed or transported vertically will be those which have a high exchange rate with the sea water. Those which will settle out to the ocean floor will be those which are strongly bound to sediments such as skeletal or tissue material. This biological influence upon the distribution of isotopes in the marine environment depends in large part upon the species of organisms and upon the elements considered. Such biologic activity could easily result in the concentration of radioisotopes in inshore and estuarine waters. As a result, the radioactive concentration of these waters could conceivably be greater than the radioactive concentration of the source water.

The water of enclosed basins such as harbors and estuaries does not as a rule mix rapidly with the water in the open sea. Therefore, the rate of dilution of a contaminant in these enclosed basins is a great deal less than the dilution rate in the open sea. Also, if the entrance to the basin is shallow, there will develop in the basin, below this entrance depth, a body of water which stagnates seasonally or permanently. Here, then, contaminants can easily accumulate and create a potentially hazardous situation to man (60).

On the other hand, radioactive materials discharged to the ocean will experience almost complete mixing in about 28 hours in the upper layer of water (surface to depths of 10 to 200 meters). This surface (mixed layer) or upper layer is separated from the lower layer by a belt of high density water called the thermocline.

Although there is good mixing in this upper zone, it is not homogeneous throughout due to concentration by the biologic factors.

It is also known that when such radioactive wastes are discharged to the near-surface layer, they are transported away by surface currents. These currents extend in general through the entire depth of the surface layer. Some currents move at the rate of 40 to 140 miles per day. Hence, vast quantities of surface water are capable of making contact with other surface waters of the ocean.

Since the surface layer is separated from the lower layer by the thermocline, then wastes discharged to the surface waters will not generally be diluted by the deeper waters nor will the waste discharged to the deeper waters be diluted by the surface waters too readily. It is possible, however, for the areas to become contaminated by organisms crossing from one layer to the other and also by particulate matter settling down to the bottom (8, 9).

In coastal areas where precipitation exceeds evaporation, there is a seaward drift of diluted surface water with a landward subsurface drift of water from the open sea. Therefore, if radioactive wastes were discharged in the coastal waters, the surface flow would carry it seaward but that portion of the material which mixes with the deeper waters will return towards shore. This coastal and estuarine circulation is rapid as exemplified by the fact that the water along the coast is a mixture of which 90 per cent is sea water even off large rivers such as the Hudson and Delaware (51, 85).

Generally man is not concerned with the contamination of the ocean as a whole, but only with the water along the coast, within an arbitrary distance of 12 miles from shore. Within these waters, contamination by land-based operations and also from marine vessels, could occur.

Of course, contamination could also happen out in the open sea where the dumping of packaged wastes will take place, but if this is done discretely in the proper area, the high potential dilution factor afforded by the ocean can be realized. Since the entire continental shelf which is the submerged rim of the land mass sloping from the beach seawards to a depth of about 200 meters and then extending precipitously into the deep sea, is essentially the ocean farmland, the disposal of wastes here must be done with extreme caution. It is from these ocean farming areas where almost the entire harvest of oysters, scallops, clams, and seaweed is realized. These organisms, as studies have indicated, concentrate certain elements by large factors (60). It is important to reduce the contamination and it is perhaps possible to accomplish this by using seaweeds. In such instances, the plant could act as the protector of the more edible organisms and thereby reduce serious contamination in the inshore waters.

Seaweeds grow quite luxuriously on these marine farms. Walker (81), in a seaweed survey conducted along eleven miles of the Scottish coast bordering the North Sea found that 15 tons (wet weight) of weeds per acre, primarily Laminaria cloustoni, were thriving in water ranging between 0 and 7 fathoms (13 meters). Between the 0 and 5 fathoms depth (9 meters) 19 tons per acre were found. No measureable quantity was found in depths greater than 13 meters.

In another survey (82), Walker found 93 per cent of the area covered predominantly by L. cloustoni with an average seaweed density of 26.5 tons per acre. The area surveyed was in the 1 to 7 meters depth and included 4,800 acres supporting 127,200 tons of weed.

Marine organisms receive their nutrients from the surrounding ocean water which throughout all its expanse has approximately the same inorganic concentration. Even in the littoral zone, where there is some dilution by fresh water from the land, the salt concentration, although much diluted, remains relatively unchanged. The inorganic material present in the ocean is chiefly in ionic form with some in colloidal form as well. The form of some elements in sea water is not known and since the ocean waters are in a constant state of dynamic equilibrium, a complete picture of the ocean's chemical composition is as yet not possible (79). Harvey (32), however, has made an analysis of the salt content of sea water. Although this analysis may not be completely and precisely correct, it nevertheless serves adequately for most marine studies. A portion of Harvey's analysis may be found in Appendix I.

CHAPTER V

EXPERIMENTAL PROCEDURE

Introduction

Perhaps the most important single aspect in conducting this present study was the acquisition of algal species to be used.

Before any research study was begun, several field trips were made to the coastal waters off Cedar Key, Florida, to locate healthy growing algal specimens. Growths of Sphacelaria sp., Gracilaria sp., and Enteromorpha intestinalis were found in scattered unattached patches during the summer months of 1959, and it was thought these algae representing three different groups would be ideal with which to work. However, during subsequent field trips during the winter of 1959 and into 1960, very few healthy algal growths were noted. This indicated that a reliable yearly growth of the desired algae was not available at Cedar Key. Attempts were made to culture, under laboratory conditions, the few good growths which were obtained from that locale. It was no simple task to raise, successfully, algae under laboratory conditions. Such culturing attempts failed. Dr. Lackey, Professor of Sanitary Science at the University of Florida, procurred cultures of Sphacelaria sp. from the University of Indiana culture collection and under his guidance, a large, fast growing, healthy growth was realized (71). As for the other two organisms, the green and the red, luxurious healthy growths were found in the coastal waters off Marineland, Florida.

Beds of Enteromorpha prolifera and Gracilaria foliifera were found in excellent growing condition all year round. These organisms were found attached to formations of coquina rock located approximately one mile south of Marineland (73).

Special trips were made to the Marineland area for fresh supplies of both sea water and the green and red algae. Sea water, having a salt concentration of about 36 grams per kilogram of sea water (36 $^{o}/_{oo}$), was transported in a 350-gallon stainless steel tank and stored in an outside translucent fiber glass-covered concrete tank -- a facility of the Earle B. Phelps Sanitary Engineering Research Laboratory where all the experimental work for this study was performed. Upon arrival at the laboratory, the algae were examined and only the most healthy segments were placed in several glass aquaria holding fresh filtered sea water. These specimens were in a continuously lighted 20°C constant temperature room until such time as they were needed. The algae were usually held under these conditions for no more than three days before they were used.

Experimental Procedure

The experimental work concerning these algae consisted of several different items of interest and was conducted as follows:

1. The determination of how the algal uptake of Zn^{65} was affected by changes in both light and temperature.
2. The development of the relationship between growth and uptake of algae.
3. The examination of the retention capacity of the algae for Zn^{65}.
4. The ascertainment of whether the algal uptake was achieved by adsorption, absorption, or both.
5. The measurement of the translocation ability of the algae.

In determining the effects of light and temperature upon the uptake by these algae, three different experimental rooms were employed concurrently. One room was kept at ordinary room temperature of approximately 25°C, another at 18°C and the third room was a specially constructed refrigerator to house the experiments at 5°C. In each room, the lighting conditions were easily altered so as to produce the desired light variation. The lighting variations were arbitrarily designated as bright, moderate, and dim. These variations were controlled by the use of a Weston light meter, model 703, type-6A, which measured the intensity of light incident upon the aquaria containing the algae. A meter reading of approximately 500 foot-candles was selected as the bright condition, 10 and 1-2 foot-candles were selected as the moderate and dim conditions respectively. The readings were achieved by placing Sylvania F, cool-white fluorescent lamps close to or away from the aquaria containing the photosynthetic algae (33). It was further considered that the differences in these light intensities were large enough to create the desired significant changes in the uptake.

In any one given room under a given light condition, the uptake phase of the experiment was accomplished in the following manner: Sixteen glass aquaria of at least eight liters capacity each were arranged in separate groups of fours, as shown in Figure 1. Each group of aquaria was filled with filtered sea water until the group's total capacity was about twenty-three liters. With the exception of the control groups, the first aquarium in each group or the elevated aquarium on the far right in both Figures 1 and 2 was to contain the algae and hence receive the light, while the other three aquaria served merely

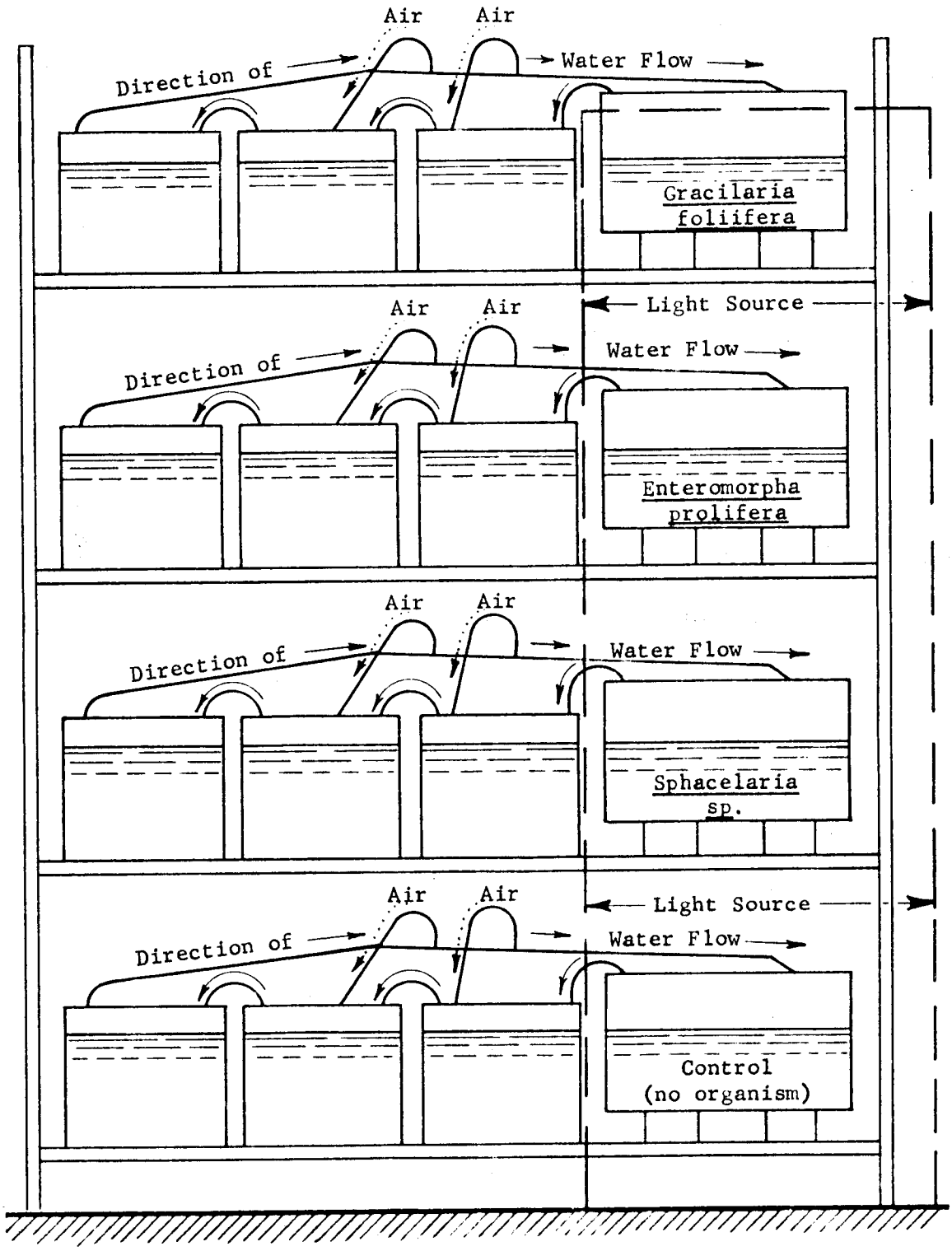

Fig. 1.- Schematic for the arrangement of aquaria, light source, and algae for a given uptake experiment.

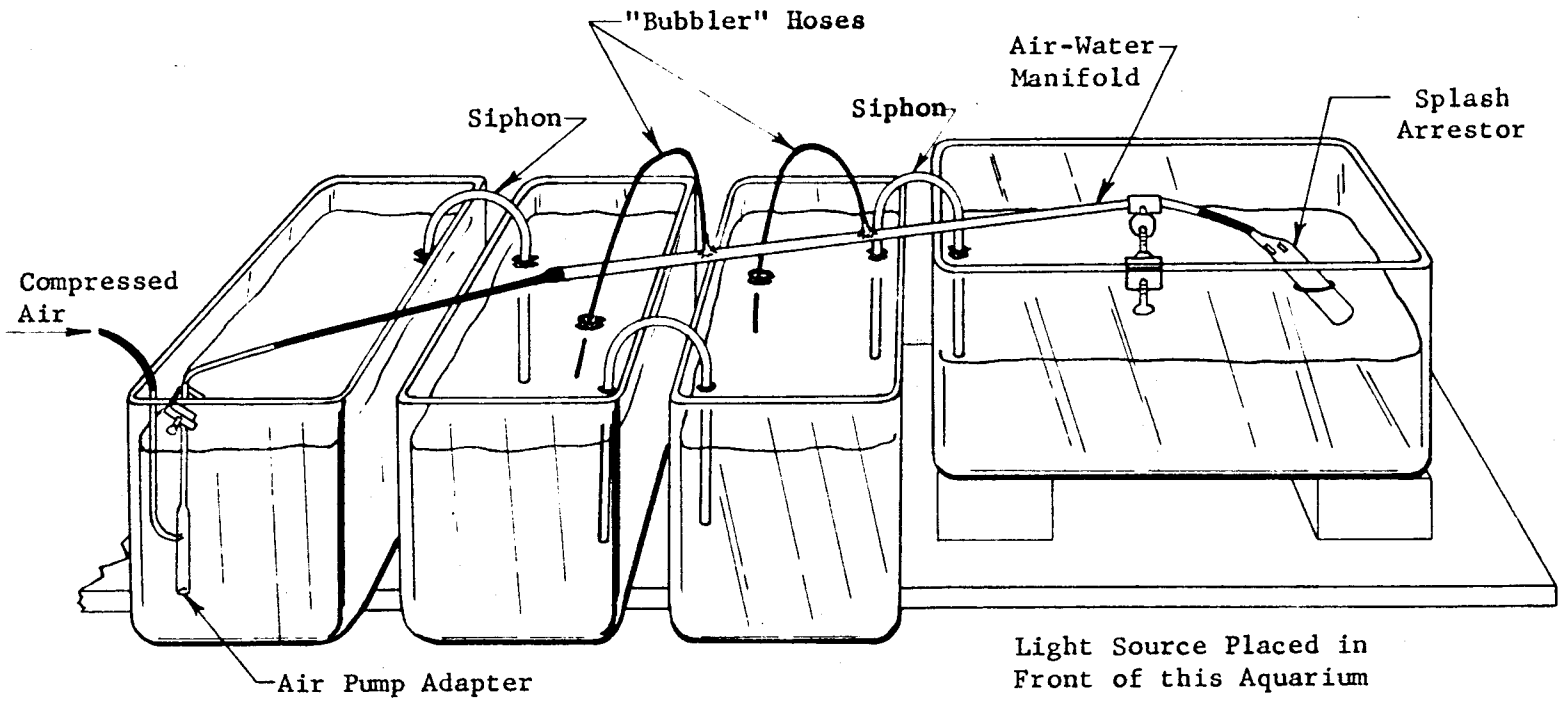

Fig. 2.- Arrangement of glass aquaria and appurtenances in the water recirculation system for the uptake experiments.

as reservoirs for the sea water. So that all the algae in any one of the lighted aquaria would receive approximately the same amount of light, these particular aquaria were positioned so that the incident light would penetrate the narrow width of each aquarium. All four aquaria in each group were connected in a series fashion by polyethylene siphons. Water was pumped from the aquarium on the far left by means of the air pump through the glass manifold and into the first aquarium containing the alga. The pumping was facilitated by elevating the first aquarium. As the pumping began to raise the water level in the first aquarium and reduce the level in the last, the siphon effect was brought into play, continuously keeping the water levels in all four aquaria at the same height. In this manner, the alga located in the first aquarium was not involved in just five liters of water -- the total volume contained in the first aquarium -- but rather subjected to the entire twenty-three liters contained by the chain of four vessels. Under the experimental conditions, this recirculation system was perhaps the simplest way of maintaining a sufficient quantity of water as might be required by the algal portions so as to adequately sustain them for the experimental period of about eighteen days. This system provided the added advantage of using the same radioactive-dosed water throughout the life of any one separate experiment.

After the recirculation systems were operating properly, the desired radioactive dose of Zn^{65} in the form of $Zn\ Cl_2$ was placed in the first aquarium of each group and the pumping was allowed to continue operating to make certain that the radioisotope was equally distributed throughout the systems. To aid in this distribution, "bubbler"

hoses were designed so that they would cause an occasional bubble to agitate the otherwise quiescent water. The algae to be used in a certain experiment were taken out of the 20°C storage room and placed in freshly filtered undosed sea water. The algae were then subjected to the desired experimental condition for approximately one week in order to acclimatize them to the specific experimental situation. At the end of this period, the algae were then placed in the appropriate aquaria containing radioactive-dosed sea water, thus marking the beginning of the uptake experiments.

For the first few days of the experimental period, samples of both algae and water were taken and examined for Zn^{65}. The results were immediately plotted in order to select sampling times more judiciously. When the plotted graph indicated that the desired results had been obtained, the sampling was discontinued and the recirculating system was dismantled, thoroughly cleaned, and reassembled for the next experiment. When all the algae had been successfully studied under the three different light and temperature conditions, the uptake phase was deemed completed. Both pH and salinity measurements were made before, during and after the experiments merely to make certain that no great differences existed during these tests. As a check for a portion of the uptake experiments, some replication experiments were made.

So that the interpretation and analysis of the various uptake curves could be made more clearly, algal growth rates were determined. Since only two different basic uptake curves were found for the three algae, it was decided that the growth rate of only one alga responsible for each different curve would suffice. It was for this reason that

Sphacelaria and Enteromorpha were selected for the growth rate determinations.

The growth rate of these organisms was ascertained by plotting the percentage increase in weight of the organisms against the time at which the weight was determined. An analytical balance was used in order to accomplish this measurement. Each specimen used for weighing was first surface dried by being placed between the folds of thick absorbent paper. A constant pressure of about 2 grams per cm^2 was applied to the sandwich of absorbent paper and alga for an arbitrary time of 1 minute. This was done in order to procure reliable reproducible results. After the dried alga was removed from the paper folds, placed in a tared weighing bottle, stoppered and weighed to the nearest 0.1 milligram (mg), it was returned to the aquarium and this entire weighing process was repeated until three successive weighings were completed. The average of these three weighings was taken as the weight of the alga for that particular weighing period.

In another phase of experimentation, the retention capabilities of the algae were measured. This was accomplished by first allowing the algae to concentrate the radioactive Zn^{65} and then placing the organisms in not only plain sea water, but also in plain sea water of which the non-active zinc content had been increased by the addition of 5 mg of zinc metal per liter of sea water. Zinc metal was added after it had been dissolved in 0.2 milliliters (ml) of concentrated HCl. This acid solution made no detectable pH change in the strongly buffered sea water. Before the contaminated algae were placed in the non-dosed sea water, they were first washed off with the plain sea water so as to

remove any radioactive material which might have been contained in the water covering the outer algal surfaces. These retention experiments were carried out under 500 foot-candles of light at 18°C and in the same equipment used to determine algal uptake. Both algae and sea water were sampled and a graph plotted to observe whether or not, with time, the algae's activity decreased as the water's increased.

The next phase of this study was to determine where in the algae the radioactive nuclide became concentrated. This determination was made by employing the autoradiograph technique (5, 18). The only alga that was suitable from a physical standpoint in this particular phase of the experiment was Gracilaria foliifera. Gracilaria was used because it was of sufficient size so as to be easily handled in this experimental phase. The other two algae were morphologically smaller in size and were composed of several filamentous threads, only fractions of a millimeter in diameter, ranging from 2 to 4 cm in length and all emanating from one common holdfast.

Pieces of Gracilaria grown in Zn^{65}-dosed sea water were washed lightly with ordinary sea water, patted dry with absorbent tissues, gently sandwiched between two sheets of saran wrap and then sealed (30). Several such sandwiches were made and were carefully placed in contact with a no screen backing, A A industrial X-ray film. These contact exposures were allowed to stand for varying periods of time in light-proof photographic envelopes under conditions of controlled temperature and humidity. It was only by trial and error that the proper exposure could be ascertained. After a successful autoradiograph had been made, the algal specimens used for that purpose were dissected and the

dissections were examined for radioactive content so that the autoradiograph could be read quantitatively.

Since algae in their natural habitat are generally completely submerged in water, it seemed valid to assume that they perhaps take up nutrients through all their surfaces, and hence preclude the action of translocation. If, however, the organisms could translocate nutrients, then their effective uptake capacity would still continue while the algae were almost completely out of water. The determination of the algae's capacity to translocate nutrients can be proven by placing one end of the organism in a sea water solution of Zn^{65} and noting if the other end which is not in the Zn^{65} solution contains this nuclide. Such an experiment should be set up so that the only possible way for the Zn^{65} to get to the opposite end would be by way of the plants' ability to translocate the isotope. Many methods, all in non-active sea water, were tried to test the feasibility of this experiment. One was to place one end of the alga in a vessel containing water and allow the other end to remain in air out of contact with the water. The portion kept out of water desiccated in a matter of a few minutes. Then two opposite ends were bridged between two vessels containing plain sea water and because the small bridged portion of necessity had to be out of water, it soon desiccated. Bridging under an atmosphere containing 100 per cent relative humidity caused the exposed portion of the alga to become coated with water droplets and thus prevented desiccation but created the following new problem. Under the high humidity condition, the transfer of Zn^{65} from one portion of the plant to the other portion could occur by way of surface action along the

moisture laden bridged portion, thereby defeating the purpose of the experiment. The ideal solution to solving this problem would be to place the alga completely in water and have the lower portion of the plant in sea water dosed with Zn^{65} and the upper portion in plain sea water and have the two waters completely separated. This ideal situation was in effect accomplished as shown in Figure 3. It is seen that two beakers were used, one an ordinary tall form 400 ml vessel and the other a 400 ml beaker modified by making a funnel opening in its base. Since, as it was explained previously, the Gracilaria species was of fairly workable size, it was used as the test organism.

From this experimental set-up only the water in the top beaker was assayed for a period of one week, as this was the maximum time which the alga could survive under the imposed restriction. After that time, the upper and lower portions of the alga were severed and assayed for activity. The complete translocation experiment included six separate examinations. Two were concerned with the alga's holdfast being submerged into the Zn^{65} solution, and four with other portions of the alga submerged in the Zn^{65}-dosed water. This was done to see if the holdfast was necessary for uptake.

Sample Preparation

The algae were cut up to a minimum size so that forty pieces of each alga were available for sampling purposes in any one uptake experiment. It was felt that this number of pieces would not create a crowded condition in the experimental aquarium and would be enough to make three replicate samples for each of twelve days with four extra portions included as a precautionary measure against loss.

