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UPTAKE OF Zn^{65} AND PRIMARY PRODUCTIVITY IN MARINE BENTHIC ALGAE[1]

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ABSTRACT

The rate of uptake of Zn^{65} from natural sea water and the primary production of 6 species of marine benthic algae were measured in light and dark bottles suspended in a running sea water aquarium under constant light and temperature conditions. Uptake and production of one species (*Chaetomorpha Linum*) were also measured in bottles suspended at different depths in a marine pond in order to achieve a gradient of light intensities. Definite uptake occurred in the light, but no measurable uptake occurred in the dark. The initial rate of uptake in the light was proportional to the gross oxygen production which varied with the species. The apparent equilibrium uptake rates in *Chaetomorpha* at different light intensities were proportional to the net oxygen production. The data indicated that in marine seaweeds zinc is taken up in proportion to the gross oxygen production and accumulated in proportion to the net oxygen production. Consequently Zn^{65} has possibilities as a tool for the measurement of primary productivity.

Zinc occurs in natural sea water as a minor constituent of the total salt content. In a weekly series of determinations over a period of a year at Beaufort, N. C., Chipman *et al.* (1958) found a range of 1.2 to 19.6 μg/L with a yearly average of 10.8 μg/L. The potential biological importance of zinc in the sea may be great since there is evidence that it is an essential micronutrient element for the growth of plants. A lack of available zinc in soils has long been indicated as a factor in certain crop diseases (Thorne 1957) while Arnon (1958) has demonstrated its importance in culturing experiments with terrestrial plants. In a review of algal physiology, Myers (1951) cites evidence which indicates that zinc is a growth requirement for some species of algae.

In light of the potential importance of zinc as a trace metal in the sea the following series of experiments were executed to determine the pattern and rates of uptake of zinc by marine macroscopic benthic algae. Such information is of interest in connection with the basic zinc cycle in the sea and the possible fate of radioactive contaminants. Also, if the rates of uptake of this element are related to the metabolic activities of the algae, it might be possible to adapt zinc uptake determinations to the measurement of metabolism.

The gamma-emitting isotope, Zn^{65}, was utilized as a tracer in these experiments since it provided a sensitive means of measuring uptake at the low concentrations involved in the sea. The benthic marine

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algae served as convenient test materials due to the availability of several different species and the ease with which they could be utilized in a simple experimental set-up. These experiments are a sequel to similar work with P^{32} carried out by Odum *et al.* (1958), and constituted one of the staff-student research projects which were a part of the 1959 Marine Ecology Course at the Marine Biological Laboratory, Woods Hole, Massachusetts. We are indebted to Dr. T. R. Rice for important suggestions concerning interpretation of the data.

METHODS

In all experiments approximately 0.5 g oven-dry weight of fresh, actively growing algae were placed in 250 cc glass-stoppered bottles along with freshly collected sea water from the Woods Hole harbor. The Zn^{65} tracer solution was obtained from the Oak Ridge National Laboratory on July 12, 1957, as "Zn-65-P Processed, High Specific Activity" at which time it had a specific activity of 789 mc/g of zinc. Allowing for about two years radioactive decay, the amount of tracer introduced into each bottle was about 3.7 μc, and the amount of total zinc added about 37 μg.

In the first type of experiment the bottles were suspended in a running sea water aquarium at constant temperature (approximately 22°C) and were allowed to swing in the current in order to achieve a gentle mixing of the contents. Illumination was provided from the sides by 6 fluorescent tubes which produced an intensity of about 350 ft-c. Dark bottles were suspended in the same manner except that they were covered with aluminum foil in order to exclude all light. In the second group of experiments the bottles were suspended at different depths in a marine pond in order to obtain a gradient of light intensities. Uptake was interpreted as the loss of radioactivity from the water after preliminary experiments showed that there was no measurable adsorption by the glass surfaces of the bottles. With the exception of some of the initial experiments, which were run for several hours, the water samples were taken at 0, 0.5, 1.0, 2.0, and 3.0 hr. At each period the bottles were removed from the bath or pond, shaken gently to insure through mixing, and three aliquots of 0.5 cc each were removed with a syringe and the activity counted with a well scintillation counter. Under these conditions the measured uptake could include actual uptake by the plant cells, uptake by the epiphytic bacteria, or adsorption by the surfaces of the algae.

Net productivity, respiration and gross productivity for the three-hour period were determined by measuring the oxygen concentration by the Winkler method at the beginning and the end of the experiment. After each small withdrawal of water for radioactivity determination, the loss was replenished with an equal amount of sea water in order to avoid the formation of an air space in the bottle.

RESULTS

A measurable uptake of Zn^{65} occurred only in those bottles which were exposed to light. The dark bottles showed no significant change in the activity of the water not only in the 3-hr experiments, but also in those which lasted from 6 to 13 hr. In contrast, those bottles exposed to the light in the aquarium or in the pond showed a marked decrease in the activity of the water. Removal of the aluminum foil from the dark bottles and subsequent exposure to the light resulted in an uptake typical of those algae which were illuminated from the start.

The data from a long-term experiment with *Chaetomorpha Linum* in a light bottle are presented in Figure 1 and can be considered to be representative of the type of curves obtained. The rate of change of activity in the water was greatest at the start of the experiment and continued to decrease until it appeared to approach a constant value. Unfortunately, as these values approach the background rate of the counter (300 cpm) it becomes impossible to determine if a constant value was ever reached.

To compare the uptake rates between different experiments objectively, it would be desirable to know what factors determine the shape of the curve and consequently

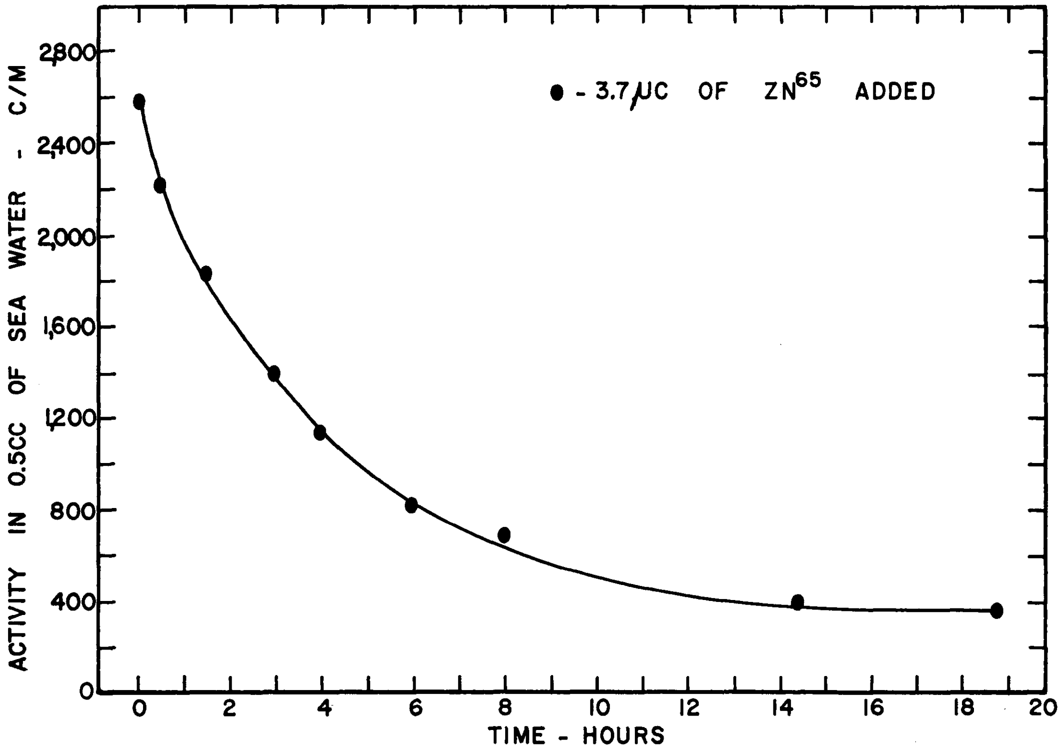

FIG. 1. Typical uptake curve showing loss of Zn^{65} from sea water in a 250 ml "light" bottle containing about 0.5 g (dry wt.) of *Chaetomorpha Linum.*