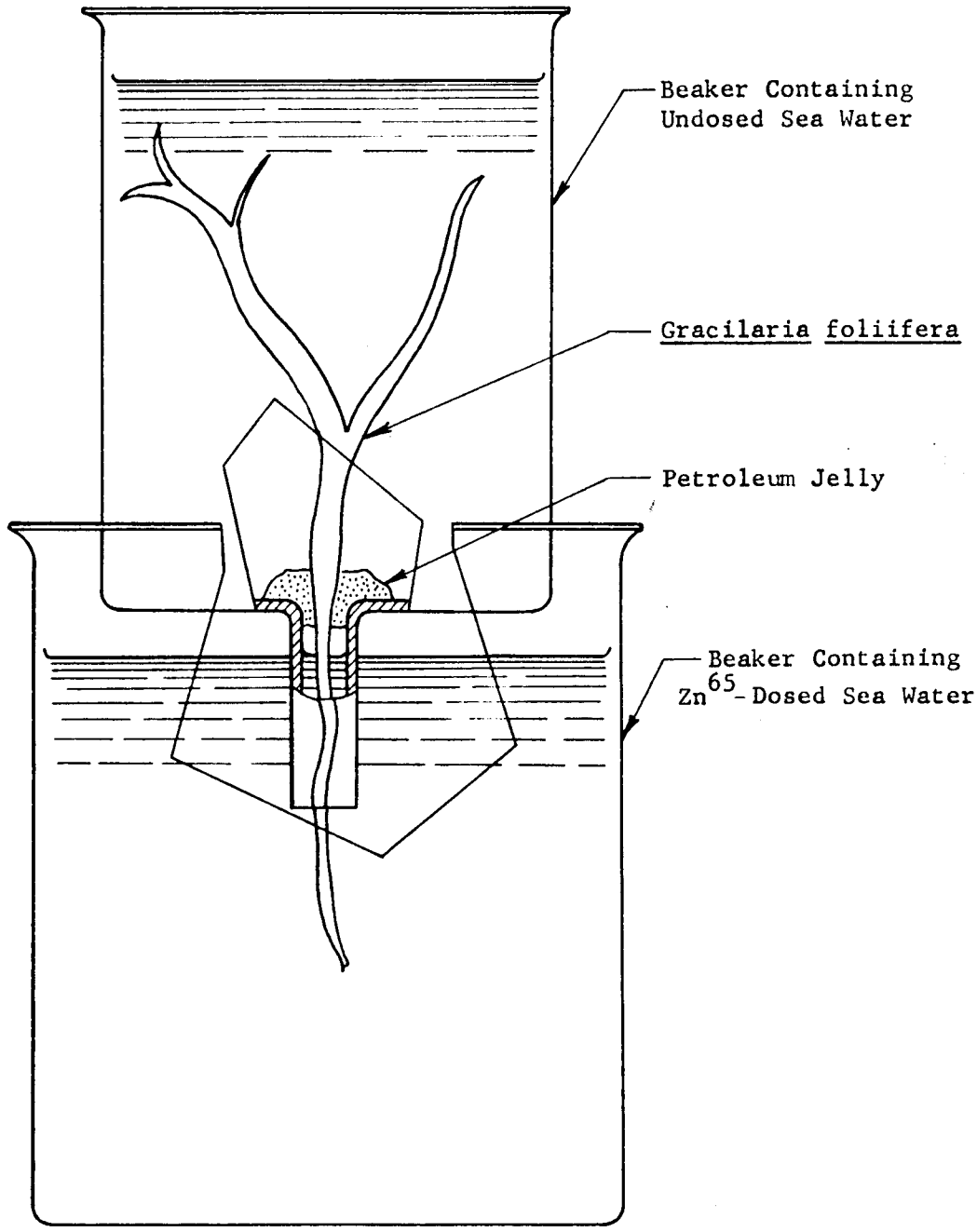

Fig. 3.- Arrangement of beakers and alga in translocation experiment.

When samples were taken for analysis, they were first cleansed lightly with distilled water to wash off any loosely attached radioactive matter, then each single algal sample was placed in a separate mortar and oven dried at 103°C for forty minutes. When sufficiently cool to handle, these dried organisms were ground to a fine powder and then made into a very loose paste by the addition of about 1.5 ml of distilled water. The paste was transferred to prelabled and weighed planchets and spread out evenly over the entire planchets' area which measured approximately 8.0 cm^2. The average dry weight sample density was about 5 mg/cm^2. After having slowly and thoroughly dried these plancheted samples under heat lamps, they were placed in a desiccator and then weighed to the nearest 0.1 mg on a balance. After weighing, the algal material on the planchets was fixed with a lucite binder (containing 0.5 mg lucite per ml of acetone) and then counted. The counting of sea water samples was accomplished after an 0.8 ml-water sample was pipetted onto each of several marked planchets, dried under heat lamps, and fixed with the lucite binder.

Counting Techniques

Since all samples in this study were placed on aluminum planchets and all were counted in the same Nuclear-Chicago Model D-47 Proportional Flow Counter, no correction for backscattering or for geometry was necessary. "PR" counting gas under slight pressure was used in the counter and counts were recorded on a Nuclear-Chicago Model 186-P Scaler. Since only relative activity figures were desired, no absolute counting was done and no such counting correction factors were applied.

One factor applied to the counting data was the correction factor to normalize the data from variations as caused by the counter's changing daily characteristics. This normalizing correction factor was determined by comparing the value of the standard as counted at the time when the first sample was counted to the running average of the same standard counted throughout the experiment. All counting data were corrected for background and the only other factor applied was a self-absorption correction for sea water. This factor will be more fully discussed in Chapter VI.

An examination of the specific activities in Appendices V through VII will indicate that despite a wide range of algal weights used (5 mg to 50 mg per planchet), self-absorption corrections for these organisms were of no concern. Since the counter had a resolving time of 8.0 microseconds and the highest gross activity recorded in any experiment was approximately 1×10^{4} counts per minute, the dead time amounted to only 8×10^{-2} seconds per minute and was quite negligible.

Counting statistics were maintained so that no greater than 5 per cent error in counts would be realized. As a general rule, no sample was counted for less than one minute nor counted for a gross count of less than 500. Mathematical examples concerning the application of these statistics can be found in Appendix III.

CHAPTER VI

RESULTS AND DISCUSSION

In this study, the counting data (activity) were not corrected to their true disintegration values. Since all radioactive samples were counted under similar counting conditions and by the same proportional flow counter, the relation between the counted activity and the true disintegrations was virtually a constant. Application of such a constant correction factor to all the data would certainly change the absolute values of these data but in no way would this change the relative magnitude of one value as compared to another. Because it was essentially this comparative type of data that was sought in this research, no conversion of actual counts to true disintegration was made. The constancy between the actual counts and the disintegration was maintained not only by maintaining the same counting conditions as mentioned above and as described in Chapter V, but also by making certain that self absorptions of all samples were corrected either by proper plancheting or by the application of self-absorption correction factors. For example, the weight of each plancheted algal sample was generally maintained at less than 50 mg in order to avoid the self-absorption effect. Table 1 shows this specific relationship between weight and activity for the alga Sphacelaria, while a similar relationship existed for Enteromorpha and Gracilaria.

TABLE 1

SPECIFIC ACTIVITY OF ZN^{65}
CONCENTRATED BY SPHACELARIA

Weight (mg)	Activity (cpm/mg)
3.1	44
5.6	46
11.9	47
30.6	45
36.9	46
47.6	47
50.7	46
>50	<40

Self-absorption correction factors, however, were applied to each counted sea water sample. These corrections are listed in Table 2 and were calculated by the use of the graph shown in Figure 4. From this graph, it can be seen that the activity varies linearly with volume for each of the three different activity ranges and exhibits no self-absorption effect below the 0.6 ml volume reading. The extrapolated straight line plot continued beyond the 0.6 ml mark represents the activities as if they were ideally not affected by self-absorption. The actual activities, however, were affected by this absorption and are clearly noticed as the sample volume increases from the 0.6 ml up to the 1 ml mark. This effect was evidently caused by too much sample material on the planchet. The material thereby formed such a thick sample that

TABLE 2

SELF-ABSORPTION CORRECTION FACTORS FOR ZN^{65} IN SEA WATER

Water Volume (ml)	Correction Factors			Average Correction Factor
	High Activity Range	Medium Activity Range	Low Activity Range	
0.1	10.0	10.0	10.0	10.0
0.2	5.0	5.0	5.0	5.0
0.3	3.3	3.3	3.3	3.3
0.4	2.5	2.5	2.5	2.5
0.5	2.0	2.0	2.0	2.0
0.6	1.7	1.7	1.7	1.7
0.7	1.6	1.6	1.6	1.6
0.8	1.5	1.5	1.5	1.5
0.9	1.4	1.4	1.4	1.4
1.0	1.2	1.2	1.2	1.2

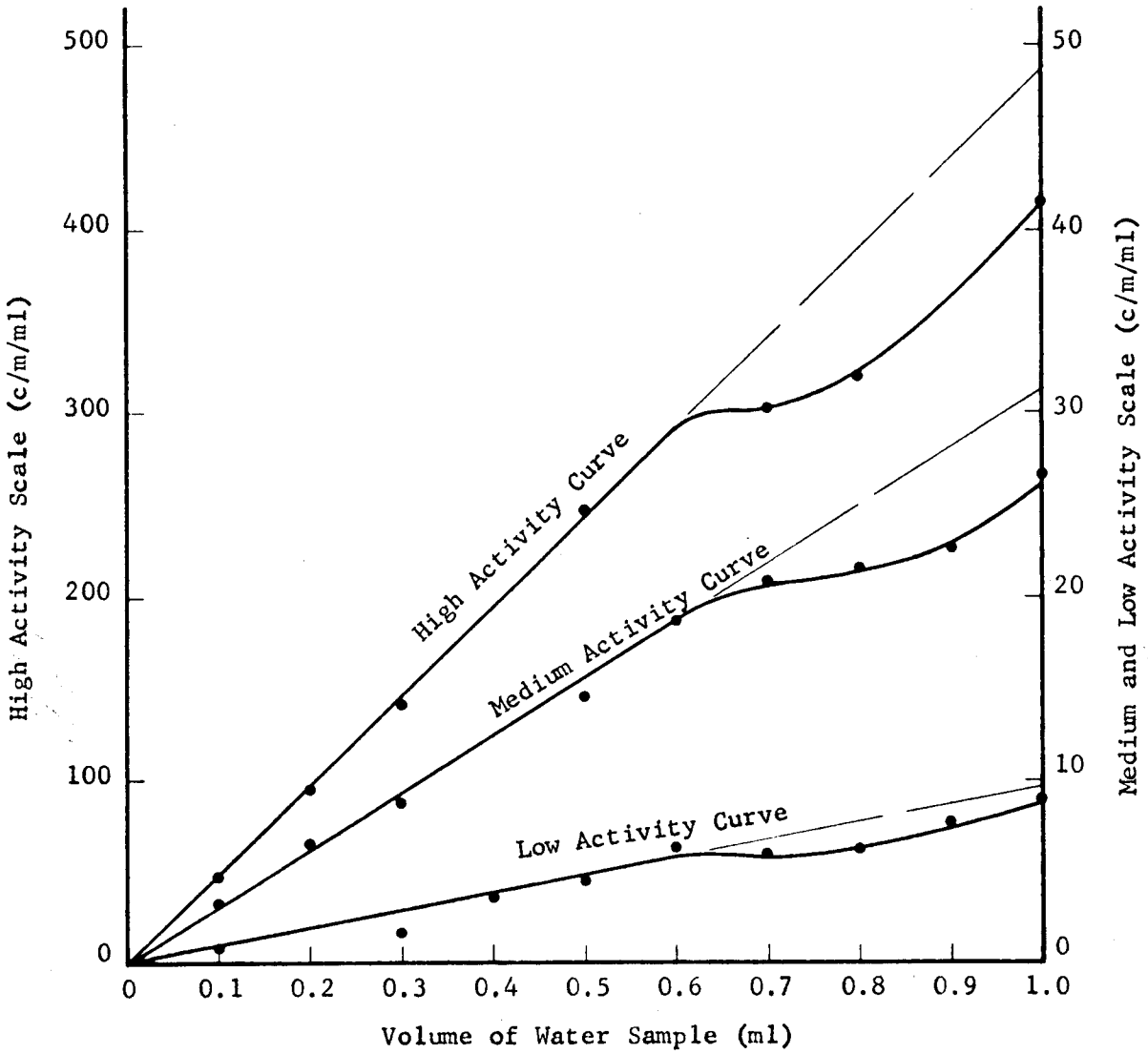

Fig. 4.- The self-absorption effect and correction curves for sea water of approximately 35 $^{o}/_{oo}$ salinity for three concentrations of Zn^{65}.

almost all the weak Zn^{65} positrons (0.03 Million electron volts - Mev) from within the sample were not counted whereas the strong (1.12 Mev) gammas not being impeded by the material were detected (3). Therefore, the deviation of each activity curve from the extrapolated line represents the loss of the positron and the further almost linear rise represents the affect of the unimpeded accumulating gammas. Obviously, to avoid significant self-absorption, a sample volume of 0.7 ml or less would have had to been taken. Although this would have reduced the self-absorption, such a small sample might have easily led to significant sampling errors; for to convert the activity of such a small volume to its equivalent 1 ml volume activity would have required a correction factor equal to the reciprocal of the volume fraction sampled. For example, if a water sample of 0.8, 0.5, 0.2, and 0.1 ml were taken and assayed, then to determine the activity for 1 ml, a volume conversion factor of 1.25, 2.00, 5.00, and 10.0 respectively would have to be applied. It is seen then, that the smaller volumes require the larger factors, hence, any error in sampling or in counting would be magnified in the conversion process. On the other hand, a sample volume greater than 0.8 ml would almost always have resulted in a poorly plancheted sample because the sea water salts usually crawled over the edges of the planchet in spite of the application of grease to these edges. It was experimentally found that a sample of 0.8 ml was small enough so as not to cause poorly plancheted samples and it was large enough so that its conversion factor would have less tendency to magnify any errors. It was for this reason that 0.8 ml sea water samples were used. A glance at Figure 4 will readily show that the 0.8 ml was greatly

affected by self-absorption, hence, a factor for self-absorption and one for volume conversion had to be applied to obtain the equivalent 1 ml activity as represented by the straight line extension.

Further study of Figure 4 indicates that the self-absorption occurred at about the same volume for the three different ranges of activity shown and that it is therefore probably independent of radioactive concentration insofar as it concerns Zn^{65}. Employing Figure 4, the following numerical example will show how these correction factors were obtained for the 0.8 ml sample in the high activity range.

1. Correction factor from actual curve to extrapolated straight line $\frac{390}{325} = 1.20$.

2. Correction factor from 0.8 ml mark on extrapolated line to the 1.0 ml mark on this same line $\frac{1.0}{0.8} = 1.25$.

3. Total correction from 0.8 ml mark on actual curve to 1.0 ml mark on extrapolated straight line $(1.25)(1.20) = 1.5$.

One of the first considerations in this investigation was the determination of the concentration ability of macroscopic algae for the radioactive Zn^{65} isotope. This ability was measured by the concentration factor (C.F.). The factor is a measure of the organism's ability to take up a specific isotope and for any one given organism, the factor usually varies with the nuclide in question and with the environmental conditions to which the organisms are subjected. It was because of such variations that the three algae used in this study were subjected to changes of both temperature and light. The C.F. used here is the ratio of the activity detected in one gram (dry weight) of alga as compared

to the activity of one gram (approximately 1 ml) of the ambient sea water. As previously mentioned in Chapter III, the C.F. also represents the number of milliliters of water necessary to cause by dilution the same reduction in radioactivity as caused by the uptake of one gram of alga. For example, suppose that one gram of an alga was placed in 20,000 ml of dosed water having an activity of 40 counts per minute per milliliter (c/m/ml). Assume that after a definite period of time the activity of the water was measured and found reduced to 10 c/m/ml. This reduction in activity represents the uptake of 600,000 c/m for the one gram of alga $\left[(40 \text{ c/m/ml} - 10 \text{ c/m/ml})(20{,}000 \text{ ml}) = 600{,}000 \text{ c/m}\right]$.

Now, at this time when the water has an activity of 10 c/m/ml, the alga has a C.F. of $\frac{600{,}000 \text{ c/m/g}}{10 \text{ c/m/ml}} = 60{,}000$. The one gram of alga is therefore equivalent to 60,000 ml of dosed water having an activity of 10 c/m/ml. If this 60,000 ml of equivalent water were added to the 20,000 ml of water containing the 10 c/m/ml, there would then result a total volume of 80,000 ml having 10 c/m/ml. Under this condition then, no radioactive matter would have been removed from the original 20,000 ml of 40 c/m/ml. In other words, if 60,000 ml of plain water were added to the original 20,000 ml of radioactive water, the latter volume would increase to 80,000 ml with the simultaneous dilution of the water activity from 40 c/m/ml to 10 c/m/ml -- the same change in activity that was effected by the one gram of alga.

Uptake Studies

All the uptake studies were investigated in terms of the C.F. and not in terms of actual uptake values of the organisms. This approach

was used because the actual uptake varied primarily with the concentration of radioactive isotopes present in the water medium. Under such circumstances then, an organism indicating a high uptake value could be in fact a poorer concentrator than one showing a lower uptake value. The C.F., however, negates the effect of varied concentration and yields a truer comparison of the concentration abilities of the organisms.

As previously mentioned, the C.F. is the ratio of the activity of one gram of alga to the activity of 1 ml (approximately one gram) of the ambient water. However, this definition is not wholly true when the C.F. is determined from the dosed water contained in glass aquaria as employed in this study. From actual experimental work in this research it was found that Zn^{65} was absorbed significantly on glass surface. Hence, a substantial decrease in Zn^{65} concentration occurred by the action of the glass surfaces alone. No specific studies were made in this regard but it was noted that when, for a desired range of activity, a calculated amount of Zn^{65} had been added to the undosed water, the subsequent assayed Zn^{65} activity in the water varied as much as 30 per cent from the calculated value. Some of the Zn^{65} reduction was probably caused by microbial uptake, but it was felt that most of this reduction was due to the glass since the radioactive drop-off began within a matter of ten minutes after dosing. It was for this reason that a larger than calculated dose was added to the water to insure that the desired activity of about 25 c/m/ml was maintained. The concentration of Zn^{65} so added to the sea water was about 1×10^{-11} times that of the natural stable zinc ordinarily present in sea water. It is now apparent that by using such aquaria the algal C.F. for Zn^{65} could increase substantially with only a

slight uptake by the algae. The increase in the C.F. ratio could be caused merely by the decrease in water activity attributed to the glass effect. In order to negate this effect, a period of approximately five days was allowed for the glass and Zn^{65} to equilibrate as much as possible before the algae were placed in the dosed water. Hence, time "0" for the experiment was about five days after the water had been dosed. Complete equilibrium did not occur for there was almost always a slight drop-off in water activity, about 0.3 c/m/ml/day, as perhaps still caused by the glass effect. Only an insignificant amount of this reduction was caused by the natural decay of the Zn^{65} since its half life was about 250 days and the life of the experiment was never longer than 20 days. During this period, the decay would have reduced the activity by about 0.05 per cent (42).

The C.F.'s were determined by taking the ratio of the graphed-uptake activity per gram of alga to the corresponding graphed-medium water activity per ml. The C.F. quantities were not derived directly by the ratio of the assayed raw data. The plotting of a few C.F. curves by using the raw assayed data were attempted, but it was found that such points were highly erratic and the trend of any potential curve was lost. However, the algae uptake values as recorded in Appendices V through VII produced fairly smooth plots; the same was generally true with the sea water activity values. The data taken from these graphs produced C.F.'s which plotted quite smoothly and delineated curves with distinct forms.

In the display of experimental uptake results, the C.F. curves for the algae have been arranged according to the three different light conditions in one figure representing one temperature condition.

This was done for each alga for each temperature condition. Accordingly then, the C.F. curves for Gracilaria foliifera shown in Figures 5 through 8 indicate that for any of the three temperature conditions used, the C.F. was greatest under the condition of bright light with moderate and dim light following in that order. The highest C.F. was achieved by Gracilaria under bright lights at 18°C. This, however, is a special case and will be explained later. For the non special case, the highest C.F. was achieved under bright light at 25°C. Under this latter temperature, the C.F. dropped from a high value of approximately 5,300 under bright light to about 520 and 220 for moderate and dim light respectively. These curves in themselves point out the fact that the more light there is available for photosynthesis, the more will be the resulting uptake. This finding is not in conflict with the accepted understanding that red algae grow best in deeper waters where primarily the blue end of the light spectrum reaches them. Under bright light conditions, there is obviously more blue light reaching the red alga than under moderate or dim light conditions. Consequently, intense light containing more blue component should cause more metabolic uptake than light of lesser intensity. The C.F. curves for Gracilaria show also that the lower the temperature, the lower the algal uptake and moreover since the C.F.'s for these low-valued curves were fairly constant throughout almost their entire length, the indication is that only the adsorption mechanism had been operating. Figure 8 indicates that under bright light condition a reduction in temperature from 25° to 5°C effected a marked decrease in the C.F.'s. For the same temperature drop there was almost no change in the C.F.'s for the other two lighting conditions.

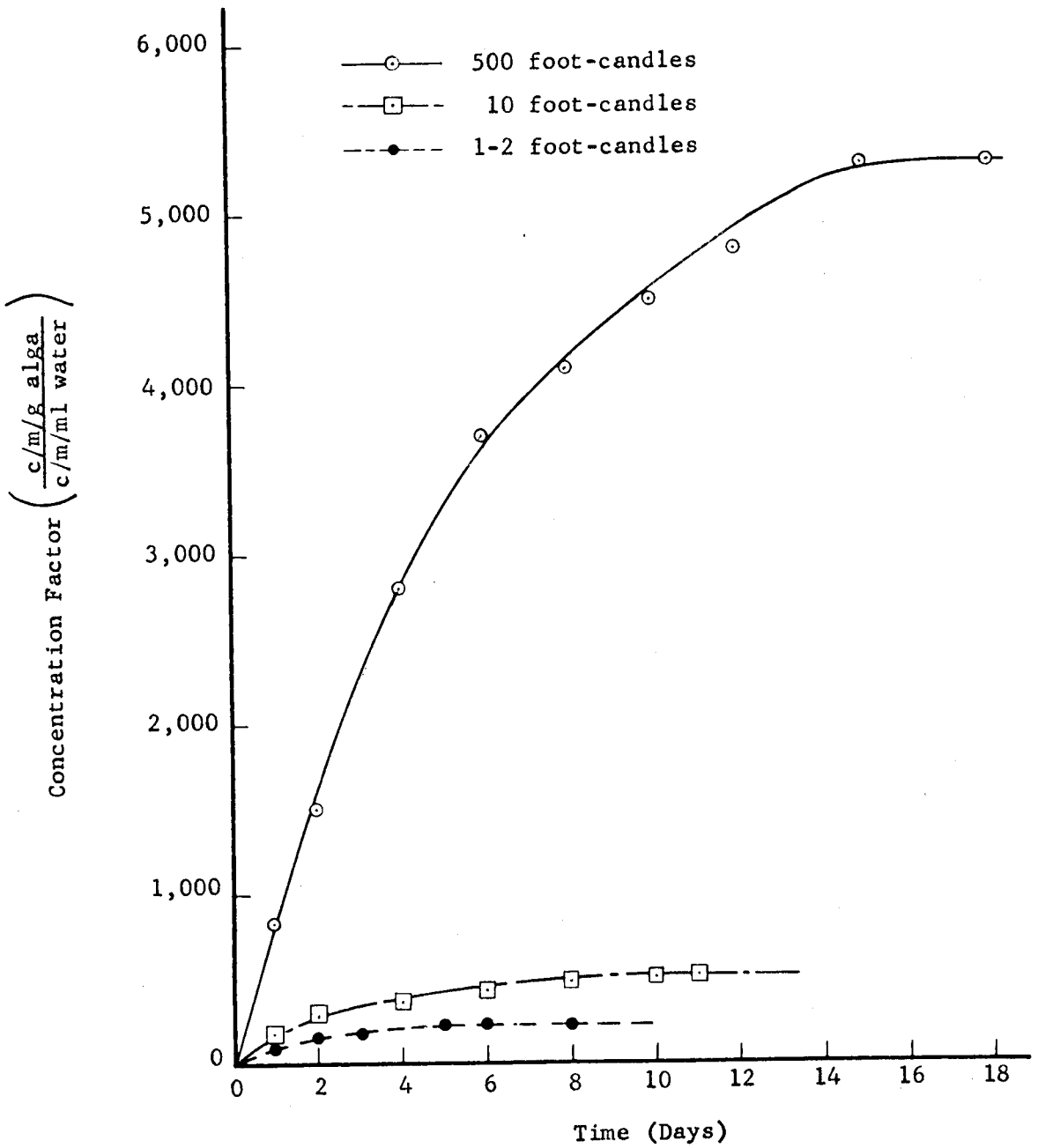

Fig. 5.- Concentration factors for Gracilaria foliifera in Zn^{65}-dosed sea water under various lighting conditions at 25°C.