what parameters are being measured. One possibility would be that zinc is taken up in direct proportion to its concentration in the water. If this were the case, a semi-logarithmic plot of the activities against time would yield a straight line. It was found, however, that in all cases such a plot yielded a curve which was concave (Fig. 2). Another alternative would be that the rate of uptake of zinc was constant during the experimental period, but that a back diffusion of zinc from the algae was reducing the specific activity (defined as the ratio of radioactive to non-radioactive atoms) of the zinc solutions. If a portion of this backflow were Zn^{65} being recycled, this could account for the shape of the curves found. While this theory cannot be conclusively proved, it was found that a backflow of labeled zinc did exist. This was shown by taking pieces of *Ceramium rubrum* which had been labeled with Zn^{65} and placing them in two bottles of fresh sea water. One was placed in the light and the other was covered with aluminum foil. Initial water samples showed some radioactivity in the water at the start of the experiments which was presumably carried over by the water wetting the plants at the time of transfer. During the next 14 hr the activity of the water was reduced in the light bottle while that in the dark bottle showed a constant increase.

In the light of the above information it was assumed that the changing slopes of the uptake curves were due in part to a back flow of zinc from the plants. Since no information was available on the sizes or turnover rates of the zinc pools within the algae, a simple comparison between the curves could not be made. Under the experimental procedure used, however, the initial specific activities of the sea water solutions should have been the same in all of the experimental bottles. The slopes of the uptake curves at time zero should thus provide the most objective values for compar-

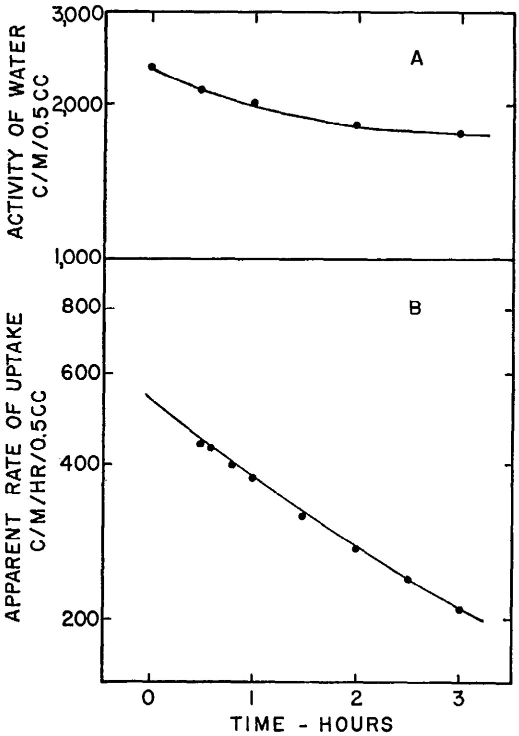

FIG. 2. Graphical method used to estimate the initial rate of uptake of Zn^{65}. Curve A represents the experimental points with a fitted curve. Curve B is a plot of the apparent rates of uptake against the times for which they were computed. The extrapolated rate at time zero (530 c/m/hr/0.5 cc) was considered to be the best estimate of the initial rate of uptake.

ing the uptake curves between different experiments, since the slopes would be directly proportional to the rates of uptake of zinc. Estimates of these initial slopes were made in the following manner. The uptake curves were plotted on semi-logarithmic paper and a line was fitted to the points to form a smooth curve. Lines were then drawn from the activity at time zero to a series of points on the line. The slopes of these lines were then computed as the apparent rates of uptake. These rates were then plotted against the times for which they were calculated, and since as the time approaches zero the rate will approach the initial rate, an extrapolation of a line fitted to these points should yield an estimate of the initial rate. The value for the initial rate was then divided by the weight of algae used to arrive at a relative value for comparative purposes. This procedure is illustrated in Figure 2.