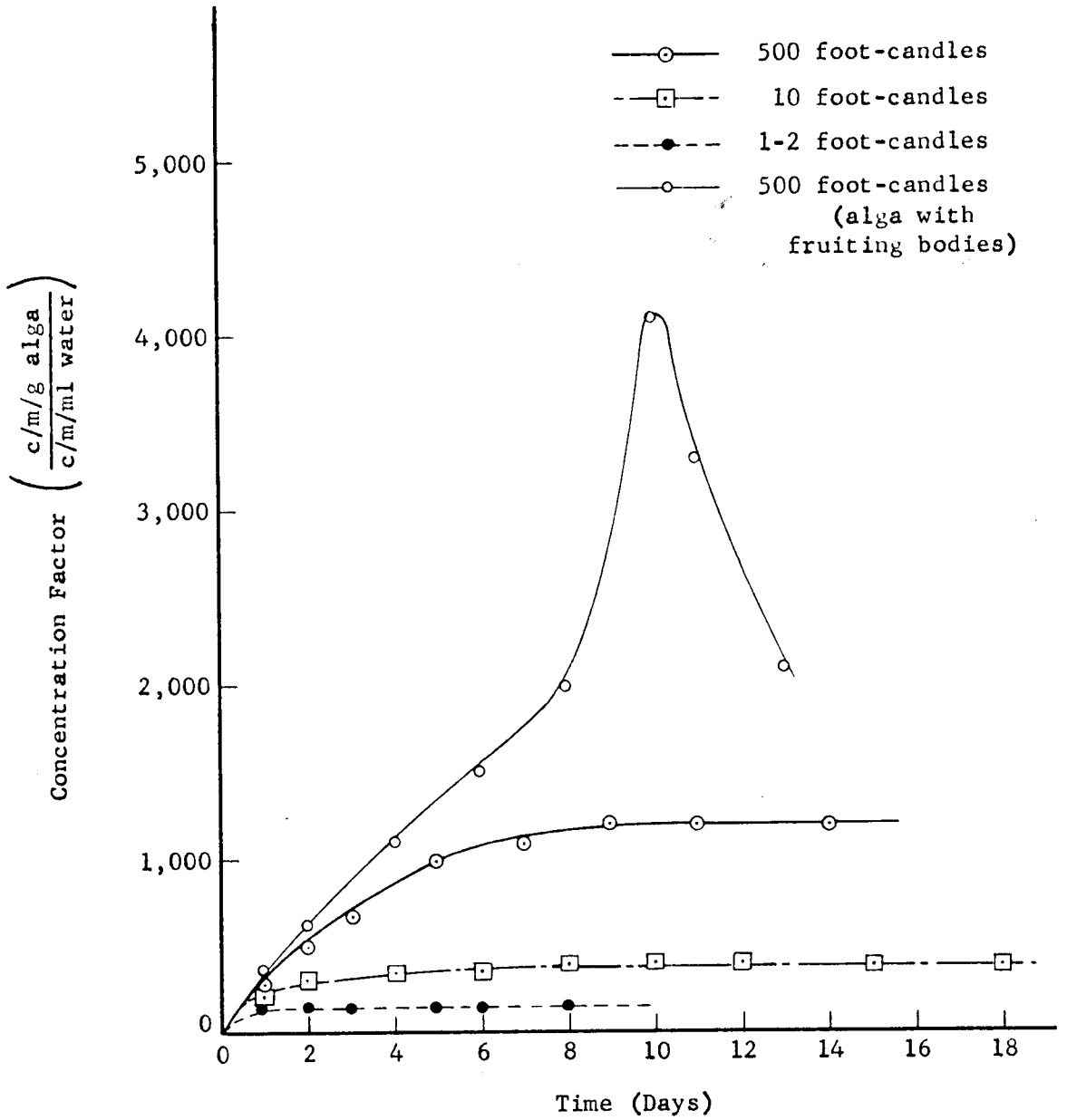

Fig. 6.- Concentration factors for Gracilaria foliifera in Zn^{65}-dosed sea water under various lighting conditions at 18°C.

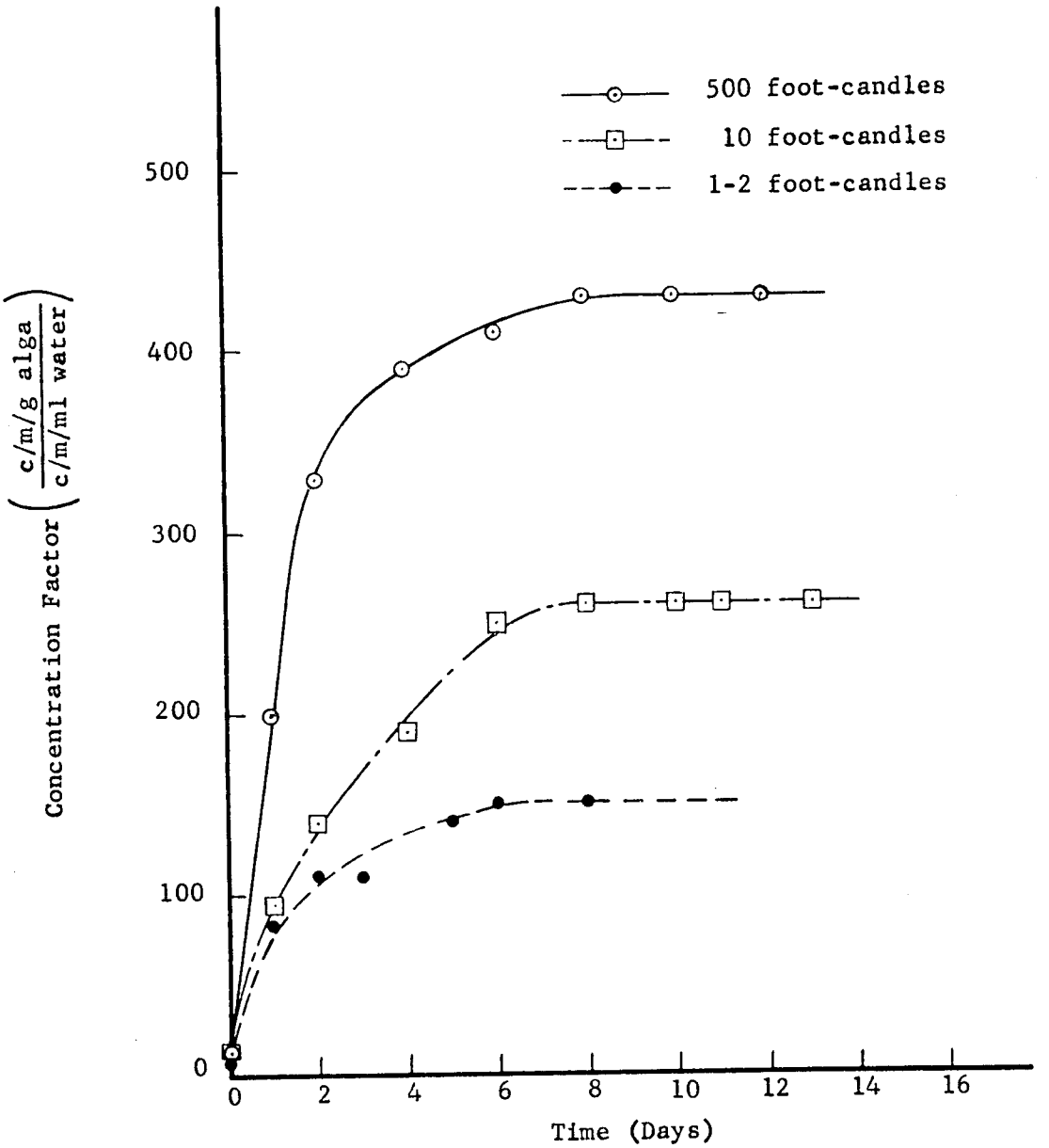

Fig. 7.- Concentration factors for Gracilaria foliifera in Zn^{65}-dosed sea water under various lighting conditions at 5°C.

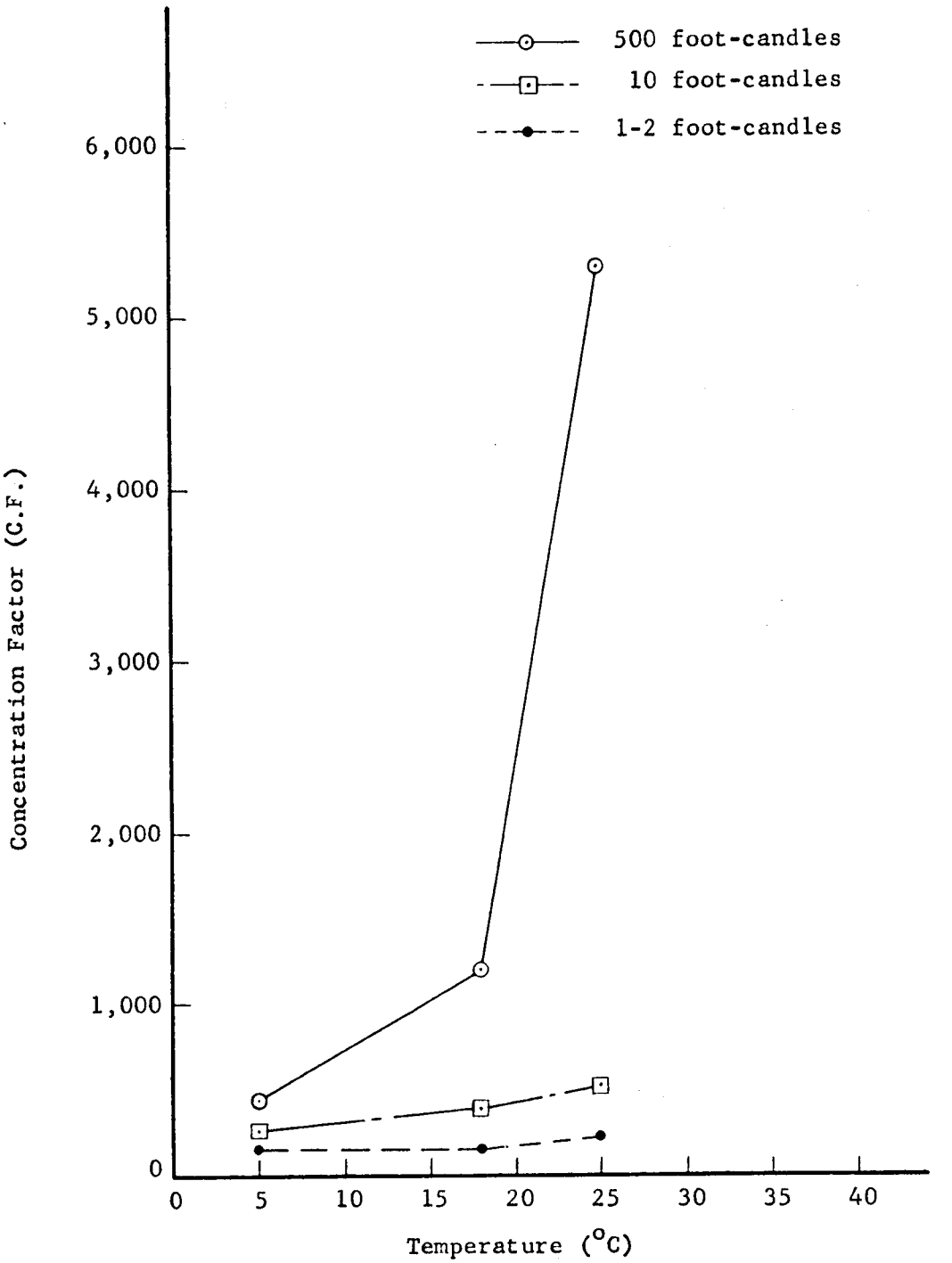

Fig. 8.- The approximate maximum concentration factors of Gracilaria foliifera for Zn^{65} in dosed sea water under different lighting and temperature conditions.

This would indicate that the C.F.'s for both moderate and dim light were not based upon the alga's uptake as caused by an active metabolic process influenced by light, but rather by adsorption to the external surfaces of Gracilaria. An examination of all the Gracilaria curves under the lesser light conditions will show that all these C.F.'s were approximately the same. The effect of too low a temperature was to reduce metabolic uptake with the result that concentration was achieved primarily by adsorption. It seems that the red alga was quite sensitive to a temperature change as shown by both Figure 8 and Table 3 and was also quite responsive to reduction in light intensity.

TABLE 3

THE APPROXIMATE MAXIMUM CONCENTRATION FACTORS FOR ZN^{65} TAKEN UP BY THREE MARINE ALGAE UNDER VARIOUS CONDITIONS OF LIGHT AND TEMPERATURE

Conditions of Uptake	Concentration Factor (C.F.)		
	Enteromorpha	Sphacelaria	Gracilaria
500 foot-candles of light			
@ 25°C	1,100	9,000	5,300
18°C	1,200	13,000	1,200
5°C	760	4,000	430
10 foot-candles of light			
@ 25°C	1,100	8,700	520
18°C	700	4,700	390
5°C	1,400	3,500	260
1-2 foot-candles of light			
@ 25°C	720	3,000	220
18°C	630	2,800	150
5°C	560	2,800	150

The C.F. curves for Enteromorpha prolifera show uptake variations quite similar to those of Gracilaria. Referring to Figures 9 through 12 and Table 3, it is noted that the dim light produced little change in C.F. and, therefore, the uptake was achieved mainly by way of non-metabolic processes, which may be assumed as adsorption only. The variations of the C.F. under moderate light condition as shown in Figure 12, fluctuated oddly as the temperature decreased. It is believed, as will be brought out later, that this moderate-light curve should have decreased along the path represented by the dotted line and not increased as experimentally found. With reference to Table 3, it is noteworthy to see that under the bright light condition the green alga showed far less uptake than Gracilaria. One reason for the higher uptake by the red alga could be that it had in addition to chlorophyll at least one more photosynthetic pigment -- a phycobilin, probably r-phycoerythrin (23). It is now known that such an accessory pigment can aid in the process of photosynthesis by absorbing light quanta and then transferring this absorbed energy perhaps to chlorophyll a, the principal photosynthetic pigment which then functions as if it had absorbed the original light quanta. It is then possible for light of such wave lengths that cannot be absorbed by chlorophyll to become active photosynthetically by this means (70). Hence, it is very possible that the phycobilin in Gracilaria was a more effective absorber of light energy than the chlorophyll in Enteromorpha and this could account for the higher Gracilaria uptake and therefore higher C.F. However, the higher C.F. for Enteromorpha under the moderate and dim light conditions as compared to Gracilaria for the same conditions could be attributed primarily to the fact that

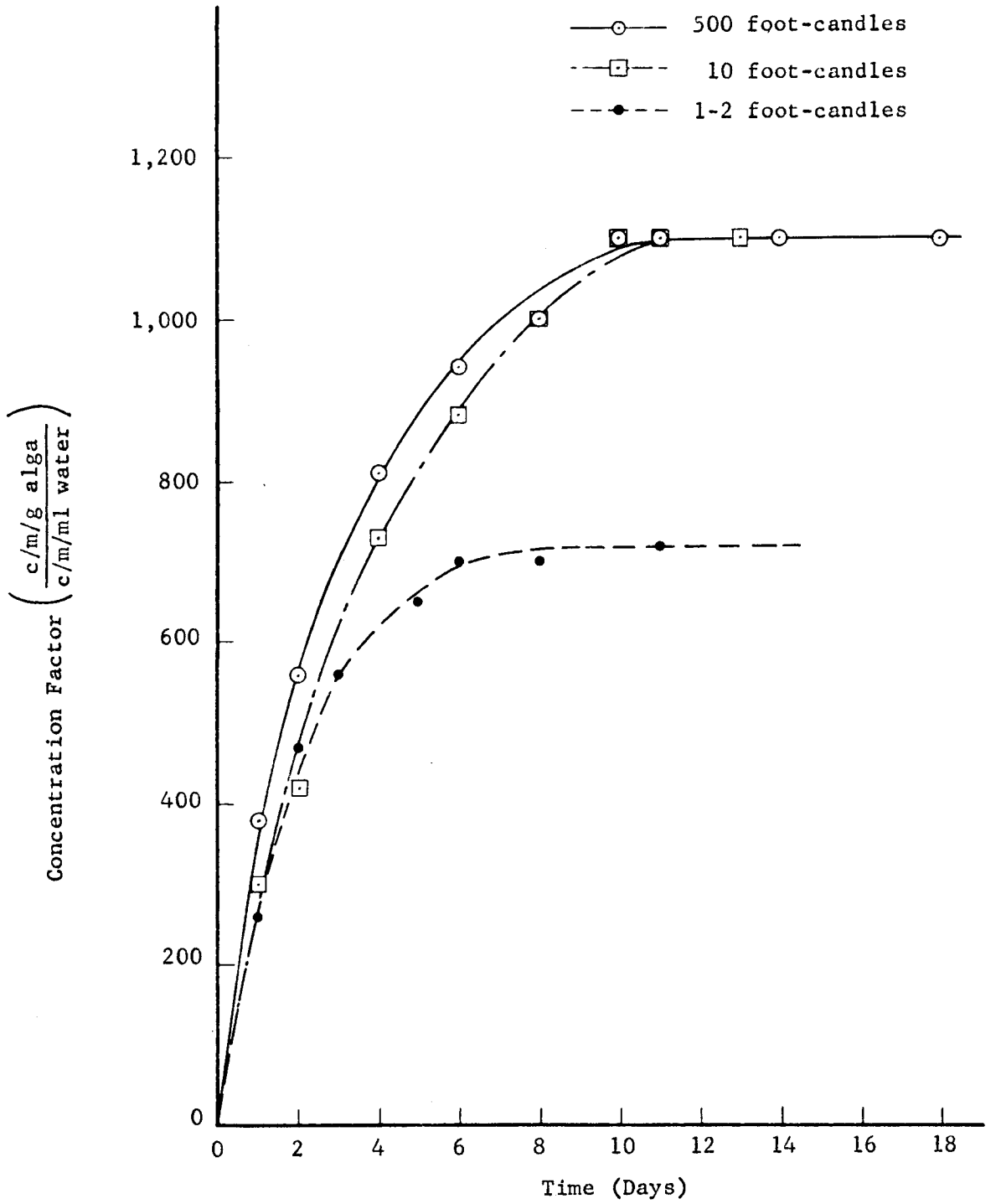

Fig. 9.- Concentration factors for Enteromorpha prolifera in Zn^{65}-dosed sea water under various lighting conditions at 25°C.

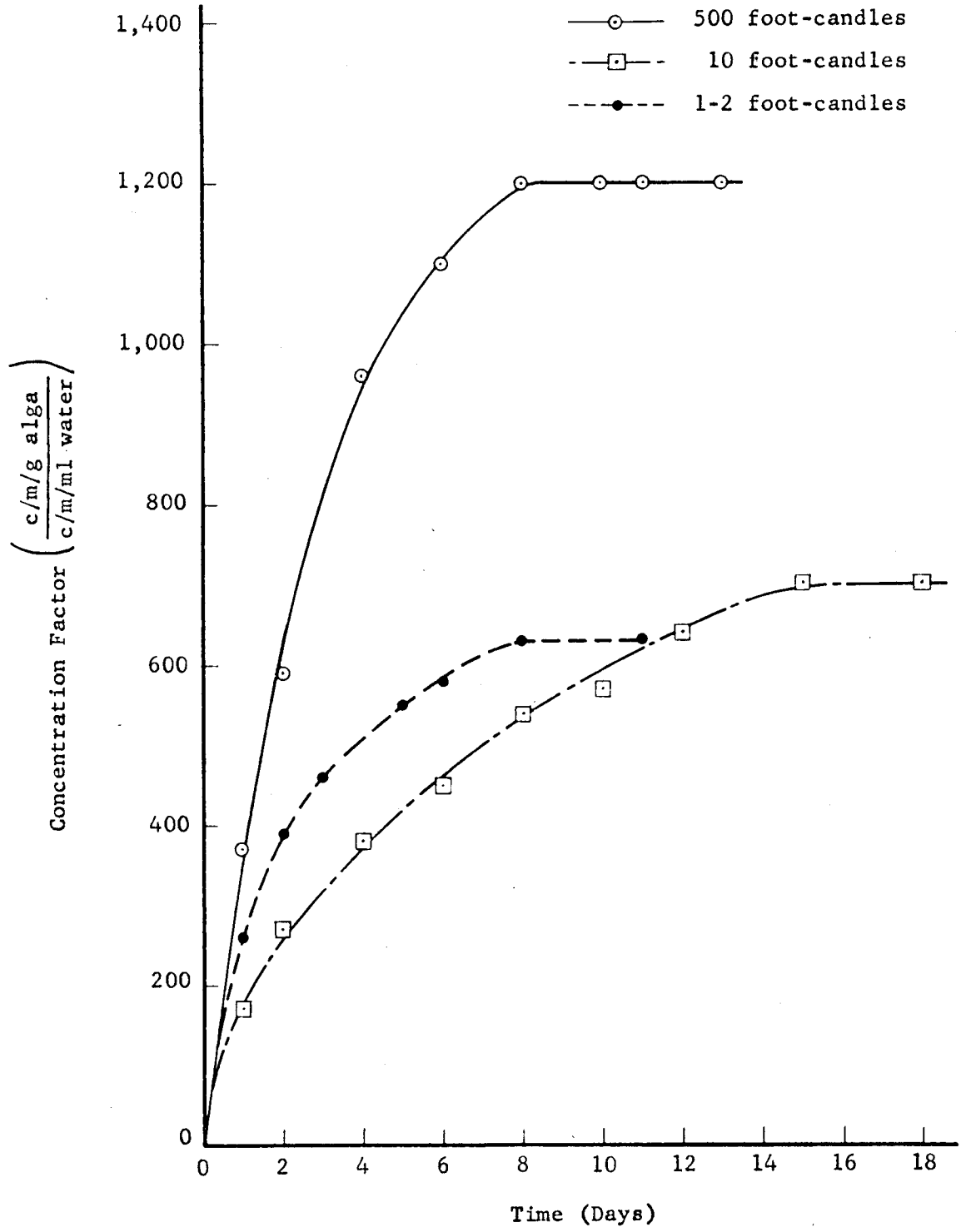

Fig. 10.- Concentration factors for Enteromorpha prolifera in Zn^{65}-dosed sea water under various lighting conditions at 18°C.

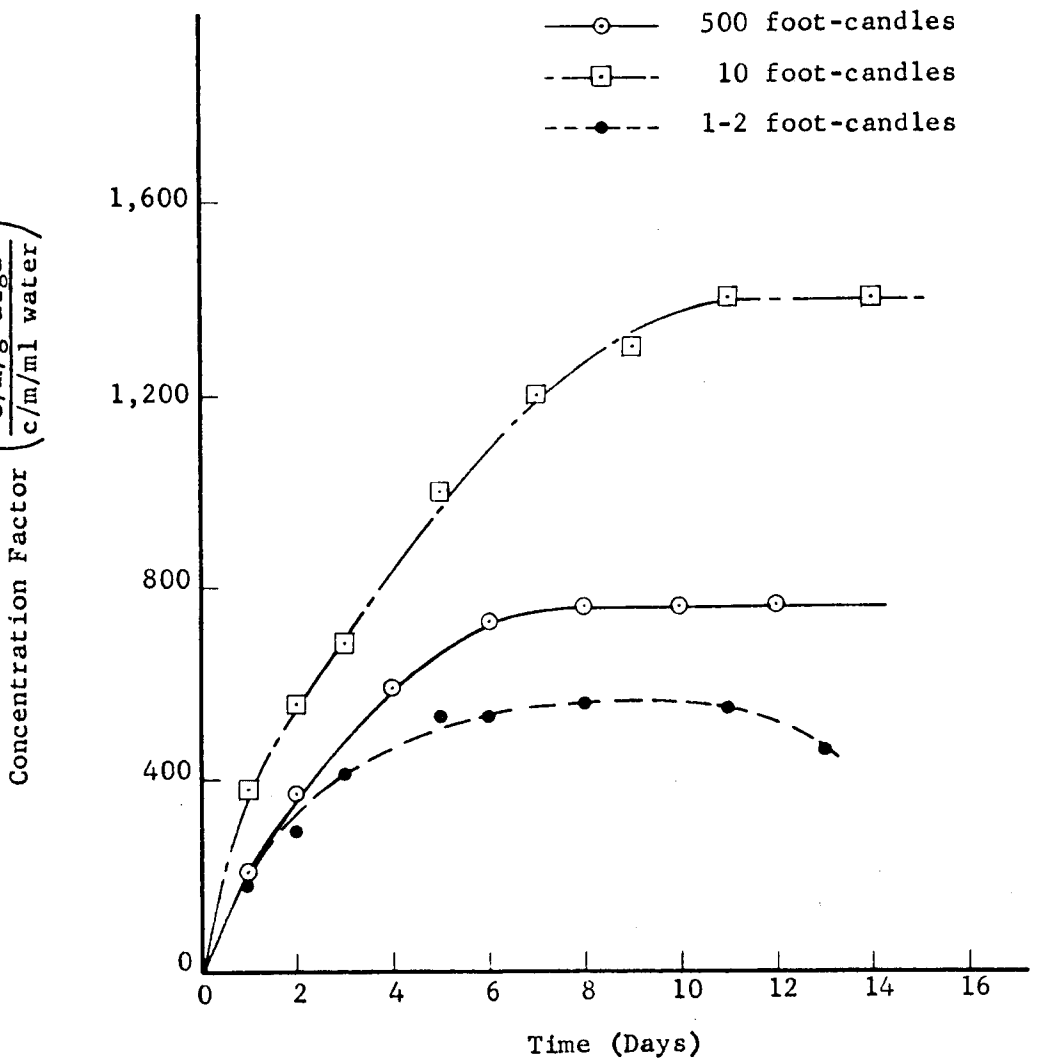

Fig. 11.- Concentration factors for Enteromorpha prolifera in Zn^{65}-dosed sea water under various lighting conditions at 5°C.

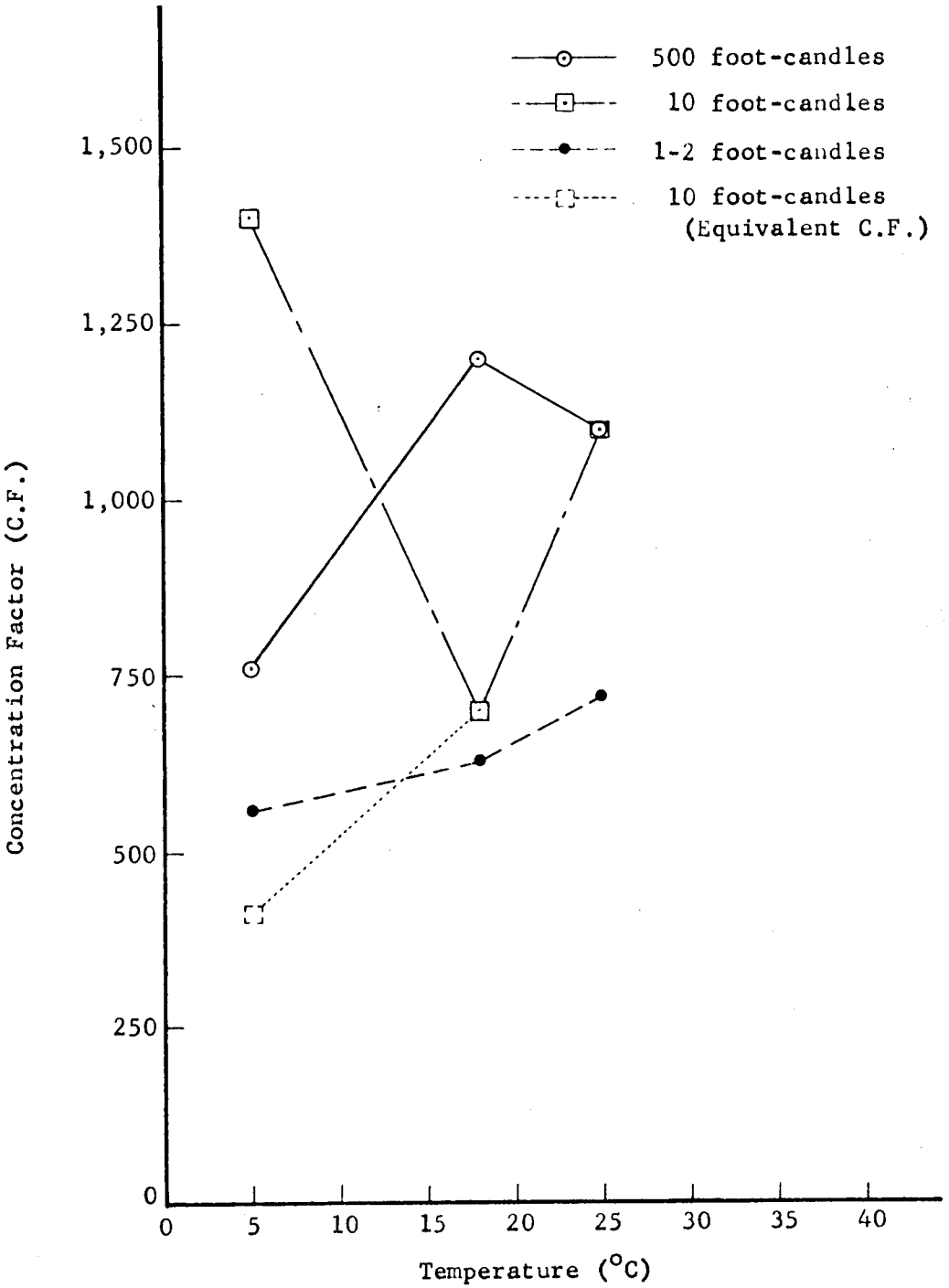

Fig. 12.- The approximate maximum concentration factors of Enteromorpha prolifera for Zn^{65} in dosed sea water under different lighting and temperature conditions.

Enteromorpha had more surface area per unit weight of organism and was therefore capable of more uptake by adsorption. So under the poor lighting situations where photosynthetic pigments of both algae were somewhat ineffective, the alga with the more area per unit weight yielded the higher C.F.

The Sphacelaria curves as shown by Figures 13 through 16 depict C.F.'s which are very high as compared to those of either Enteromorpha or Gracilaria. It is also seen that the Sphacelaria curves are quite irregular in shape as compared to the other two algae. Both the high values of the factors and the irregularities of the curves can be attributed to the growth rate and surface area of the brown alga.

As already seen, any one of the C.F. curves for Enteromorpha and all but one for Gracilaria are smooth curves. This, for the most part, was caused by the fact that these latter algae had small surface areas per unit weight of the organisms. From measurements made by using a microscope fitted with a calibrated ocular micrometer, the average diameter of the individual (nearly cylindrical) branches of Enteromorpha were found to be approximately 300 microns (μ). Those of Sphacelaria were measured as nearly 25 μ. Accordingly then, as calculations in Appendix VIII show, the surface area of a given weight of Sphacelaria was in the order of 12 times as large as the surface area of an equal weight of Enteromorpha under similar growing conditions. Although the growth rate of the green alga was about 1.5 times as great as that of the brown (see Figure 17) its surface area increase was calculated to be slightly greater than one-tenth that of the brown alga. For all practical purposes then, the brown alga increased its surface

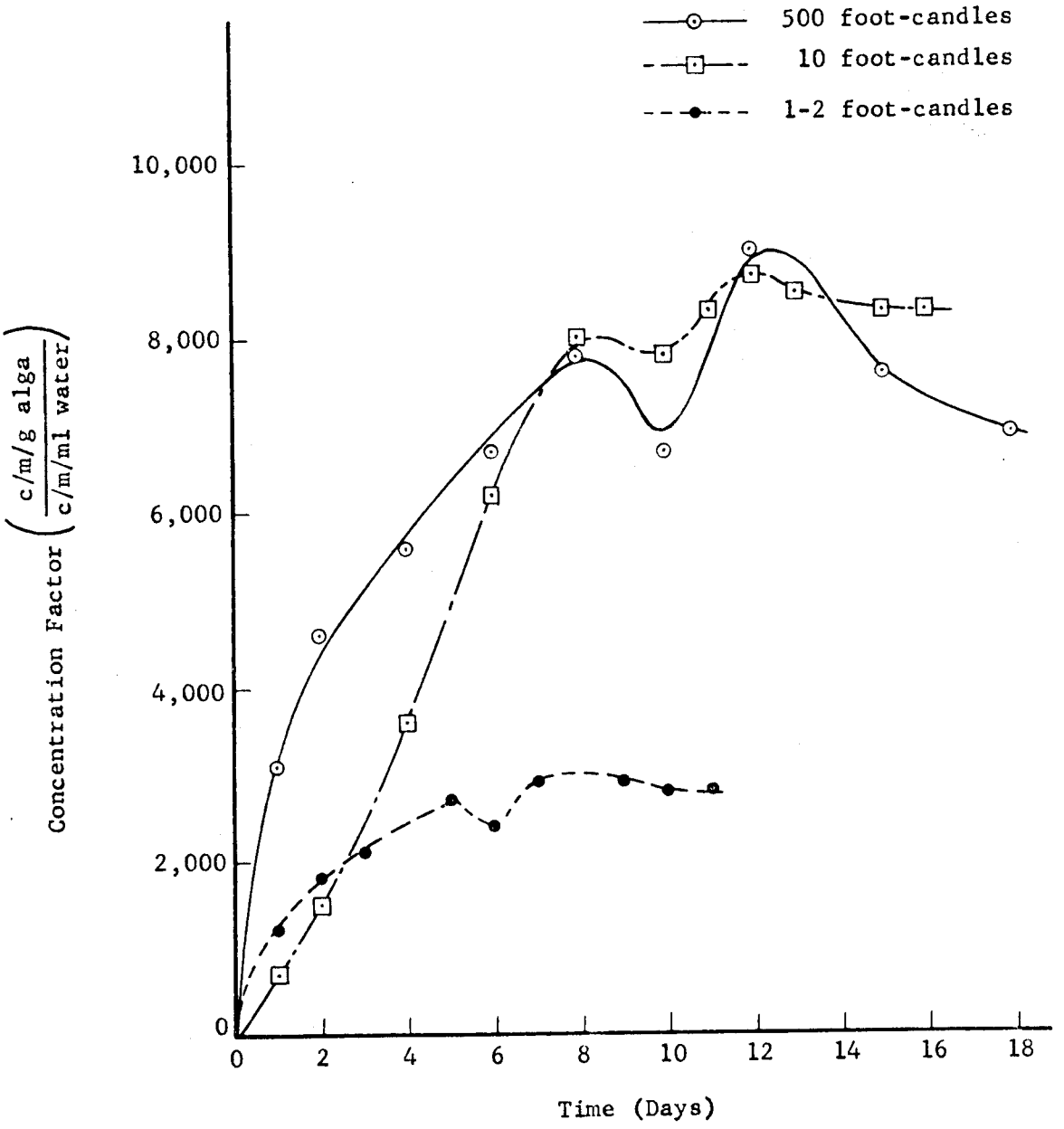

Fig. 13.- Concentration factors for Sphacelaria sp. in Zn^{65}-dosed sea water under various lighting conditions at 25°C.

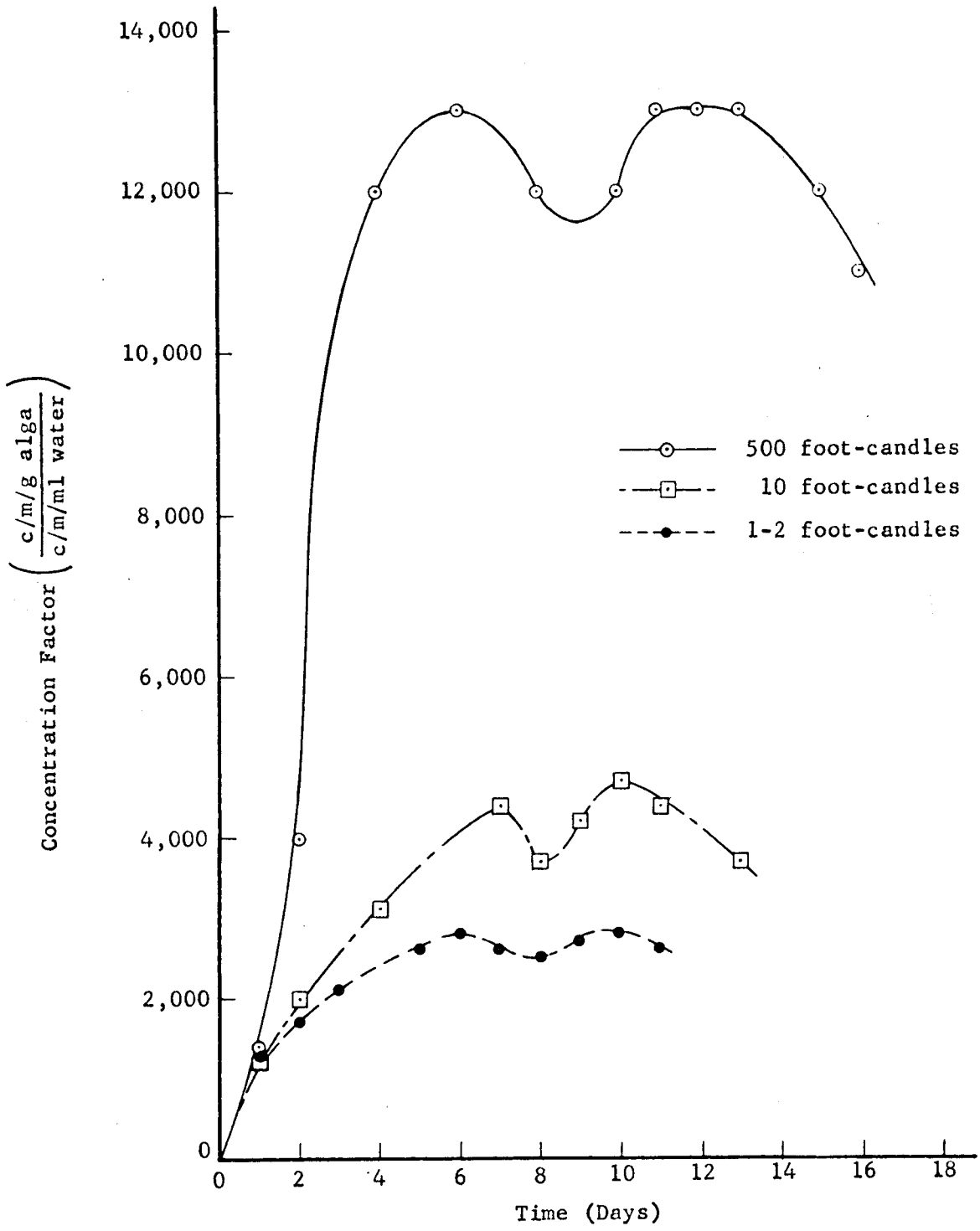

Fig. 14.- Concentration factors for Sphacelaria sp. in Zn^{65}-dosed sea water under various lighting conditions at 18°C.

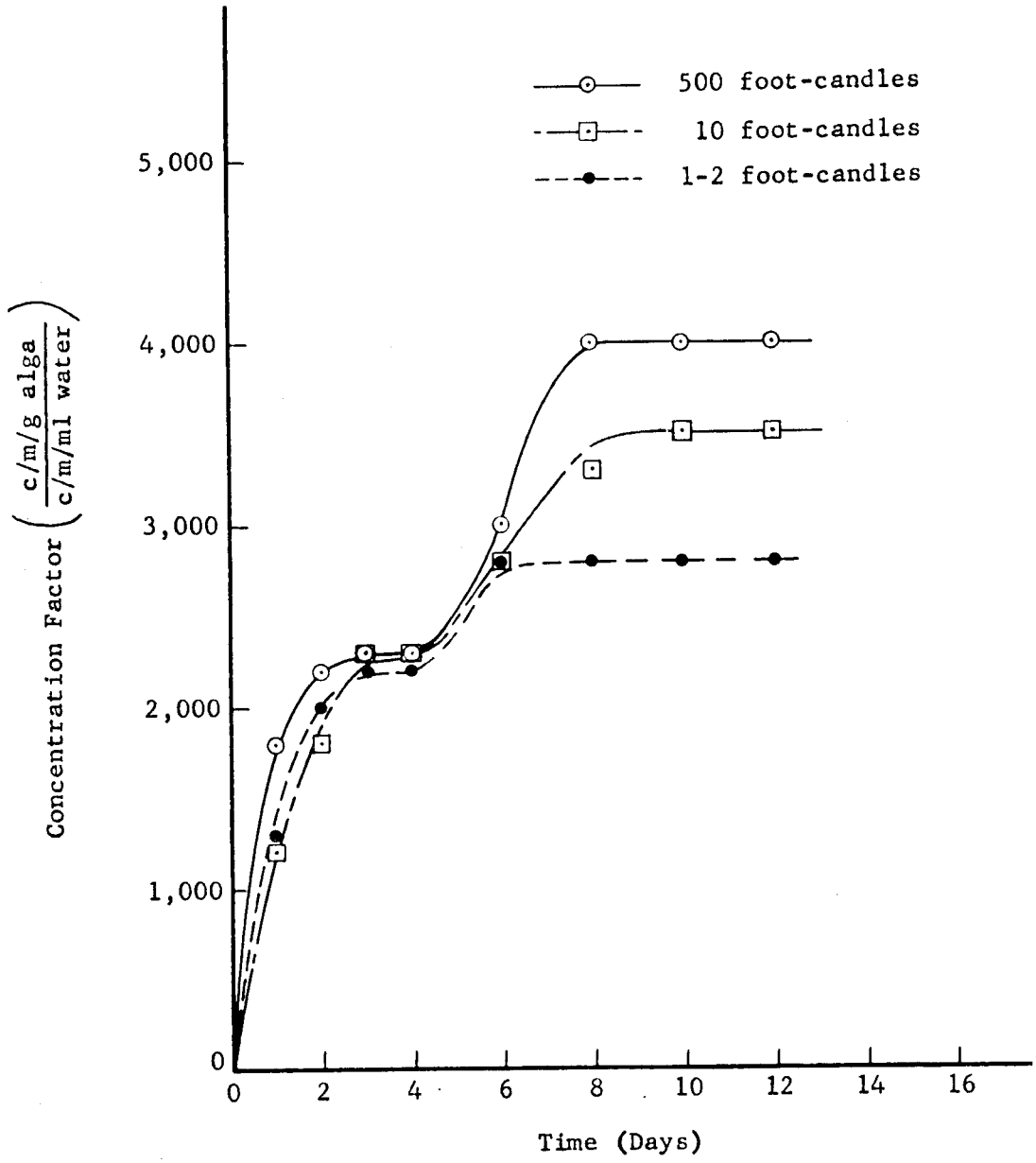

Fig. 15.- Concentration factors for Sphacelaria sp. in Zn^{65}-dosed sea water under various lighting conditions at 5°C.

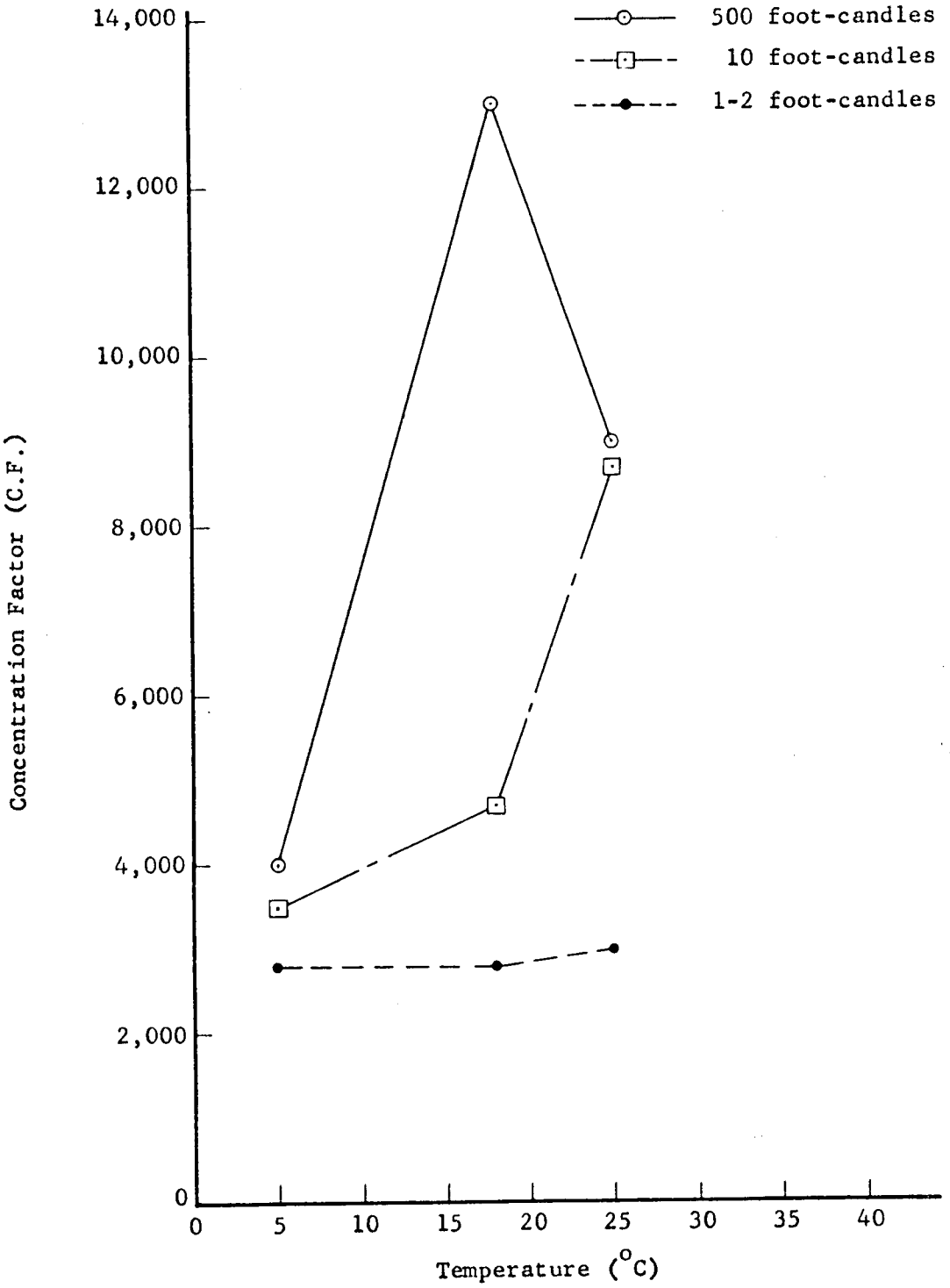

Fig. 16.- The approximate maximum concentration factors of Sphacelaria sp. for Zn^{65} in dosed sea water under different lighting and temperature conditions.