TABLE 1. *Summary of Zn^{65} uptake and metabolism of 6 species of marine benthic algae at 22°C and 350 ft-c illumination*

Species	Zn^{65} uptake c/m/gm/hr /0.5cc	Metabolism ml of O_2/g/hr Net production	Respiration	Gross production
Ceramium rubrum	4746	4.49	1.30	5.79
	5058	6.00	1.30	7.30
Fucus vesiculosis	1641	1.00	0.96	1.96
	1867	1.05	0.96	2.01
Porphyra umbilicalis	2960	3.04	0.89	3.93
	2792	2.63	0.89	3.52
Chordaria flagelliformis	1916	2.22	0.37	2.59
	2322	2.50	0.37	2.87
Chaetomorpha Linum	3197	1.71	1.45	3.16
Laminaria sp.	2565	1.65	1.27	2.92

The above method of analysis was applied to the results of 10 3-hr experiments utilizing *Ceramium rubrum, Fucus vesiculosis, Porphyra umbilicalis, Chordaria flagelliformis, Chaetomorpha Linum,* and *Laminaria* sp. The calculated uptake rates and oxygen production values are given in Table 1. The rates of zinc uptake and gross oxygen production for each experiment are plotted in Figure 3. On the basis of the apparent straight line relationship between these two variables a regression line was calculated by the least squares method. A coefficient of correlation was also calculated which gave a value of 0.968. It thus appeared that some type of relationship might exist between the rates of zinc uptake and the gross oxygen production with these taxonomically diverse species.

There is the possibility, however, that these two variables are independent of each other but are dependent on some common factor which varies between species. In order to check this point a single species, *Chaetomorpha Linum,* was on two occasions subjected to a range of light conditions in the field in order to produce a range of metabolic rates in the same species. The data for

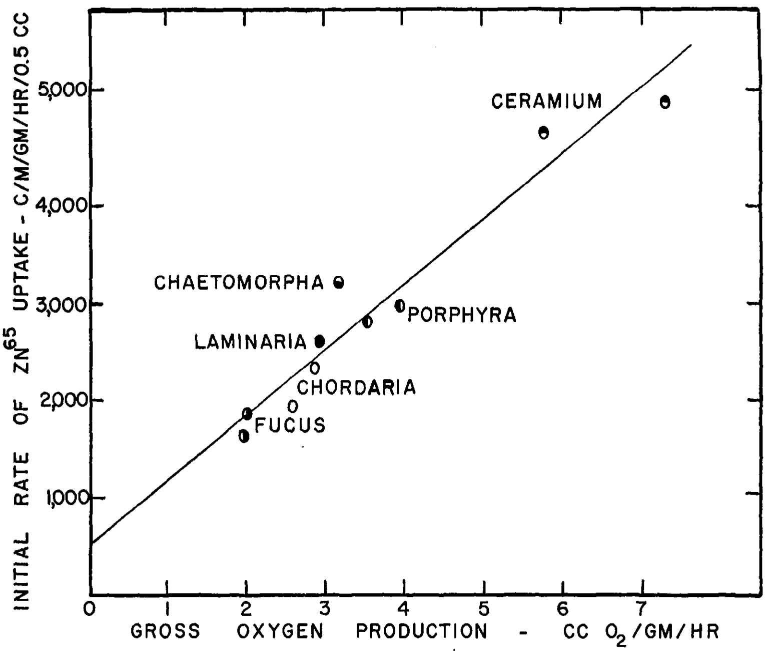

FIG. 3. Relationship between the initial rate of uptake of Zn^{65} and the gross oxygen production of 6 species of marine benthic algae. The least squares line is represented by the equation $Y = 663.8 X + 513.5$.