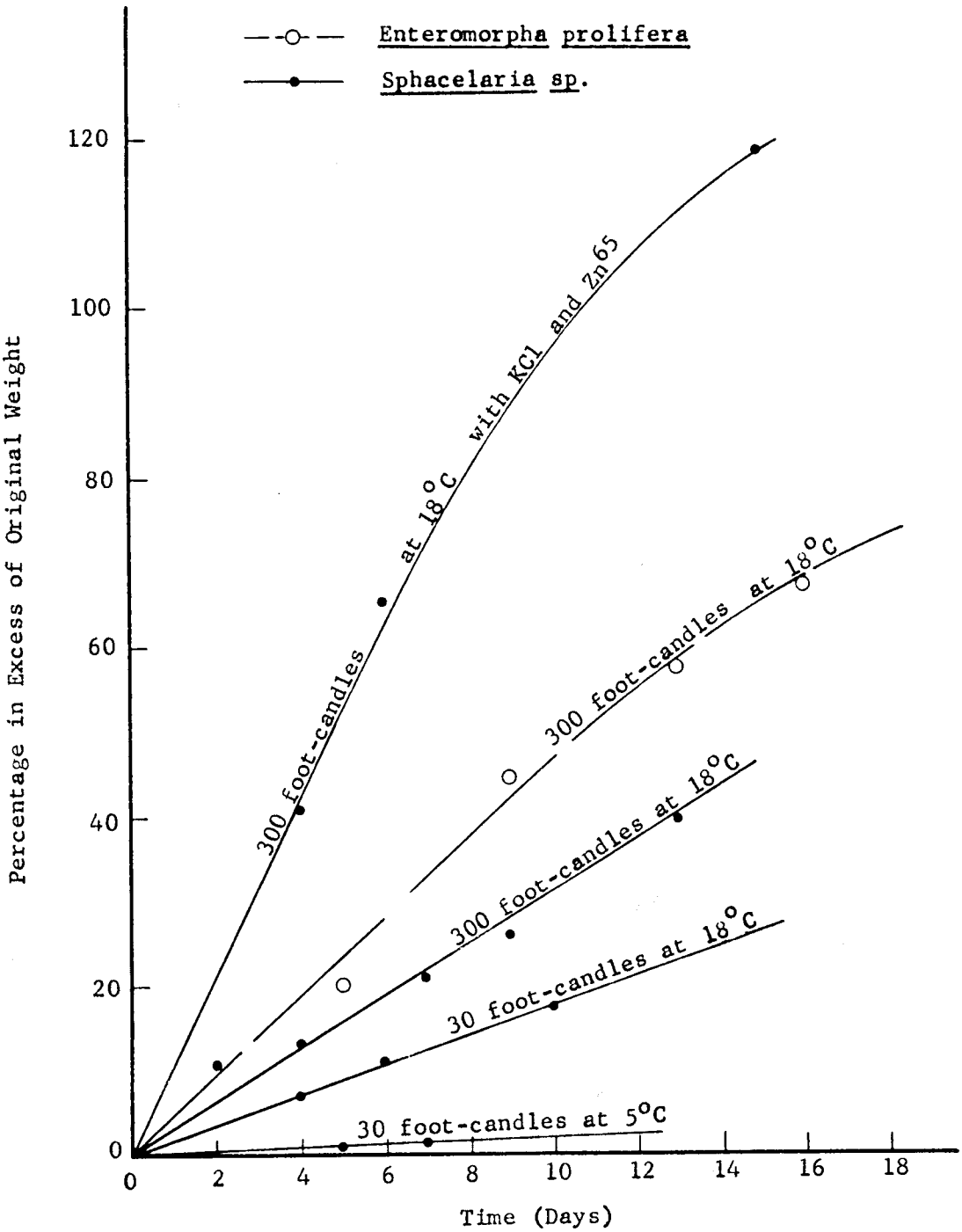

Fig. 17.- Growth rate curves for Sphacelaria sp. and Enteromorpha prolifera under various conditions of lighting, temperature, and medium enrichment.

area ten times faster than the green alga. It is felt that the peculiar dips in the C.F. curves for Sphacelaria were caused by this rapid surface area growth. A glance at Figure 17 will indicate readily that the uptake by Sphacelaria was not directly related to its growth rate. That is, the dips in the C.F. curves were not caused by dips in the growth rate curves. If nothing else, the growth curves do indicate that for a variety of conditions, the alga continually gains weight by growing and does not lose weight. In analyzing the dips, there are perhaps two possible mechanisms whereby an organism which is increasingly accumulating a radioisotope could possibly lose such an accumulation. An organism can lose its concentrated radionuclide by simply releasing it back to the water, or it can appear to have lost it by growing so rapidly that the new growth had not accumulated its share of the radionuclide and hence a dilution effect is experienced. In other words, the activity per gram of the growing organism gets smaller without any radioactive substances actually being released by the organism. This explanation for the apparent radioactive reduction seems more feasible than the one concerning an actual loss of the radioisotope. For Sphacelaria, this explanation is supported by the fact that the alga was continually growing in a positive direction. The following is an attempt to explain the formation of the dips in the C.F. curves. When the brown alga was first placed in the medium containing Zn^{65}, a large portion, perhaps 70 per cent, of its uptake was caused primarily by adsorption to the surface with the rest being absorbed. As the surface area increased, radioactive substances were adsorbed more rapidly than they were being absorbed. This was feasible since the adsorption process was concerned

with the surface and not with the plant's interior. The growing continued and adsorption continually increased until per unit portion of alga there was a fairly large segment which had adsorbed activity but comparatively little absorbed activity. Then, per unit weight of the alga the activity began to level off then decrease. That part of the alga which up to this time had not fully utilized its absorption capacity for Zn^{65} probably began to increase its absorption process. The increase could have been brought about by the fact that the alga had experienced a sufficient contact time in the Zn^{65}-dosed medium to effect the absorption. From this point on, the activity per unit weight of alga began to climb, level off, and then resume dropping. This second peak and subsequent depression was caused undoubtedly by the continued growth of the plant with increasing adsorption and lagging absorption. It was felt that the uptake would continue in this fashion so long as the plant remained in a good growing condition. This did not happen with the green or red alga probably because the rate of surface increase with weight was small, hence both absorption and adsorption were either likely in phase or their differences were negligible.

The dipping fluctuations of the C.F. curves appeared not to be a direct result of comparable fluctuations in the alga's metabolism because the dips usually occurred about the same time. If the dips had occurred as a result of dips in the variations of metabolic processes, then the metabolic dips must have cyclically occurred for the alga in the undosed water during the conditioning period just prior to the alga's being placed into the Zn^{65}-dosed medium. Now the question arises, why,

if the algal uptake varied directly with metabolic fluctuations, did not the C.F. dips occur haphazardly at different times instead of almost uniformly at the same time? Why could not the plants, for example, have been removed from the non-active water while they were just metabolically cycling into a dip, placed in the dosed water and so indicate the start of the uptake curve with a dip? It is for these arguments that the growth rate idea is preferred. To further substantiate this idea, it was assumed that if the organism could be made to grow faster, then the dips should occur much sooner and perhaps be more severe. This very idea was accomplished by adding approximately 3 grams of KCl to a Zn^{65} uptake experiment and plotting the C.F. curve. Figure 18 depicts such a curve with sharp dips and peaks which do occur earlier as postulated. To make certain that this was truly caused by the growth rate, three pieces of Sphacelaria were grown under similar conditions of the above-mentioned uptake experiment and their growth rates plotted. Figure 17 shows that the brown alga grown under these conditions had a growth rate which was about 8 times greater than the growth rate found when no KCl was used. Since the growth rate was 8 times as fast, then the first dip in the C.F. curve should have generally occurred at about 1/8 of the usual time or in about 1 to 2 days. Figure 18 shows that the dip occurred between the second and third days. This experiment, it was felt, substantiated the growth-rate theory. Further, the C.F. curves shown in Figure 15 indicate by their dips that the algal metabolic uptake must have been somewhat arrested by the low $5^{\circ}C$ temperature and, hence, the immediate C.F. increase from time "0" to time "2 days" in all probability was caused by adsorption. The absorption of Zn^{65} was, perhaps, retarded

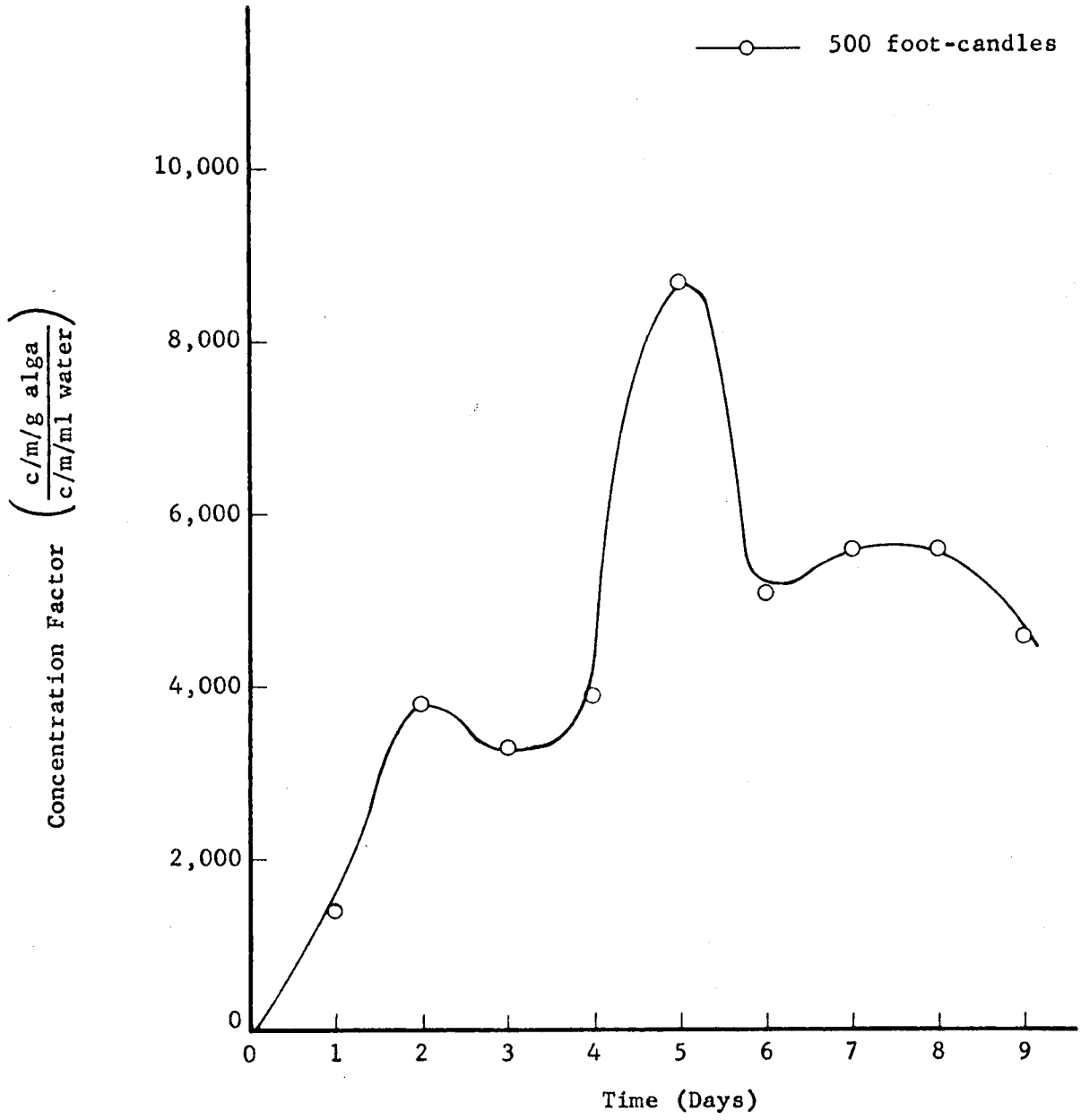

Fig. 18.- The concentration factors for Sphacelaria sp. at 25°C in Zn^{65}-dosed sea water enriched with KCl.

by the cold and, as the plant grew slowly it was adding on weight without adding on its proportionate amount of radioactive uptake. It was assumed, that because of this, the C.F.'s leveled off only to rise when probably both adsorption and absorption again became operable. The growth rate curve for Sphacelaria at 5°C shown in Figure 17 indicates a very shallow weight increase which was compatible to the low concentration factors found at the low temperature. The growth rate for Sphacelaria under bright light and 18°C temperature was quite high and this also was compatible to the C.F. curve in Figure 14.

After studying the C.F. curves developed from the uptake experiments, it was desired to ascertain what made one alga a better concentrator than another. To do this at least two algae, one having a higher C.F. value, had to be mutually compared. The organism having the higher value was then, by the application of certain assumptions, made equivalent to the one having the lower C.F. value. Having thus made this transition, the C.F.'s for the equivalent alga were calculated and compared to those of the actual alga. The above transition was accomplished by converting Sphacelaria to an equivalent Enteromorpha by the following approach. Of the probable factors which caused Sphacelaria to concentrate so much more Zn^{65} than Enteromorpha the two prime factors were, perhaps, surface area and the different photosynthetic pigments. Not only had the brown alga a larger surface area than the green, as previously calculated, but it also had, in addition to the chlorophylls at least one more photosynthetic pigment which is common to almost all Phaeophyceae, namely, fucoxanthin (23). Assume that the concentration of Zn^{65} was accomplished by both adsorption to the algal surface and

absorption within the alga. (These assumptions will subsequently be proven feasible.) To equalize the surface area effect of Sphacelaria with that of Enteromorpha, the uptake of the brown alga was first separated into its adsorbing and absorbing fractions. The adsorbing fraction, which was assumed to be at least 10 times greater than that of the green, was then reduced by this amount. Actually, the surface area had been calculated as being 12 times as great, but not knowing exactly how this difference operated, the lesser factor of 10 was considered a valid assumption. Further, the absorption was no doubt influenced by the surface area and again owing to uncertainty, about 1/4 of the calculated difference was used. This factor for absorption was, therefore, assumed to be 3. Now, how much of the absorption was caused by the fucoxanthin? From Fogg's work (23), it was assumed for the approximate light spectrum range as utilized by plants ($4{,}000 \times 10^{-9}$ cm to $7{,}000 \times 10^{-9}$ cm) that both the chlorophyll and fucoxanthin share the light absorption equally. Hence, for this property a factor of 2 was considered. To summarize briefly: the adsorbing fraction of the brown alga's uptake was divided by 10 and the absorbing fraction was divided by 6 (3 x 2). The corrected uptake was then used in the calculation of the C.F. These new C.F.'s determined from the Sphacelaria data should then resemble the actual C.F.'s for Enteromorpha. Table 4 shows these comparative data which indicate the proximity of the C.F.'s figured for both the equivalent and actual organisms. Based on these data, it would seem that in Figure 12 the C.F.'s for Enteromorpha in the low temperature range should perhaps have decreased along the dotted line instead of increasing as found experimentally.

TABLE 4

COMPARISONS OF THE APPROXIMATE MAXIMUM CONCENTRATION FACTORS OF THE EQUIVALENT ALGA TO THOSE OF THE ACTUAL ALGA

Conditions of Uptake	Concentration Factor (C.F.)		
	Actual Sphacelaria	Equivalent Enteromorpha	Actual Enteromorpha
500 foot-candles of light			
@ 25°C	8,900	1,100	1,100
18°C	13,000	1,600	1,200
5°C	4,000	460	760
10 foot-candles of light			
@ 25°C	8,700	1,000	1,100
18°C	4,700	600	700
5°C	3,500	410	1,400
1-2 foot-candles of light			
@ 25°C	3,000	380	720
18°C	2,800	330	630
5°C	2,800	320	560

At least one uptake replication for each alga was made under various conditions of light and temperature. The replications were made only to provide an approximate check on the initial experiments and not meant to increase the accuracy of these experiments. The comparisons of the replicates to the originals are listed in Table 5 and indicate a reasonable variation.

The relationship between an alga's C.F. and the amount of decontamination afforded by the alga was approximately ascertained. There seemed to be an interrelationship between the C.F. and the weight of alga used. It was pointed out that the C.F. was numerically equivalent to a volume of dilution water which would reduce the activity level of the dosed water by the same amount as would be realized by the uptake

TABLE 5

COMPARISONS OF THE APPROXIMATE MAXIMUM CONCENTRATION FACTORS AS DERIVED FROM ORIGINAL AND REPLICATED UPTAKE EXPERIMENTS

Organism and Conditions of Uptake	Concentration Factor (C.F.)	
	Original	Replication
Gracilaria		
500 foot-candles of light @ 5°C	430	390
Sphacelaria		
500 foot-candles of light @ 25°C	9,000	7,600
Enteromorpha		
500 foot-candles of light @ 5°C	760	720

of one gram of alga. It is then apparent that more than one gram of alga would have a greater reduction effect. Also, it seems very probable that 100 grams of an alga having a low C.F. could cause more reduction of an active water than perhaps one gram of an alga having a slightly higher C.F. Therefore, a large C.F. value per se is not indicative of the amount of reduction possible. The ultimate radioactive reduction is apparently a function of both C.F. and quantity of alga used. In support of this theory the following general comparison was made.

From each uptake experiment, an approximate dried weight of the total alga used was averaged from the many weighed samples assayed. Using this very approximated average weight, and the average C.F. value from the appropriate C.F. curve, an approximate total dilution effect as caused by the entire amount of alga was determined. This dilution value was then compared to the dilution value as determined by the ratio of initial water activity to final water activity. Of course these latter values must represent the activities of the water as it was affected by the algae only and not by the glass. In order to correct for any glass effect, the actual uptake of the glass had to be determined. As an example as to how this determination was made, the water data for Sphacelaria under the condition of 500 foot-candles at 18°C will be used. These data as compiled in Appendix VII are graphed in Figure 19. With reference to the upper half of this figure, there are plotted three distinct curves. The theoretical activity level curve represents the level of activity that would probably be present in the water if no uptake of any kind were realized.

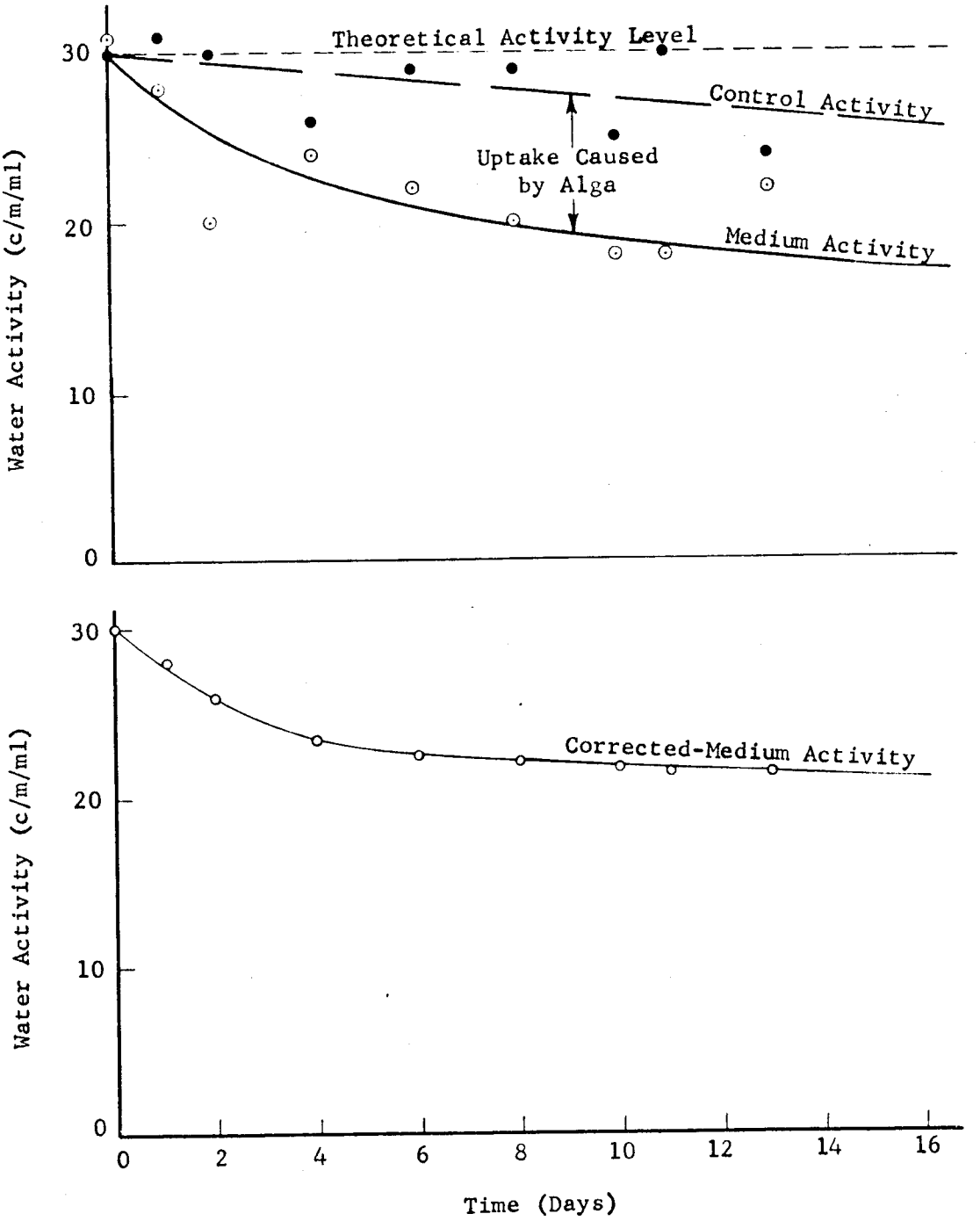

Fig. 19.- Typical working graphs depicting the determination of the corrected-medium curve.

The control curve represents the radioactivity change in the water as caused by all possible effects other than the alga under consideration. The medium curve represents the radioactivity change in the water as caused by all possible effects plus the uptake capacity of the alga. Therefore, the difference between the medium curve and the control curve represents in all probability the uptake achieved by the alga. Now, if this uptake amount is subtracted from the theoretical curve, there will result the so-called corrected-medium activity curve. This latter curve insofar as is practical should represent the true activity in the water as it is affected only by the uptake of the alga and nothing else. It is the activities as represented by this curve that were used in the calculations of the dilution factor. If all involved factors in the uptake studies had been accurately and precisely determined, then the dilution effects as developed by the two methods previously mentioned should be similar. The total dry weight of alga approximated from the C.F. data of each uptake experiment for any one of the three algae was found to be: 1.1, 1.4 and 3.6 grams for Enteromorpha, Gracilaria, and Sphacelaria respectively. Table 6 records these dilution factors and indicates fairly closely the relationship between the C.F. and algal weight in the dilution factor determination.

Retention Studies

In a prior section, it was mentioned that approximately 69 per cent of the uptake was achieved by adsorption while the remaining 31 per cent was by absorption. These data were based upon the retention curves

TABLE 6

COMPARISONS OF THE APPROXIMATE DILUTION FACTORS OF THE MARINE ALGAE AS DETERMINED BY THE CONCENTRATION FACTOR EQUIVALENT VOLUME AND BY THE RATIO OF INITIAL AND FINAL WATER ACTIVITY

Conditions of Uptake	Dilution Factor Determined by Method of Average Concentration Factor Equivalent Volume			Dilution Factor Determined by the ratio: initial water activity/ml / final water activity/ml		
	Enteromorpha	Sphacelaria	Gracilaria	Enteromorpha	Sphacelaria	Gracilaria
500 ft-c						
@ 25°C	1.0	1.4	1.6	1.1	1.2	2.2
18°C	1.1	1.6	1.2	1.1	1.4	1.2
5°C	1.0	1.2	1.2	1.1	1.1	1.2
10 ft-c						
@ 25°C	1.0	1.3	1.0	1.1	1.3	1.1
18°C	1.0	1.2	1.0	1.1	1.1	1.1
5°C	1.1	1.2	1.0	1.1	1.2	1.1
1-2 ft-c						
@ 25°C	1.0	1.2	1.1	1.1	1.3	1.1
18°C	1.0	1.1	1.0	1.1	1.2	1.1
5°C	1.0	1.1	1.0	1.0	1.1	1.1

shown in Figures 20 and 21. The two curves in Figure 20 indicate a definite, almost immediate release of the Zn^{65} contained by the brown alga. Since a surface reaction has a more favorable chance of occurring before an internal reaction, it is likely that this immediate reduction was caused by the loss of the alga's adsorbed fraction of Zn^{65}. The further release of Zn^{65} progressed along at a much slower rate until it appeared to have attained an equilibrium. This eventual leveling off was probably caused by a gradual release of the unsequestered absorbed Zn^{65}. It is interesting to note that the alga placed in the sea water containing an excess of zinc released more Zn^{65} initially than the alga placed in the sea water not containing excess zinc. This was expected as it was felt that the exchange between the adsorbed Zn^{65} on the alga and the zinc in the water would proceed faster where an excess of zinc was present. The retention curves show also that after the initial drop, the release of more Zn^{65} proceeded at a faster rate in plain sea water than in stable zinc-enriched water. After a period of approximately 13 days, both curves began to show equal rates of loss for Zn^{65}, and the algal retention from that time on seems to decrease at a constant rate of 1 c/m/mg/day. The rates in the first two days were about 45 c/m/mg/day which decreased to 3 c/m/mg/day then decreased to the constant rate. A likely explanation for the greater algal retention in the zinc sea water is that the Zn^{65} absorbed within the alga could not be released to the water rapidly because the direction of the zinc concentration gradient was from the water towards the alga. Since there was an excess of zinc outside the algal cells, the tendency for the passage of zinc ions would not be from alga to water but rather the

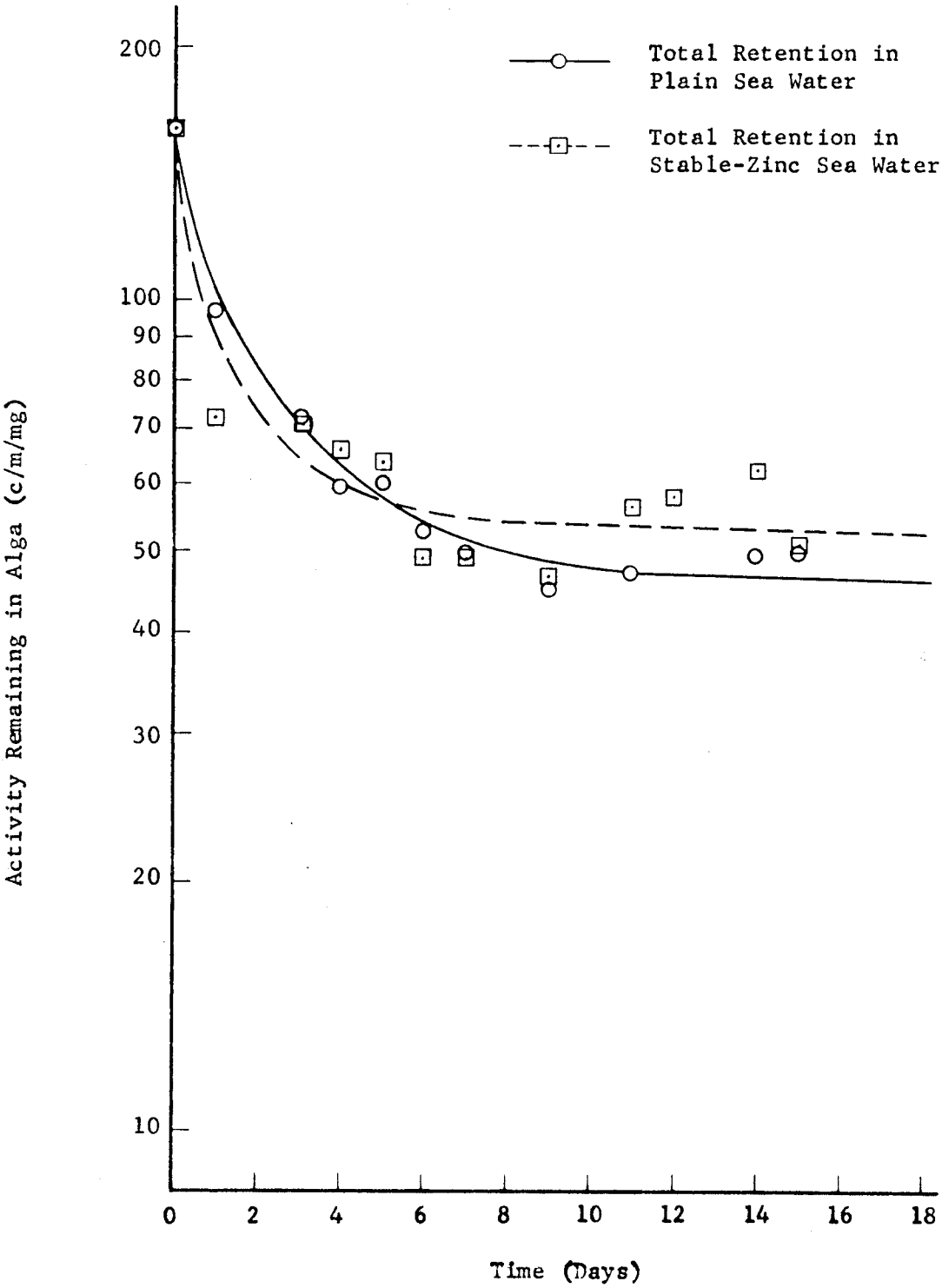

Fig. 20.- Retention of Zn^{65} by Sphacelaria sp. at 18°C, under 500 foot-candles of light, in plain sea water and in sea water enriched with stable zinc.

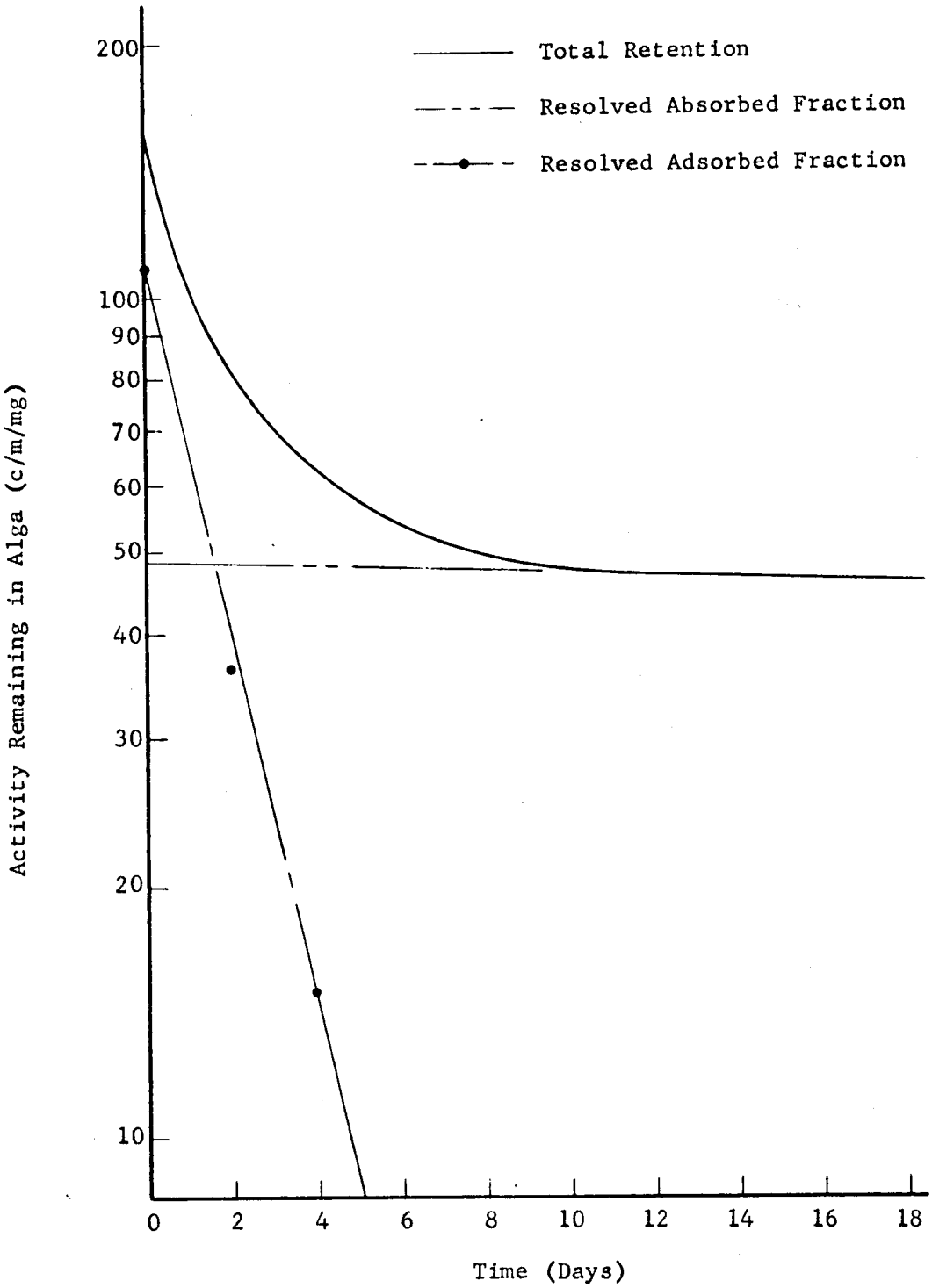

Fig. 21.- Resolution of the retention curve for Sphacelaria sp. at 18°C, under 500 foot-candles of light, and in plain sea water.

reverse. Therefore, after a certain time period when the zinc ions, both active and non active, had approached a certain concentration in both alga and ambient water, then the exchange was able to continue on as it did in the plain sea water situation. In other words, both retention curves are identical with the exception that the excess zinc curve lags 13 days behind the plain sea water curve as a result of the retarding effect caused by the excess zinc. That about 13 days were probably required for an apparent equilibrium to occur between the zinc in the alga and in the ambient water is in good harmony with the alga's C.F. curves. These latter curves show that the maximum uptake required about 13 days at which time the zinc in the water and alga were probably at some close mutual balance. At the time when about 30 per cent of their original uptake still remained, two pieces of the alga were removed from their retention experiment and placed into the uptake setup from which they had originally been taken. The purpose here was to see if the alga once having concentrated Zn^{65} and then having released it could reconcentrate it as much as its previous C.F. level. The results as tabulated in Table 7 indicate that the alga could repeat its prior C.F. quite closely. The shape of both retention curves indicate that they are of a logarithmic decay form and are also capable of being resolved into two components. This resolution was done as shown by Figures 21 and 22.

Approximating the half life (the time required for the loss of one-half of the original concentrated Zn^{65}) it was found directly from the developed resolution curves that the adsorbed portions of the excess zinc and plain sea water curves were 0.4 day and 0.7 day respectively.

For the absorbed fraction, the half-life period was approximately 230 days for the two experimental conditions examined. The latter half lives were calculated by the expression:

$$T_{1/2} = \frac{0.693}{\lambda}$$

where: $T_{1/2}$ = half life in days

0.693 = a constant

λ = slope of absorbed curve in units of $(day)^{-1}$ when the curve is plotted with the natural logarithm of activity vs. time in days.

TABLE 7

COMPARISONS OF ASSAYED ALGAL CONCENTRATION FACTORS IN MEDIUM OF HIGHER ACTIVITY AS COMPARED TO REFINED CONCENTRATION FACTORS IN MEDIUM OF ABOUT 25 COUNTS PER MINUTE PER MILLILITER

Conditions of Uptake for Assayed Algae	Assayed C.F.		Refined C.F.	
	Sphacelaria	Gracilaria	Sphacelaria	Gracilaria
Reuptake: 500 ft-c @ 25°C for 7 days in water of 40 c/m/ml	5,300		7,400	
Reuptake: 500 ft-c @ 25°C for 8 days in water of 40 c/m/ml	5,700		7,800	
Original uptake: 500 ft-c @ 18°C for 8 days in water of 500 c/m/ml		1,500		1,200

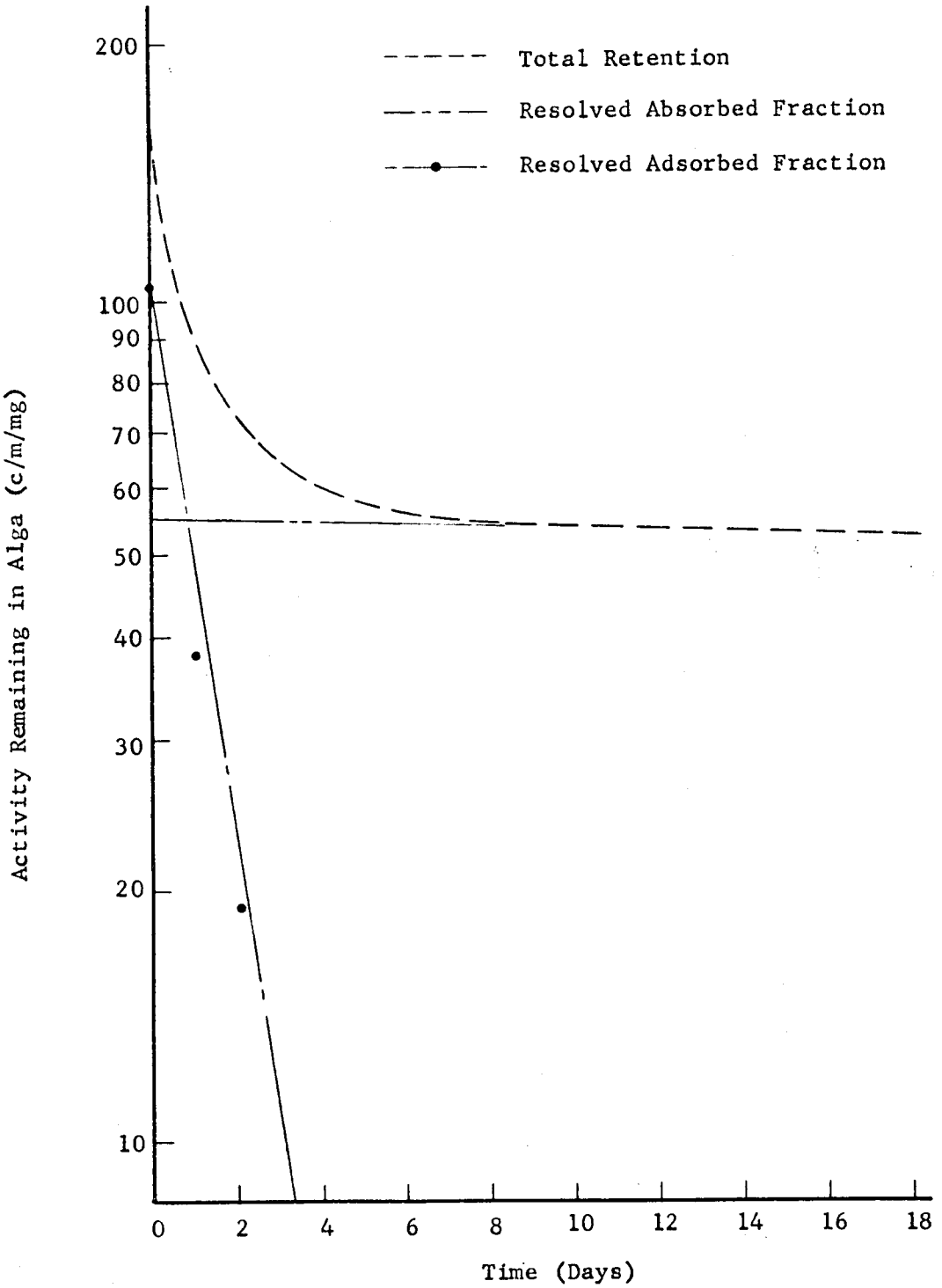

Fig. 22.- Resolution of the retention curve for Sphacelaria sp. at 18°C, under 500 foot-candles of light, and in sea water enriched with stable zinc.

These retention curves show quite well that approximately 69 per cent of the Zn^{65} was adsorbed while the remaining uptake was absorbed. Only a cursory investigation was made concerning the retentive ability of Gracilaria and it was found that approximately 70 per cent of its uptake was achieved by absorption -- just the reverse of Sphacelaria. Since this had not been meticulously investigated, neither data nor curves are recorded here. The intimated results, however, seem feasible because there is reason to believe that a large portion of the red alga's uptake was due to absorption.

It has been shown in this chapter that Sphacelaria's greater uptake as compared to Enteromorpha's was probably caused by the brown alga's larger ratio of surface area to weight and to some degree by the combination of fucoxanthin and chlorophyll. Likewise, from visual inspection, it appeared that Enteromorpha's area to weight ratio was larger than Gracilaria's, hence, the green alga by adsorption alone could possibly have taken up more Zn^{65} than the red alga. The C.F. curves, however, show that under bright light conditions Gracilaria had the higher average value. It is probable then, that the higher C.F.'s for the red alga were caused primarily by absorption and not by adsorption. To further strengthen this point of view, reference is made to the autoradiographs in Figure 23. These are the pictures of two pieces of Gracilaria, one literally covered by fruiting bodies and one without fruiting bodies. It is noted that in the organism containing no fruiting bodies, the thicker lower part of the plant appears, by its lighter contrast, to contain more radioactive substances than any other portion of that plant. Now, if uptake by Gracilaria were achieved

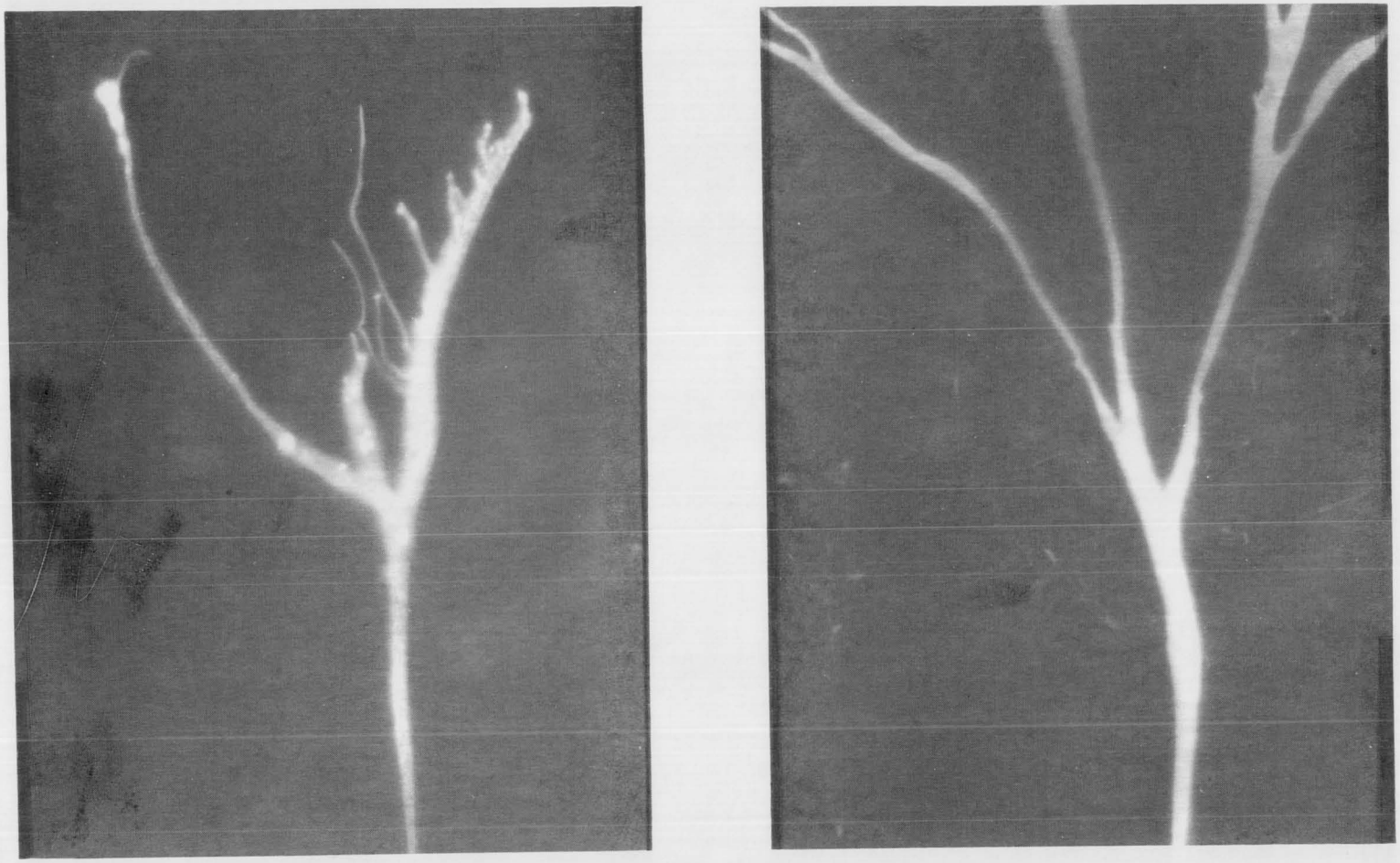

Fig. 23.- Autoradiographs of concentration sites for Zn^{65} taken up by Gracilaria foliifera under 500 foot-candles at 18°C. Left organism with fruiting bodies, right without fruiting bodies.

primarily by adsorption, then the concentration of the radioactive substances everywhere on the plant's surface should have been almost the same and should therefore have had about the same effect on the X-ray film used in making the exposure. Since this was not the case, the higher concentration in the lower portion of the alga must have been caused by the absorbed uptake. Further, when the specimens used in preparing the autoradiographs were dissected into their apparent more active and less active parts and when these parts were assayed separately, the data so derived as tabulated in Table 8 show that all portions of the non-fruiting plant had approximately the same specific activity. The indication from this is that the lighter areas of the autoradiograph were, in all probability, a result of the larger quantity of Zn^{65} concentrated by absorption into the larger mass present in the thick portion of the plain alga. This idea is supported further by the fact that the lower part of the red alga was thicker than the upper branching part. By measuring other pieces of red alga, it was determined that the thicker parts were about 860 microns as compared to 350 microns for the thinner branches. Therefore, the uptake must have been primarily by absorption with some adsorption.

The data in Table 8 for the Gracilaria covered with the fruiting bodies show that these bodies had concentrated Zn^{65} at a somewhat higher rate than the rest of the organism. The uptake achieved by the red alga having fruiting bodies was different from the red having no fruiting bodies. This difference is readily discernible by comparing the peaked C.F. curve in Figure 6 with the curve of the non-fruiting alga plotted immediately below it.