the August 29, 1959, experiment are shown in Figure 4. It can be seen that these curves are characterized by a very rapid drop within the first hour, and, with the exception of the 3 m bottle, an apparent linear decrease in activity during the next two hours. Since there were no samples taken at the end of the first half hour, the initial shape of the curve during the first hour is uncertain so that one cannot apply the same methods of analysis which were used in the laboratory experiments. The form of these curves suggested that under the light intensities found in the field the rate of zinc uptake was of such a magnitude as to bring the specific activity of the exchangeable zinc in the algae into equilibrium with that in the sea water within the first hour. The straight line decrease could thus represent a net uptake of zinc in the algae. Going on this assumption, the data were analyzed in the following manner. A straight line was fitted to the last three points and extrapolated to the zero time axis. The slope of the line was then determined and expressed as a percentage of the extrapolated value for comparative purposes. Percentage uptake for both pond experiments are given in Table 2 together with the gross and net oxygen values. It was found that if the uptake rates were plotted against the gross oxygen production in each experiment, they formed a straight line

TABLE 2. *Summary of zinc uptake and metabolism of* Chaetomorpha Linum *suspended at different depths in a marine pond*

Date	Depth m	Zinc uptake %/g/hr[1]	Metabolism ml of O_2/g/hr Net production	Respiration	Gross production
VIII-21-59	0	29.5	1.54	0.95	2.49
	0.5	41.2	2.07	0.95	3.02
	1.0	27.0	1.48	0.95	2.43
	1.5	21.9	1.23	0.95	2.18
VIII-27-59	0.5	26.3	1.34	1.32	2.66
	1.0	22.6	1.07	1.32	2.39
	1.5	29.0	1.20	1.32	2.52
	2.0	15.5	0.84	1.32	2.16
	3.0	16.9	0.79	1.32	2.11

[1] See text for method of calculation.

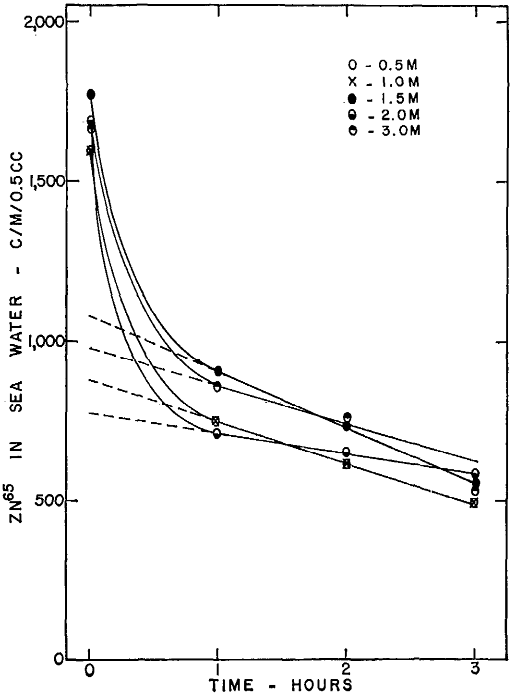

FIG. 4. Uptake of Zn^{65} by *Chaetomorpha Linum* suspended at the indicated depths in a marine pond on August 27, 1959. Values not corrected for the weights of algae used.

which intercepted the oxygen axis at or near to the experimental value for respiration. Thus the apparent net uptake of zinc appears to be related to the net oxygen production. A plot of these values for both experiments is given in Figure 5 along with a calculated regression line. In this case there was a coefficient of correlation of 0.955.

DISCUSSION

The loss of Zn^{65} from the sea water could be due either to physical adsorption on the surfaces of the algae and their associated epiphytes or to actual uptake into the algal cells. It is probable that the latter was what was measured in these experiments due to the facts that no uptake could be detected in the dark bottle controls and that rates of uptake were correlated with oxygen production indicating that a metabolic process was involved. The extent to which adsorption took place was obscured by the relatively large amount of non-radioactive zinc present in the tracer solution. In the experiments described this had the effect of increasing the zinc concentration from about 10 μg/L to about 150 μg/L. Thus the adsorption of 5 μg of zinc would result in only a 3% loss in activity which could not be distinguished from normal sampling and counting errors. When carrier free Zn^{65} was used by Chipman *et al.* (1958) to measure uptake in the planktonic diatom, *Nitzschia closterium*, it was found that about 80% of the zinc was taken up in the first hour both in the light and in the dark. In their case it is indicated that surface adsorption played an important role in zinc uptake since by means of repeated washings of labeled cells a large percentage of the Zn^{65} could be removed with the amount removed increasing as the zinc content of the washing medium was increased.