TABLE 8

ACTIVITY OF SPECIFIC PORTIONS OF GRACILARIA FOLIIFERA USED IN THE PREPARATION OF THE AUTORADIOGRAPHS

Organism	Portion	Activity (c/m/mg)
Alga with Fruiting Bodies	Fruiting Bodies	510
	Plain Portion	455
Alga with no Fruiting Bodies	Plain Outer Branches	480
	Thick Lower Branches	500

The algae prepared for both the autoradiographs and for the previous retention experiment had achieved their uptake in waters containing a higher radioactive dose than that used in the uptake experiments. It was decided then to determine the momentary C.F. for these algae and see how they compared with the C.F.'s determined with the lower dose of the uptake experiments. These momentary values were not refined as were the other C.F. values but were approximate and used only to see in a general way what effect concentration had on the C.F. determination. In the same vein of thought the C.F. for the above mentioned fruiting alga was determined and compared to the refined C.F. as found in the peaked graph of Figure 6. These data found in Table 7 point out the fact that the C.F. is apparently independent of concentration and this is in keeping with similar observations made by other investigators as previously discussed in the literature review.

The last portion of this study, dealing with the translocation capacity of algae proved negative. Of course, the negative data might pertain just to the organism used or to the method. Nevertheless, the examination was conducted six separate times and each test indicated no translocation of any kind.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The atomic industry, like most industries, has a waste disposal problem which, at present, shows signs of future augmentation. However, unlike most wastes of other industries, the atomic wastes by nature of their ionization potential are generally deleterious to man and most other biologic entities. Therefore, the need to handle these wastes with more purpose is not uncommon. For the young atomic field, the capacity to care for its radioactive wastes efficiently is most vital for its productive expansion. Without adequate disposal methods, the atomic industry can reasonably expand insofar as the environment can handle its radioactive wastes with no deleterious effect upon man or his immediate surroundings. Increasing the environment's capacity for accepting such wastes safely would no doubt abet the expansion of the atomic field, much to man's benefit.

Several possible methods for the disposal of these wastes have been mentioned and partially discussed. These various methods were all essentially the same in that they, in principle, advocate containment and/or treatment of the active wastes. For high-level wastes, such disposal practices are absolutely essential. On the other hand, these methods are not generally needed for low-level wastes which can be safely disposed of by dilution methods. One such dilution medium is the ocean. Here, both the packaged wastes from land and raw wastes from marine and land installations are cast into these vast waters.

Prior to the present time, it was thought that once the packaged wastes were committed to their remote watery site, they were locked out of man's environment for virtually hundreds of years. The current philosophy being accepted generally in this regard is that there is a possibility of these wastes returning to man via both subsurface and surface ocean currents. Raw wastes passed into the ocean can also be returned to man's coastal waters by these currents. This latter situation appears more probable in the light of recent advances and application of atomic power to marine vessels.

It was because of these weaknesses in ocean disposal that the present study was conducted. Since there is some possibility that a potential contamination of the coastal waters might occur, it was felt that certain denizens of these waters; namely macroscopic algae, might naturally aid in mitigating any such contamination. Generally, many radioactive uptake studies had been made concerning algae and a few specific studies were made concerning the macroscopic species. Most of these studies led to the unequivocal result that algae can generally take up radioactive nuclides in amounts that exceed by a factor of several thousands the concentrations of these nuclides in the ambient water. The tremendous concentrative power displayed by these algae made them look appealing as a potential decontaminating agent and further they also displayed a feasibility as an indicator of radioactivity (10).

Not too much was known about these algae and their relationship to radioactive decontamination. Paucity of such information led to the particulars undertaken in this research. The field of such research

is unquestionally large and this dissertation could possibly satisfy only a small portion of that research.

The particular algal interests that were under investigation concerned the effect of temperature and light upon the uptake capacity of three different algal species. The algae used were Enteromorpha prolifera (green), Sphacelaria sp. (brown), and Gracilaria foliifera (red). The algae were studied also for retention capacity and translocation ability. Additional studies were conducted in the search for relationships between algal growth and uptake and for determination of sites of concentration in algae.

Separate uptake experiments were conducted by subjecting each of the different algae, contained in twenty-three liters of Zn^{65}-dosed sea water, to the varying conditions of light and temperature. The algae were periodically sampled and assayed concurrently with a sample of their ambient water. The uptake data so gathered were then converted to what is commonly known as the concentration factor, this being the activity in counts per minute per gram of dried alga divided by the activity of one gram (1 ml) of the concurrently sampled sea water. To all these data, pertinent counting corrections, such as geometry, background, backscatter, dead time, self-absorption, and detector efficiency were considered and applied where applicable.

To explain some of the behavioral tendencies as shown by the plotted C.F. values, growth curves for the green and brown algae were ascertained. As a further aid, specific size dimensions for all three algae were determined by methods of microscopy.

For the retentive capacity of these algae, only the brown, Sphacelaria was used while only a token amount of the red, Gracilaria was so examined. The results with the red alga were mentioned in only a general fashion, but those with the brown alga were plotted and more fully discussed. Analysis of these curves revealed to a certain extent the nature of the concentration ability of the brown alga.

Autoradiographs were made by using the red alga since it was readily suitable for such an experiment by virtue of its large size. This experiment resulted in a possible answer for the uptake nature of the red alga. It strengthened the results found for the token retention study and also seemed to shed some light on the nature of the green alga's uptake.

The last phase of study was the translocation ability of the red alga. Again this alga was chosen for the experiment because of its suitable morphological characteristics. This study indicated that Gracilaria probably could not translocate Zn^{65} appreciably from one position of the plant to another.

From all these studies, conclusions were gleaned and are, for the most part, not complete nor absolute. Much further work needs to be done to fully understand the potential of the macroscopic algae in the atomic field. The conclusions are as follows:

1. It has been generally accepted that one major factor attributing to the large metabolic uptake by some microscopic algae is their large surface area to weight ratio. Likewise, from the concentration factor and growth curves, it seems that Sphacelaria sp. could have acquired its high concentration capacity primarily by having a large

surface area to weight ratio. The concentration factors of microscopic algae as discussed in the literature review, were generally in the order of 50,000 to 200,000 whereas the macroscopic algae in this study indicated approximate maximum concentration factors of 1,200 for Enteromorpha prolifera, 13,000 for Sphacelaria sp., and 5,300 for Gracilaria foliifera.

2. The concentration factors of Sphacelaria are reasonably independent of the concentration of Zn^{65} in the ambient sea water. This is true for the present study where there was a large abundance of stable zinc as compared to Zn^{65}.
3. From the reasonably high concentration factors of the macroscopic algae, it can be concluded generally that these organisms have a potential for decontaminating coastal waters where they grow luxuriously.
4. Under certain environmental conditions, Sphacelaria and Gracilaria species containing other pigments in addition to chlorophylls are able to concentrate more Zn^{65} metabolically than Enteromorpha which contains only chlorophylls.
5. Rapidly growing fruiting bodies of Gracilaria, not only concentrate more Zn^{65} than the normal cells of this red alga, but also concentrate it about 10 times faster.
6. Metabolic uptake by algae continues as long as the plant grows. Once the growth ceases, uptake is achieved primarily by adsorption.
7. Low temperatures, like reduced light intensity (both within the ranges as used in this research), have about the same general effect on algal uptake. It had been found from algal growth curves that

the growth rate of Sphacelaria was reduced from 1.8 mg/day at 20°C to 0.17 mg/day at the 5°C temperature. This, then, indicates that the effect of low temperature and perhaps of dim light upon the plant is one of growth inhibition and, hence, reduces uptake.

8. There seems to be definite optimum growth conditions which are conducive to maximum uptake. For Enteromorpha and Sphacelaria species, bright light of 500 foot-candles at 18°C were found to be best; for Gracilaria, the best conditions were the bright light at 25°C.

9. Results from this study indicate that multicellular organisms, such as Gracilaria foliifera achieve approximately 70 per cent of their uptake probably by metabolic absorption while the remaining 30 per cent is accomplished by adsorption. However, multicellular filamentous plants like Sphacelaria sp. seem to achieve approximately 70 per cent of their uptake by adsorption and about 30 per cent by absorption.

10. Retention capacities of organisms which achieve their uptake primarily by adsorption are probably not as good as the retention capacities of organisms which achieve most of their uptake by metabolism. The results with Sphacelaria indicate a half life for retention of about 0.7 day for the alga's nearly 70 per cent-adsorbed uptake, whereas Gracilaria retention studies indicate an approximate 230 day half life for the plant's almost 70 per cent-sequestered uptake.

11. Since some algae can retain a portion of their uptake for about 230 days, they can be used as indicators of past or present radioactive contamination. Such a use seems more appealing when it is known that most macroscopic algae are firmly attached to a given section of the ocean floor and, hence, any uptake would generally

be from the waters in their respective areas. This would preclude, for the most part, the concentration of radioisotopes contained in some other part of the ocean as would be the case if these organisms were free floating.

12. For the experimental conditions as used in this study, the average period for maximum uptake by the red and green algae is approximately 8 to 10 days. For the brown it is about 10 to 12 days. With the addition of KCl it was found that for the brown alga the time required for maximum uptake can be reduced to about 5 days.

13. Retention capacity of Sphacelaria is affected to only a slight degree by inducement of the ion exchange reaction. In sea water of high stable zinc concentration (approximately 1 x 10^{13} times greater than that of Zn^{65}), the release of Zn^{65} from Sphacelaria amounted to about 10 per cent lower, within a three-day period, as compared to normal sea water for the same period.

14. As indicated by experiments with Sphacelaria, once an organism has taken up Zn^{65} and is allowed to release its uptake, the same organism can possibly be used for reconcentrating Zn^{65}. Of course, all these conditions are so, providing that the alga is always maintained in a viable condition.

15. Uptake of Zn^{65} by algae indicates the possibility of radioactive contamination being introduced into man's food chain.

16. Because some of these macroscopic algae can be controlled to concentrate Zn^{65} more rapidly than normal and also made to release it more rapidly, there is some potential here for the use of such organisms in decontaminating controlled flows of radioactive-contaminated water.

17. The ultimate decontaminating efficiency of a macroscopic alga is a function of its concentration factor and the total weight of alga used.

APPENDICES

APPENDIX I

COMPOSITION OF SEA WATER

Harvey's composition of sea water is given below and lists the amounts of the known constituents of sea water having a salinity of 35 parts per thousand (32).

Constituent	Grams per Kilogram of Sea Water
Total salts	35.1
Sodium	10.77
Magnesium	1.30
Calcium	0.409
Potassium	0.388
Strontium	0.010
Zinc	$\sim$ (9-21) x 10^{-6}
Chloride	19.37
Sulphate as SO_4	2.71
Bromide	0.065
Boric acid as H_3BO_3	0.026
Carbon:	
Present as bicarbonate, carbonate and molecular carbon dioxide	0.023 at pH 8.4 0.025 at pH 8.2 0.026 at pH 8.0 0.027 at pH 7.8
As dissolved organic matter	0.001 - 0.0025
Oxygen (where in equilibrium with the atmosphere at 15°C)	0.008
Nitrogen (where in equilibrium with the atmosphere at 15°C)	0.013
Other elements	0.005

APPENDIX II

ALGAE CULTURE MEDIUM

The alga, Sphacelaria sp., which was cultured under laboratory conditions was successfully grown in Guillard's sea water medium (29). Nearly 0.05 gram of the alga, represented by 8 to 10 pieces, was placed in each of several aquaria holding one liter of this medium. Approximately once every six days, the alga was removed from the old medium and placed in fresh medium. As each algal clump became fairly large, it was cut into two or three smaller size clumps and these were then allowed to grow. As these newer portions grew to larger sizes, they too were reduced to smaller clumps and so on until the desired number of algal portions was attained. It seemed that as the clumps were made smaller, they grew faster than when the clumps were left in their larger forms.

The medium used in the above culturing procedure was a modified version of Guillard's medium. Below is listed Guillard's medium with the modifications designated.

Constituent	Concentration (mg/liter)
Guillard's:	
Sea Water 25-27 $^{o}/_{oo}$ Salinity	
NH_4Cl	50 (unless specified)
NaH_2PO_4	20
Ferric Sequestrene (NaFe EDTA, 13 per cent iron)	10

Constituent	Concentration (mg/liter)
Cu	0.005
Zn	0.01
Co	0.005
Mn	0.1
Mo	0.005
Thiamine	0.2
Biotin	0.001
B-12	0.001

Modification:

Sea Water 35-37 $^{o}/_{oo}$ Salinity

NH_4Cl	None used
Folic acid	0.002
p-Aminobenzoic Acid	0.010
Nicotinic Acid	0.1
Inisotol	1.0
Calcium Pantothenate	0.2
Pyridoxine, HCl	0.1

APPENDIX III

STATISTICS FOR COUNTING RADIOACTIVE SAMPLES

In statistics the standard deviation σ is usually taken as the $\sqrt{\bar{N}}$ where $\bar{N}$ is the average population. However, it is not $\bar{N}$ that is of concern in counting but rather it is the counting rate N/t where N is the total gross count actually recorded in time t.

Hence, the standard deviation that is desired is the one that relates the counting with time:

$$\sigma = \sqrt{\frac{\frac{N}{t}}{t}} \qquad = \sqrt{\frac{N}{t^2}} \qquad = \qquad \frac{\sqrt{N}}{t}$$

where t is the time in which the total count N was recorded.

The following examples will show that for the same gross counts, σ changes as t changes.

1. N = 500 counts
   t = 1 minute
   $$\sigma = \frac{\sqrt{500}}{1} = 22.4$$

2. N = 500 counts
   t = 2 minutes
   $$\sigma = \frac{\sqrt{500}}{2} = 11.2$$

3. N = 500 counts
   t = 5 minutes
   $$\sigma = \frac{\sqrt{500}}{5} = 4.48$$

It is seen that if σ depended only upon N, then σ in all the above examples would have been 22.4. This is evidently not true, and that for the same gross count more accuracy is obtained by a longer counting time.

Furthermore, 100 counts in one minute is not the same as 500 counts in five minutes, as shown by the following σ values:

$$\sigma = \frac{\sqrt{100}}{1} = 10$$

$$\sigma = \frac{\sqrt{500}}{5} = 4.48$$

The gross count of 500 in one minute or more would insure that σ would be less than 22.4 and that the percentage error would correspondingly be less than 5 per cent.

APPENDIX IV

SELF-ABSORPTION DATA FOR SEA WATER HAVING SALINITY OF APPROXIMATELY 36 $^{o}/_{oo}$

One milliliter pipets, graduated to tenths of a milliliter and a pipetor were used for sampling the Zn^{65}-dosed water. Prior to taking the actual sample, each of the medium waters was stirred thoroughly with the sampling pipet. While stirring, the pipet was cured by filling it three times with the medium water and discharging it three times. The final 0.8 ml sample was then taken, discharged on to an aluminum planchet, dried slowly (about ten minutes) under an infrared heat lamp, counted and yielded the following data for three different concentrations of Zn^{65}.

Volume (ml)	High Activity	Medium Activity	Low Activity
0	0	0	0
0.1	49	3	1
0.2	96	7	
0.3	141	9	2
0.4			4
0.5	248	14	5
0.6		18	6
0.7	303	21	6
0.8	320	21	6
0.9		22	8
1.0	415	26	9

APPENDIX V

TABLE 9

GRACILARIA UPTAKE DATA AT 25°C

Light Intensity 1-2 ft-c
pH Range 8.2 - 8.4
Salinity 36 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29*	0.29	32	37	33	33	9
1	9	4	5	6	3	31	35	33	33	91
2	7	6	6	6	5	36	37	33	33	150
3	4	6	6	5	6	30	35	33	33	180
5	8	9	8	8	7	32	36	32	32	220
6	6	7	6	6	7	34	37	32	32	220
7					7	35	34	32	32	220
8	4	4	4	4	7	31	32	32	32	220
9					7	30	29	32	32	220
12					7	31	37	30	30	230

*This background value for Gracilaria is the average of six separate samples and is assumed to be the same in all Gracilaria samples at time "0" in the uptake studies.

TABLE 9--Continued

Light Intensity: 10 ft-c
pH Range: 8.2 - 8.4
Salinity: 35 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	25	29	26	26	11
1	6	5	5	5	4	29	24	26	26	170
2	7	8	5	7	7	25	26	26	26	270
4	11	10	7	9	9	24	29	26	25	360
6	8	9	11	9	10	23	29	25	23	430
8	9	14	11	11	11	26	22	25	23	480
10	18	8	9	12	11	23	25	25	22	500
11	8	12	10	10	11	22	25	24	21	520
13					11	23	20	24	20	550

TABLE 9--Continued

Light Intensity: 500 ft-c
pH Range: 8.2 - 8.3
Salinity: 36 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	26	36	24	24	12
1	22	17	10	16	18	23	30	22	22	820
2	33	34	33	33	30	20	32	20	20	1,500
3					40	19	29	18	18	2,200
4	27	24	72	41	47	17	31	17	17	2,800
6	51	55	81	62	55	14	29	15	15	3,700
8	48	57	45	50	57	7	34	14	14	4,100
10	84	62	53	66	58	10	35	13	13	4,500
12	53	38	97	63	58	12	30	12	12	4,800
15	59	30	50	46	58	13	34	11	11	5,300
18	68	54	46	56	58	13	30	11	11	5,300

TABLE 10

GRACILARIA UPTAKE DATA AT 18°C

Light Intensity 1-2 ft-c
pH Range 8.0 - 8.2
Salinity 35 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	36	32	35	35	8
1	4	4	6	5	5	34	32	35	35	140
2	6	4	5	5	5	36	38	34	34	150
3	4	6	5	5	5	35	36	34	34	150
5	6	5	5	5	5	32	37	34	34	150
6	5	7	5	6	5	34	35	33	33	150
7					5		32	33	33	150
8	4	5	5	5	5	34	35	33	33	150
9					5	26	38	33	33	150
12					5	30	38	32	32	160

TABLE 10--Continued

Light Intensity: 10 ft-c
pH Range: 8.0 - 8.2
Salinity: 35 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	36	36	35	35	8
1	7	9	12	9	8	28	34	35	34	240
2	10	10	9	10	10			35	33	300
3					11	32	35	35	33	330
4	11	11	12	11	11			35	32	340
6	12	14	11	12	11	36	36	34	31	350
8	12	9	11	11	11	29	31	34	29	380
10	10	12	11	11	11	30	28	33	28	390
12	12	8	12	11	11	20	29	33	28	390
15	10	16	14	13	11	26	28	33	28	390
18	18	18	13	16	11	26	28	33	28	390

TABLE 10--Continued

Light Intensity: 500 ft-c
pH Range: 8.3 - 8.4
Salinity: 35 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	30	30	28	28	10
1	10	11	8	10	10	29	32	28	27	370
2	13	15	18	15	16	25	30	27	26	620
4	40	19	19	26	27	28	26	27	25	1,100
6	33	56	25	38	36	24	29	25	24	1,500
8	43	51	25	40	45	22	29	25	23	2,000
10	48	102	124	91	91	19	25	24	22	4,100
11	109	28	80	72	72	20	30	23	22	3,300
13	56	34	57	49	46	19	24	23	22	2,100

TABLE 10--Continued

(Replication)

Light Intensity 500 ft-c
pH Range 8.2 - 8.2
Salinity 36 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	24	23	21	21	14
1	6	7	6	6	6	20	20	21	21	290
2	5	6	7	6	10	20	25	20	20	500
3	7	13	13	11	13	19	23	19	19	680
4					16	20	23	19	19	840
5	16	18	19	18	18	19	24	18	18	1,000
7	17	28	18	21	19	18	24	18	18	1,100
9	17	29	24	23	20	17	25	17	17	1,200
10					20	16	22	16	16	1,200
11	17	20	15	17	20	14	25	16	16	1,200
14	16	23	24	21	20	17	26	16	16	1,200

TABLE 11

GRACILARIA UPTAKE DATA AT 5°C

Light Intensity 1-2 ft-c
pH Range 8.1 - 8.2
Salinity 35 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	42	35	36	36	8
1	3	4	2	3	3	31	32	36	36	83
2	4	3	3	3	4	34	37	35	35	110
3	3	3	3	3	4	38	44	35	35	110
5	5	5	4	5	5	34	33	35	35	140
6	3	4	4	4	5	47	34	34	34	150
7					5	32	31	34	34	150
8	5	4	6	5	5	29	34	34	34	150
9					5	36	38	33	33	150
12					5	37	31	33	33	150

TABLE 11--Continued

Light Intensity: 10 ft-c
pH Range: 8.0 - 8.4
Salinity: 35 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	25	20	21	21	14
1	4	3	3	3	2	17	17	21	21	95
2	3	3	2	3	3	17	20	21	21	140
4	2	3	4	3	4	29	20	21	21	190
6	3	4	4	4	5	16	17	20	20	250
8	5	5	6	5	5	20	18	19	19	260
10	7	6	6	6	5			19	19	260
11	5	7	6	6	5	17	19	19	19	260
13	4	7	4	5	5	18	20	19	19	260

TABLE 11--Continued

Light Intensity: 500 ft-c
pH Range: 8.2 - 8.2
Salinity: 32 $^{o}/_{oo}$

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	29	32	25	25	12
1	9	3	8	7	5	20	34	25	25	200
2	11	12	5	9	8			24	24	330
3					9	24	35	23	23	390
4	7	10	7	8	9			23	23	390
6	10	10	10	10	9	22	26	22	22	410
8	11	5	12	9	9	20	32	21	21	430
10	6	9	9	8	9	19	29	21	21	430
12	14	12	11	12	9	30	31	21	21	430

TABLE 11--Continued

(Replication)

Light Intensity: 500 ft-c
pH Range: 7.9 - 8.0
Salinity: 29 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.29	0.29	41	43	39	39	7
1	11	11	10	11	7	35	34	37	37	190
2	9	8	7	8	10	37	42	35	35	290
4	9	9	8	9	10	36	40	33	33	300
6	15	11	9	12	11	32	38	32	32	340
7	7	14	13	11	11	31	42	32	32	340
10	5	11	14	10	12	32	38	31	31	390
11	12	7	12	10	12	37	40	31	31	390
12	8	13	14	12	12	32	38	31	31	390

APPENDIX VI

TABLE 12

ENTEROMORPHA UPTAKE DATA AT 25°C

Light Intensity 1-2 ft-c
pH Range 8.2 - 8.4
Salinity 36 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27*	0.27	35	37	35	35	8
1	12	12	9	11	9	41	34	35	35	260
2	14	12	16	14	16	35	37	34	34	470
3	19	21	23	21	19	38	32	34	34	560
5	20	20	20	20	22	30	36	34	34	650
6	22	18	25	22	23	36	37	33	33	700
7					23	41	34	33	33	700
8	20	23	22	22	23	31	32	33	33	700
9					23	30	29	33	33	700
11	27	22	24	24	23			32	32	720
12					23	32	38	32	32	720

*This background value for Enteromorpha is the average of six separate samples and is assumed to be the same in all Enteromorpha samples at time "0" in the uptake studies.

TABLE 12--Continued

Light Intensity: 10 ft-c
pH Range: 8.2 - 8.4
Salinity: 35 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27	0.27	26	28	27	27	10
1	8	9	7	8	8	32	24	27	27	300
2	10	10	11	10	11	26	26	26	26	420
4	19	18	17	18	19	20	28	26	26	730
6	21	22	25	23	23	25	29	26	26	880
8	25	23	29	26	26	31	22	25	25	1,000
10	28	25	28	27	27	21	25	25	25	1,100
11	24	27	23	25	27	25	25	25	25	1,100
13	28	29	38	32	27	29	20	25	25	1,100

TABLE 12--Continued

Light Intensity 500 ft-c
pH Range 8.2 - 8.3
Salinity 36 $^{o}/_{oo}$

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27	0.27	36		32	32	8
1	20	11	21	17	12	32	29	32	32	380
2	12	11	17	13	18			32	32	560
3					23	31	28	32	32	720
4	36	28	22	29	26			32	32	810
6	33	26	32	30	30	31	28	32	32	940
8	27	30	28	28	32	29	34	32	32	1,000
10	33	37	31	34	33	36	34	31	31	1,100
11	29	35	25	30	33	34		31	31	1,100
14	35	42	31	39	34	29	34	31	31	1,100
18	32	32	32	32	34	25	29	30	30	1,100

TABLE 13

ENTEROMORPHA UPTAKE DATA AT 18°C

Light Intensity 1-2 ft-c
pH Range 8.0 - 8.2
Salinity 35 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27	0.27	36	34	34	34	8
1	13	13	11	12	9	32	32	34	34	260
2	15	8	16	13	13	31	38	33	33	390
3	14	18	15	16	15	36	36	33	33	460
5	15	19	18	17	18	35	37	33	33	550
6	18	15	17	17	19	31	35	33	33	580
7					20	33	32	32	32	630
8	18	20	24	21	20	33	35	32	32	630
9					20	31	39	32	32	630
11	19	25	22	22	20			32	32	630
12					20	32	39	32	32	630

TABLE 13--Continued

Light Intensity: 10 ft-c
pH Range: 8.0 - 8.2
Salinity: 35 $^{o}/_{oo}$

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27	0.27	36	36	30	30	9
1	8	8	5	7	5	30	33	30	30	170
2	9	5	15	10	8			30	30	270
3					9	34	35	29	29	310
4	13	10	9	11	11			29	29	380
6	11	13	12	12	13	30	36	29	29	450
8	9	12	12	11	15	24	31	28	28	540
10	16	15	20	17	16	24	28	28	28	570
12	12	18	16	15	18	26	29	28	28	640
15	18	23	16	19	19	27	27	27	27	700
18	21	24	16	20	19	25	27	27	27	700

TABLE 13--Continued

Light Intensity: 500 ft-c
pH Range: 8.3 - 8.4
Salinity: 35 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27	0.27	29	30	27	27	10
1	12	10	11	11	10	34	32	27	27	370
2	17	13	17	16	16	27	30	27	27	590
4	35	22	29	29	25	23	26	26	26	960
6	21	27	27	25	29	25	29	26	26	1,100
8	40	34	29	34	31	27	29	26	26	1,200
10	28	32	43	34	31	26	26	26	26	1,200
11	24	31	29	28	31	25	30	25	25	1,200
13	36	31	28	32	31	25	24	25	25	1,200

TABLE 14

ENTEROMORPHA UPTAKE DATA AT 5°C

Light Intensity 1-2 ft-c
pH Range 8.1 - 8.2
Salinity 35 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27	0.27	38	42	34	34	8
1	9	15	8	11	6	37	38	34	34	180
2	8	11	9	9	10	35	44	34	34	290
3	13	12	12	12	14	36	53	34	34	410
5	9	19	15	14	18	35	40	34	34	530
6	21	16	17	18	18	36	41	34	34	530
7					19	29	37	34	34	560
8	20	24	20	21	19	32	41	34	34	560
9					19	31	46	33	33	560
11	17	15	14	15	18			33	33	550
12					17	31	37	33	33	510
13	15	11	13	13	15			33	33	460

TABLE 14--Continued

Light Intensity: 10 ft-c
pH Range: 8.2 - 8.3
Salinity: 36 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27	0.27	17	23	16	16	17
1	8	6	6	7	6	12	20	16	16	380
2	7	8	6	7	9	17	25	16	16	560
3	8	9	9	9	11	14	23	16	16	690
4					13	17	23	16	16	810
5	14	12	12	13	16	16	24	16	16	1,000
7	15	19	20	18	19	18	24	16	16	1,200
9	19	18	19	19	21		25	16	16	1,300
10					21	14	22	15	15	1,400
11	29	22	24	25	21		25	15	15	1,400
14	28	16	27	24	21	19	26	15	15	1,400

TABLE 14--Continued

Light Intensity: 500 ft-c
pH Range: 8.2 - 8.2
Salinity: 32 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27	0.27	30	32	28	28	10
1	10	10	9	10	6	34	34	28	28	210
2	13	14	7	11	10			27	27	370
3					13	28	35	27	27	480
4	20	12	21	18	16			27	27	590
6	17	17	14	16	19	26	26	26	26	730
8	17	18	12	16	19	24	32	25	25	760
10	17	23	19	20	19	22	29	25	25	760
12	23	19	16	19	19	22	31	25	25	760

TABLE 14--Continued

(Replication)

Light Intensity: 500 ft-c
pH Range: 7.9 - 8.0
Salinity: 29 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.27	0.27	41	43	38	38	7
1	17	15	13	15	12	34	34	38	38	320
2	7	17	22	15	17	42	42	38	38	450
4	26	30	22	26	23	35	40	37	37	620
6	24	27	30	27	24	36	38	37	37	650
7	22	28	20	23	25	38	42	36	36	700
10	26	25	29	27	25	35	38	36	36	700
11	18	23	20	20	25	35	39	35	35	720
12	20	24	29	24	25	32	38	35	35	720

APPENDIX VII

TABLE 15

SPHACELARIA UPTAKE DATA AT 25°C

Light Intensity 1-2 ft-c
pH Range 8.2 - 8.4
Salinity 36 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13*	0.13	40	37	36	36	4
1	57	69	58	61	43	34	35	37	37	1,200
2	71	41	42	51	62	36	37	35	35	1,800
3	78	62	77	72	72	35	35	34	34	2,100
5	80	90	87	86	84	32	36	31	31	2,700
6	67	72	64	68	71	30	37	30	30	2,400
7	106	104	69	93	85	29	34	29	29	2,900
8					92	25	32	28	28	3,300
9	85	75	76	79	80	24	29	28	28	2,900
10	55	64	77	65	76			27	27	2,800
11	86	79	75	80	76			27	27	2,800

*This background value for Sphacelaria is the average of six separate samples and is assumed to be the same in all Sphacelaria samples at time "0" in the uptake studies.

TABLE 15--Continued

Light Intensity: 10 ft-c
pH Range: 8.2 - 8.4
Salinity: 35 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	29	29	29	29	4
1	24	30	25	26	18	24	24	25	25	720
2	35	31	34	33	36	24	25	24	24	1,500
4	151	52	54	86	86	20	29	24	24	3,600
6	165	138	146	150	150	24	29	24	24	6,200
8	212	192	170	192	192	23	22	24	24	8,000
10	195	203	162	187	187	23	25	24	24	7,800
11	180	260	160	200	200	25	25	24	24	8,300
12	183	198	218	200	200			23	23	8,700
13	207	166	188	187	196	25	20	23	23	8,500
15	197	219	162	193	190			23	23	8,300
16	202	181	203	195	190			23	23	8,300

TABLE 15--Continued

Light Intensity: 500 ft-c
pH Range: 8.2 - 8.3
Salinity: 36 $^{o}/_{oo}$

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	28	36	28	28	5
1	100	97	131	110	80	26	30	26	26	3,100
2	134	73	141	116	116			25	25	4,600
3					130	26	29	25	25	5,200
4	161	113	141	138	140			25	25	5,600
6	163	189	150	167	167	24	29	25	25	6,700
8	191	151	232	191	188	17	34	24	24	7,800
10	158	167	143	156	162	25	35	24	24	6,700
12	185	213	225	208	206	20	30	23	23	9,000
15	150	136	151	146	176	22	34	23	23	7,600
18	156	163	142	154	158	26	30	23	23	6,900

TABLE 15--Continued

(Replication)

Light Intensity: 500 ft-c
pH Range: 8.2 - 8.2
Salinity: 36 ‰

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	22	23	23	23	6
1	35	24	30	30	34	19	20	19	19	1,800
2	60	67	51	59	56	14	25	16	16	3,500
3	63	56	75	65	66	14	23	13	13	5,100
4	68	68	73	70	71	12	23	11	11	6,400
5					64	12	24	10	10	6,400
6	55	59	59	58	58			9	9	6,400
7					59	11	24	9	9	6,600
8	61	55	66	61	60			9	9	6,700
10	62	45	66	58	61		22	8	8	7,600
11	55	57	67	60	59	7	25	8	8	7,400

TABLE 16

SPHACELARIA UPTAKE DATA AT 18°C

Light Intensity 1-2 ft-c
pH Range 8.0 - 8.2
Salinity 35 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	38	32	38	38	3
1	43	42	59	48	46	36	32	36	36	1,300
2	57	56	54	56	59	35	38	35	35	1,700
3	73	65	72	70	70	34	36	34	34	2,100
5	84	88	69	80	87	34	37	34	34	2,600
6	95	103	89	96	94	30	35	34	34	2,800
7	99	82	73	85	85	35	32	33	33	2,600
8	73	98	79	83	81	35	35	33	33	2,500
9	87	96	90	91	90	31	38	33	33	2,700
10	71	62	79	71	91			33	33	2,800
11	97	102	86	95	85			33	33	2,600
12						36	38	33	33	

TABLE 16--Continued

Light Intensity: 10 ft-c
pH Range: 8.2 - 8.2
Salinity: 36 $^{o}/_{oo}$

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	17	17	17	17	8
1	44	17	25	29	20			17	17	1,200
2	37	25	40	34	34	18	19	17	17	2,000
4	37	46	40	41	52	11	16	17	17	3,100
5					60	17	16	16	16	3,800
7	85	62	62	70	70	17	14	16	16	4,400
8	60	60	54	58	59			16	16	3,700
9	73	66	65	68	67			16	16	4,200
10	70	54	77	67	70	12	17	15	15	4,700
11	80	49	78	69	66			15	15	4,400
13	48	59	62	56	55	20	16	15	15	3,700

TABLE 16--Continued

Light Intensity: 500 ft-c
pH Range: 8.3 - 8.4
Salinity: 35 $^{o}/_{oo}$

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	31	30	30	30	4
1	42	44	40	42	40	28	32	28	28	1,400
2	71	73	96	80	100	20	30	26	25	4,000
4	245	260	278	261	260	24	26	23	22	12,000
6	335	296	201	277	276	22	29	23	21	13,000
8	230	213	305	249	250	20	29	22	20	12,000
10	247	190	270	236	236	18	25	22	20	12,000
11	278	217	266	254	254	18	30	22	19	13,000
12	226	275	261	254	256			22	19	13,000
13	233	230	192	218	240	22	24	21	19	13,000
15	228	200	229	219	212			21	18	12,000
16	184	230	187	200	205			21	18	11,000

TABLE 16--Continued

(Induced Growth with KCl)

Light Intensity: 500 ft-c
pH Range: 8.2 - 8.4
Salinity: 36 $^{o}/_{oo}$

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	18	24	16	16	8
1	13	17	17	16	20	17	23	14	14	1,400
2	45	56	34	45	45	12	23	12	12	3,800
3	31	40	29	33	33	9	24	10	10	3,300
4	32	35	30	32	35			9	9	3,900
5	60	35	88	61	61	8	24	7	7	8,700
6	33	33	42	36	36			7	7	5,100
7	47	45	30	41	39	4	25	7	7	5,600
8	34	47	36	39	39		22	7	7	5,600
9	38	28	23	30	32	7	25	7	7	4,600

TABLE 17

SPHACELARIA UPTAKE DATA AT 5°C

Light Intensity 1-2 ft-c
pH Range 8.2 - 8.3
Salinity 36 °/oo

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	22	23	24	24	5
1	36	29	29	31	31	22	20	23	23	1,300
2	44	44	42	43	43	22	25	22	22	2,000
3	58	41	49	49	49	22	23	22	22	2,200
4	61	43	44	49	49	22	23	22	22	2,200
5					50	20	24	22	22	2,300
6	55	71	65	64	62			22	22	2,800
7					62	24	24	22	22	2,800
8	68	59	59	62	62			22	22	2,800
10	77	56	59	64	62	22	22	22	22	2,800
12	68	59	44	57	62			22	22	2,800
14						25	26	22	22	

TABLE 17--Continued

Light Intensity: 10 ft-c
pH Range: 8.2 - 8.3
Salinity: 36 $^{o}/_{oo}$

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	20	23	20	20	6
1	28	27	21	25	24	22	20	20	20	1,200
2	40	33	35	36	36	18	25	20	20	1,800
3	47	43	43	44	43	19	23	19	19	2,300
4	51	33	48	44	44	20	23	19	19	2,300
5					45	19	24	18	18	2,500
6	59	50	47	52	51			18	18	2,800
7					58	18	24	18	18	3,200
8	49	61	69	60	59			18	18	3,300
10	54	61	65	60	59	16	22	17	17	3,500
12	61	62	51	58	59			17	17	3,500

TABLE 17--Continued

Light Intensity: 500 ft-c
pH Range: 8.2 - 8.3
Salinity: 36 $^{o}/_{oo}$

Exposure Time (Days)	Uptake of Three Separate Algal Samples (c/m/mg)			Average Algal Uptake (c/m/mg)		Sea Water Activity (c/m/ml)				Concentration Factor
				Assayed	Graphed	Assayed Medium	Assayed Control	Corrected Medium	Graphed Medium	
0	-	-	-	0.13	0.13	13	23	12	12	12
1	21	19	26	22	20	10	20	11	11	1,800
2	22	27	21	23	24	10	25	11	11	2,200
3	27	27	27	27	25	10	23	11	11	2,300
4	22	27	28	26	25	10	23	11	11	2,300
5					25	14	24	11	11	2,300
6	34	32	35	34	33			11	11	3,000
7					42	11	24	11	11	3,800
8	44	55	40	46	44			11	11	4,000
10	49	38	32	40	44	12	22	11	11	4,000
12	53	53	39	48	44			11	11	4,000
14						16	26	11	11	

APPENDIX VIII

SURFACE AREA COMPARISON BETWEEN *SPHACELARIA* AND *ENTEROMORPHA*

From microscopic measurements, the average diameter of the algae were found to be 300 μ (10^{-4} cm) for *Enteromorpha* and 25 μ for *Sphacelaria*. Assuming that the algal filaments are true cylinders and that the density of both algae are approximately equal, the following expressions are made for equal weights of these two algae.

Where: L_E = length of *Enteromorpha* in cm

L_S = length of *Sphacelaria* in cm

d = density in grams/cm^3

(Volume *Enteromorpha*)(density) = (Volume *Sphacelaria*)(density)

$$\text{or } \left(\frac{\pi D^2}{4}\right)(L_E)(d) = \left(\frac{\pi D^2}{4}\right)(L_S)(d)$$

$$(3.00 \times 10^{-2} \text{ cm})^2 \, (L_E \text{cm}) = (25 \times 10^{-4} \text{ cm})^2 \, (L_S \text{cm})$$

$$\frac{9 \times 10^{-4}}{6.25 \times 10^{-6}} = \frac{L_S}{L_E}$$

$$\frac{L_S}{L_E} = 144$$

This result indicates that for equal weights of each algae, the length of *Sphacelaria* is about 144 times longer than that of *Enteromorpha*.

Therefore, the surface area of a given weight of *Sphacelaria* as compared to the same weight of *Enteromorpha* is:

$$\text{Area of } \underline{\text{Sphacelaria}} = (144 \text{ cm})(\pi)(25 \times 10^{-4} \text{ cm})$$

$$= 1.130 \text{ cm}^2$$

$$\text{Area of } \underline{\text{Enteromorpha}} = (1 \text{ cm})(\pi)(300 \times 10^{-4} \text{ cm})$$

$$= 0.0944 \text{ cm}^2$$

$$\frac{\text{Area of } \underline{\text{Sphacelaria}}}{\text{Area of } \underline{\text{Enteromorpha}}} = \frac{1.130}{0.0944} = 12.0 \text{ times as great}$$

APPENDIX IX

TABLE 18

GROWTH RATE DATA FOR ENTEROMORPHA

Growth Condition	Time Days	Weight of Alga (mg) Samples 1	2	3	Percentage Excess Over Original Average Weight Samples 1	2	3	Average Percentage Excess
300 ft-c @ 18°C in Plain Sea Water	0	84.8	131.9	68.3				
		84.8	130.3	68.0				
		84.8	131.0	68.1				
		84.8*	131.1*	68.1*	0	0	0	0
	5	107.0	158.5	78.2				
		105.5	157.4	78.0				
		106.0	158.6	78.1				
		106.2*	158.2*	78.1*	25.3	20.6	14.7	20.2
	9	122.2	191.1	98.0				
		120.4	188.0	97.5				
		121.2	192.1	97.9				
		121.3*	190.4*	97.8*	43.1	45.2	43.6	44.0
	13	132.7	207.8	110.1				
		131.5	207.0	109.8				
		131.2	206.3	110.0				
		131.8*	207.0*	110.0*	55.5	57.8	61.4	58.2
	16	138.4	221.0	116.6				
		137.5	222.4	116.6				
		138.0	219.6	117.1				
		138.0*	221.0*	116.8*	62.8	68.5	71.5	67.6

*Indicates the average.

TABLE 19

GROWTH RATE DATA FOR SPHACELARIA

Growth Condition	Time Days	Weight of Alga (mg) Samples 1	2	3	Percentage Excess Over Original Average Weight Samples 1	2	3	Average Percentage Excess
30 ft-c @ 18°C in Plain Sea Water	0	22.2	32.4	30.3				
		23.2	33.4	31.3				
		22.7	32.8	30.6				
		22.7*	32.9*	30.7*	0	0	0	0
	4	24.2	35.3	33.2				
		23.9	34.9	33.1				
		24.1	34.8	33.3				
		24.1*	35.0*	33.2*	5.72	6.39	8.15	6.75
	6	25.0	37.0	34.5				
		25.0	36.3	34.5				
		25.1	36.3	33.8				
		25.0*	36.5*	34.3*	10.3	10.9	11.7	11.0
	10	26.2	39.2	36.7				
		26.2	39.3	36.7				
		26.2	38.9	36.5				
		26.2*	39.1*	36.6*	15.4	18.8	19.2	17.8

*Indicates the average.

TABLE 19--Continued

Growth Condition	Time Days	Weight of Alga (mg) Samples 1	2	3	Percentage Excess Over Original Average Weight Samples 1	2	3	Average Percentage Excess
30 ft-c @ 5°C in Plain Sea Water	0	37.5	63.1	58.8				
		38.2	62.6	58.5				
		38.2	62.5	59.0				
		38.0*	62.7*	58.8*	0	0	0	0
	5	38.4	64.1	58.8				
		37.7	62.7	58.9				
		38.2	63.6	58.7				
		38.1*	63.5*	58.8*	0.26	1.27	0	0.51
	7	38.4	64.6	59.5				
		37.9	64.2	59.7				
		38.3	64.1	59.3				
		38.2*	64.3*	59.5*	0.53	2.55	1.19	1.42

*Indicates the average.

TABLE 19--Continued

Growth Condition	Time Days	Weight of Alga (mg) Samples 1	2	3	Percentage Excess Over Original Average Weight Samples 1	2	3	Average Percentage Excess
300 ft-c @ 18°C in Plain Sea Water	0	26.2	39.2	36.7				
		26.2	39.3	36.7				
		26.2	38.9	36.5				
		26.2*	39.1*	36.6*	0	0	0	0
	2	29.0	44.1	40.4				
		28.9	43.9	39.8				
		29.0	44.0	40.3				
		29.0*	44.0*	40.2*	10.7	12.5	9.84	10.7
	4	30.4	44.9	40.9				
		28.9	43.8	40.9				
		29.3	44.1	41.0				
		29.5*	44.3*	40.9*	12.6	13.3	11.8	12.6
	7	31.3	47.4	44.2				
		31.8	48.1	44.2				
		31.5	47.7	44.3				
		31.5*	47.7*	44.2*	20.2	22.0	20.8	21.0
	9	33.2	49.5	45.8				
		33.1	49.7	45.8				
		33.1	49.9	45.6				
		33.1*	49.7*	45.7*	26.3	27.1	24.9	26.1
	13	35.6	55.7	52.2				
		34.9	56.0	52.1				
		35.0	55.6	52.4				
		35.2*	55.8*	52.2*	34.4	42.8	42.7	40.0

*Indicates the average.

TABLE 19--Continued

Growth Condition	Time Days	Weight of Alga (mg) Samples 1	2	3	Percentage Excess Over Original Average Weight Samples 1	2	3	Average Percentage Excess
300 ft-c @ 18°C in Sea Water Dosed with Zn^{65} (50 c/m/ml) and also with KCl (0.3 gram/liter)	0	38.1	64.6	59.5				
		37.9	64.2	59.5				
		38.2	64.1	59.3				
		38.1*	64.3*	59.4*	0	0	0	0
	4	51.7	92.9	84.5				
		51.0	93.4	85.2				
		51.3	93.7	85.6				
		51.3*	93.3*	85.1*	34.7	45.2	45.0	41.6
	6	58.0	107.2	105.2				
		58.8	108.8	99.9				
		58.2	110.2	103.1				
		58.3*	108.7*	102.7*	53.0	69.1	72.9	65.0
	15	81.4	142.5	134.6				
		81.0	141.4	134.2				
		79.1	140.8	134.4				
		80.5*	141.6*	134.4*	111.3	120.2	126.3	119.3

*Indicates the average.

APPENDIX X

TABLE 20

RETENTION DATA FOR SPHACELARIA AT 18°C IN PLAIN SEA WATER

Light Intensity 500 ft-c
pH Range 8.2 - 8.2
Salinity 36 °/oo

Exposure Time (Days)	Retained Algal Uptake (c/m/mg)			Average Algal Retention	Sea Water Activity (c/m/ml)	
					Assayed Medium	Assayed Control
0	-	-	-	159*	4	2
1	114	96	88	99	4	2
3	68	83	67	73	14	2
4	73	49	57	60	8	2
5	59	66	56	60		2
6	61	44	55	53	13	2
7	45	40	64	50		2
8					9	
9	45	46	43	45		2
11	46	52	43	47		2
14	48	48	53	50		2
15	50	50	49	50	17	2

*Average of five samples.

TABLE 21

RETENTION DATA FOR SPHACELARIA AT 18°C IN ZINC ENRICHED SEA WATER

Light Intensity 500 ft-c
pH Range 8.2 - 8.2
Salinity 36 °/oo

Exposure Time (Days)	Retained Algal Uptake (c/m/mg)			Average Algal Retention	Sea Water Activity (c/m/ml)	
					Assayed Medium	Assayed Control
0	-	-	-	159*	2	2
1	72	86	62	73	7	2
3	48	88	78	71	8	2
4	22	90	90	67	13	2
5	69	61	62	64		2
6	47	39	60	49	7	2
7	40	46	61	49		2
8					10	2
9	41	48	52	47		2
10					9	
11	47	56	68	57	8	2
12	49	57	69	58		2
14	78	59	56	63		2
15	42	46	66	51		2

*Average of five samples.

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BIOGRAPHICAL SKETCH

Paul Harry Bedrosian was born on August 16, 1928, in Haverhill, Massachusetts. All of his elementary and secondary education was received in the Haverhill public schools. He graduated from Haverhill High School in 1946, and entered Northeastern University in Boston, Massachusetts. He received the degree of Bachelor of Science in Civil Engineering in June, 1951, and immediately following graduation he worked as a junior field engineer for an engineering consulting firm. He served in the United States Army from 1952 to 1954 as an engineering research assistant at Fort Monmouth, New Jersey. After he was honorably separated from the service in 1954, he was engaged by the U. S. Geological Survey as a hydraulic engineer. He left the Survey to enroll at the University of Florida in February, 1958, from which institution he received his Master of Science in Engineering in January, 1959. He continued his studies, specializing in Radiological Health, at the University towards his Doctorate. He is married and has one son.

This dissertation was prepared under the direction of the Chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Engineering and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 5, 1961

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