On the basis of the apparent straight line relationship between zinc uptake and oxygen production, it is suggested that zinc is taken up in direct proportion to the photosynthetic rate, and that it accumulates in the algae as a function of their growth or net production. Further research is thus indicated to test this hypothesis and if found true to establish a zinc to oxygen ratio in absolute terms. If such a relationship can be shown to be valid for several species over a wide range of conditions, Zn^{65} might provide still another tool for the estimation of primary production. This radionuclide would be of particular value under certain circumstances since its gamma radiations eliminate the self-adsorption problems found with other tracers commonly employed in productivity studies.

REFERENCES

ARNON, D. I. 1938. Microelements in culture solution experiments with higher plants. Amer. Jour. Bot., **25**:322–325.

CHIPMAN, W. A., T. R. RICE, AND T. J. PRICE. 1958. Uptake and accumulation of radioactive zinc by marine phytoplankton, fish, and shellfish. U. S. Fish and Wildlife Service Fish. Bull., 58(135):279–292.

MYERS, J. 1951. Physiology of the algae. Ann. Rev. Microbiol., **5**:157–180.

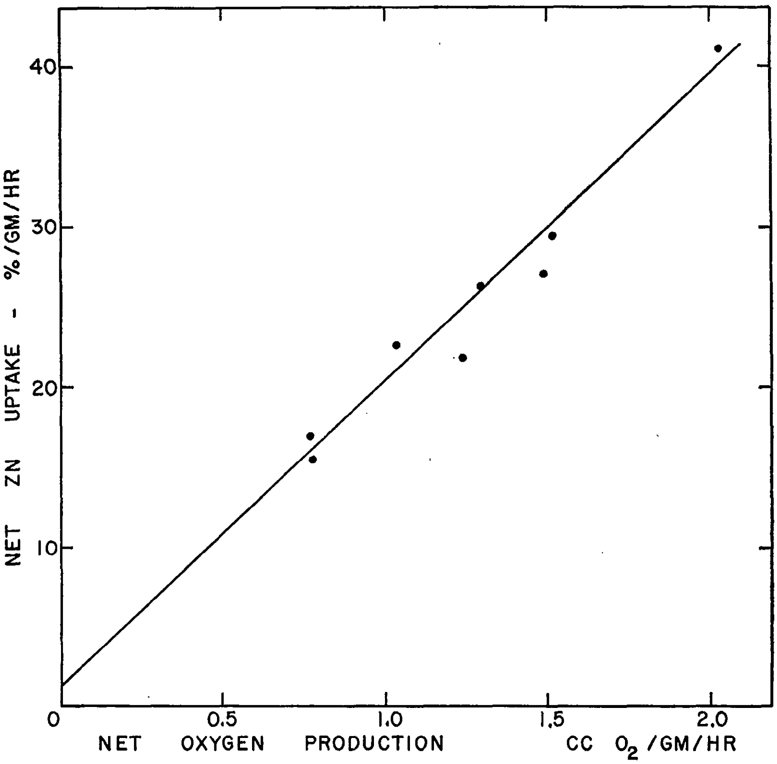

FIG. 5. "Equilibrium" uptake rates of *Chaetomorpha Linum* plotted against the net oxygen production. The least squares line is represented by Y = 18.8 X +1.4.

ODUM, E. P., E. J. KUENZLER, AND SISTER MARION XAVIER BLUNT. 1958. Uptake of P^{32} and primary productivity in marine benthic algae. Limnol. Oceanogr., **3**:340–345.

THORNE, W. 1957. Zinc deficiency and its control. *In* Advances in Agronomy, Norman, A. G. (*Ed.*). Vol. IX pp. 31–65.